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# Complementary and Alternative Medicine: Nanotechnology-I

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# PREFACE

In the swiftly evolving fields of medicine and veterinary science, nanotechnology has emerged as a cornerstone, reshaping how we approach disease prevention, treatment, and overall healthcare. *Complementary and Alternative Medicine: Nanotechnology-I* delves into the extensive potential of nanoparticles, presenting groundbreaking applications across human and veterinary medicine, alternative therapies, diagnostics, and disease control. This book offers a comprehensive guide to understanding how nanoscale innovations bring unparalleled precision and effectiveness, opening new doors for sustainable and advanced medical solutions. The book covers the multifaceted roles of nanoparticles in enhancing therapeutic approaches for various complex diseases. From advanced cancer therapies to solutions for resistant infections like tuberculosis and leishmaniasis, nanoparticles demonstrate capabilities that go beyond conventional treatments. This volume highlights their role in targeted drug delivery systems, which allow precise interaction with disease sites, minimizing side effects and maximizing efficacy. Nanoparticles' transformative role in cancer treatments, especially breast cancer, illustrates the impact of nanotechnology in oncology, offering novel, personalized approaches that were once considered science fiction. In the realm of veterinary medicine, nanoparticles bring new possibilities for disease management and growth enhancement in livestock and aquaculture. This book discusses advancements in nanoparticle-based treatments against diseases like salmonellosis and bovine mastitis, and explores how copper and silver nanoparticles can promote poultry health. Nanotechnology also revolutionizes fish medicine, helping create healthier aquatic environments and offering new methods for parasite control, which are vital for sustainable aquaculture practices. Beyond treatment, the book examines the diagnostic innovations fueled by nanotechnology, where nanoscale particles enhance imaging techniques and support the precise detection of conditions such as cancers and infectious diseases. The exploration of diagnostic imaging advancements, nanoparticle-based biosensors, and cancer screening tools illustrates how these innovations are transforming early detection and improving patient outcomes. Addressing health and wellness on a broader scale, the volume also covers the use of nanoparticles for immune response enhancement, wound healing, and regenerative medicine. Whether it's through natural product-loaded nanoparticles for wound care or inorganic nanoparticles for tissue engineering, this book illustrates the synergies between nanotechnology and complementary medicine, presenting a holistic approach to health that emphasizes both innovation and sustainability. The book also considers the potential toxicities and environmental impacts of nanoparticles, recognizing the need for ethical and safe deployment of these powerful tools. Discussions on nanoparticle-induced toxicity on human health, particularly on the nervous, immune, and respiratory systems, provide an important reminder of the responsibility that comes with such advanced capabilities. Equally important are sustainable practices, such as green synthesis methods, which leverage plant-based nanoparticles and environmentally friendly approaches to reduce potential risks. *Complementary and Alternative Medicine: Nanotechnology-I* serves as a crucial resource for students, researchers, and practitioners interested in the cutting-edge integration of nanotechnology with complementary medicine. By bridging the latest technological advancements with traditional healing approaches, this volume reflects a forward-looking perspective on healthcare that combines innovation with a commitment to ethical, holistic, and sustainable solutions. Through this exploration, readers will gain valuable insights into how nanotechnology is poised to reshape medicine, offering transformative possibilities for improving human and animal health.

Editor

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Chapter 01

# Applications and Perspectives of Nanotechnology in Veterinary Medicine

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## ABSTRACT

Nanotechnology, founded in 1974, is a rapidly evolving field with applications in various disciplines, including research, agriculture, and infection therapy delivery tests. Nanomaterials have the potential to enhance medicine delivery, improve animal health and well-being, and reduce adverse effects. Nanoparticles, substances with at least a single dimension in the nanoscale (less than 100 nm), are highly useful in biomedical fields due to their tiny dimensions and compatibility with many biological organisms. They are small enough to move around the body without interfering with normal physiological functions. Nanomaterials are categorized into four categories: zero-dimensional (0-D), one-dimensional (1-D), two-dimensional (2-D), and three-dimensional (3-D). In veterinary medicine, a vast scope of nanomaterials is utilized. It comprises carbon nanotubes, polymer nanostructures, liposomes, micelles, nanoparticles (NPs), nanofibers, nano-platelets, and nanocapsules. Nano materials are used in different aspects including; diagnosis, treatment, gene therapy, vaccines, tissue scaffold, meat packaging and poultry nutrition.

**KEYWORDS**
Drug delivery, Gene therapy, Nanomaterials, Nanovaccines, Veterinary Sciences

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## INTRODUCTION

Nanotechnology is considered as a rapidly evolving area that was founded about 1974 to assemble innovative materials ranging from 1 to 100 nm. The term "nano" is derived from the Latin word "nanus," which means "predominate" and denotes an extremely small size ($1nm=10^{-9}$) (Bayda et al., 2020). Nanotechnology is considered as another discovery with applications in a variety of disciplines, including research, agriculture, and infection therapy delivery tests. Moreover, nanomaterials may have got an impact on in-vivo and in-vitro medical specialty applications and research (Youssef et al., 2019). Most countries' economies rely primarily on animals. Despite the increase of various diseases, new significant and useful technologies are generated in the future to diagnose and treat animal diseases and finally expanding the protein provision for human feeding (Kappes et al., 2023). Veterinary medicine is important in biological studies, translational research in medicine, public health, the investigation of infectious and developing diseases, animal medication manufacture, exotic and domestic animal therapy, and managing ecosystems (Christopher, 2015). Nanotechnology has a tremendous potential to enhance medicine delivery. Animal diseases, both infectious and non-infectious, can be effectively diagnosed, treated, and prevented by using nanoscale materials and drug delivery systems (Huang et al., 2024). Veterinarians can use targeted and controlled drug delivery with nanotechnology to improve animal health and well-being by reducing adverse effects and increasing efficacy. Compared to traditional vaccinations, a new class of vaccines administered by nanovectors demonstrated noticeably greater efficacy. Furthermore, a direct evaluation of the feeds' quality was made possible by the use of nanoparticles. There are currently test systems on the market that use nanoparticles for quality assurance and inspection of various products with both plant and animal origins (Bezbaruah et al.,

2022). These cutting-edge methods have shown encouraging results in veterinary medicine, providing improved therapeutic outcomes.

**Nanoparticles**

The term nano is derived from the Greek word nanos, which meaning "a dwarf". At the 14th conference of the International Union of Pure and Applied Chemistry (IUPAC), the prefix nano was officially adopted to signify the one billionth part ($10^{-9}$) of a unit (Joudeh and Linke, 2022). Nanoparticles are substances with at least a single dimension in the nanoscale (less than 100 nm). Nanomaterials are extremely useful in biomedical fields due to their tiny dimensions and compatibility with many biological organisms. These substances have sizes similar to proteins (50nm), genes (10-100nm), viruses (20-450nm) and cells (1-100µm). Nanoparticles are small enough to move around the body with no interfering with normal physiological functions (Danchuk et al., 2023).

**Classification of Nanomaterials**

Nanomaterials are the most significant parts of nanotechnology. Nanomaterials are branched into 4 categories depending on their dimensions:

(1) Zero-dimensional nanomaterials (0-D) have the three dimensions at the nanoscale level. This includes quantum dots, fullerenes, and nanoparticles.

(2) One-dimensional nanomaterials (1-D): These nanomaterials comprise a single dimension beyond the nanoscale. These include nanotubes, nanofibers, nanorods, nanowires, and nanohorns.

(3) Two-dimensional nanomaterials (2-D): These nanomaterials possess two dimensions that extend beyond the nanoscale. This includes nanosheets, nanofilms, and nanolayers.

(4) Three-dimensional nanomaterials (3-D), also known as bulk nanomaterials, are substances that are not limited to the nanoscale in any dimension. This category includes bulk powders, nanoparticle dispersions, nanowire and nanotube arrays, and so on (Kolahalam et al., 2019; Joudeh and Linke, 2022)

**Characteristics of Nanomaterials**

Nanoparticles are preferable than other substances when it comes to of surface-to-volume bioavailability, bioactivity, ratio, reactivity, stability, regulated particle size, controlled drug release, site-specific targeting, and controlled medication delivery. Furthermore, nanotechnology provides great prospects for delivering medicines due to its capacity to enter cells, tissues, and organs rather than macroparticles, exceeding existing drugs' low bioavailability and significant cytotoxicity. Medications could be incorporated into nanoparticles or bonded to their surfaces (Patra et al., 2018; Abbasi et al., 2023).

**Synthesis of Nanoparticles**

Nanoparticle production is mostly determined by the requirements and desired applications of the final product. When employed for biological systems, consideration is also given to the reliability of the active ingredient, the toxicity of the nanoparticle, its release, and its potential impact on the living system (Ahmad et al., 2022). Nanoparticle synthesis can be broadly classified into chemical and biological methods (Fig. 1).

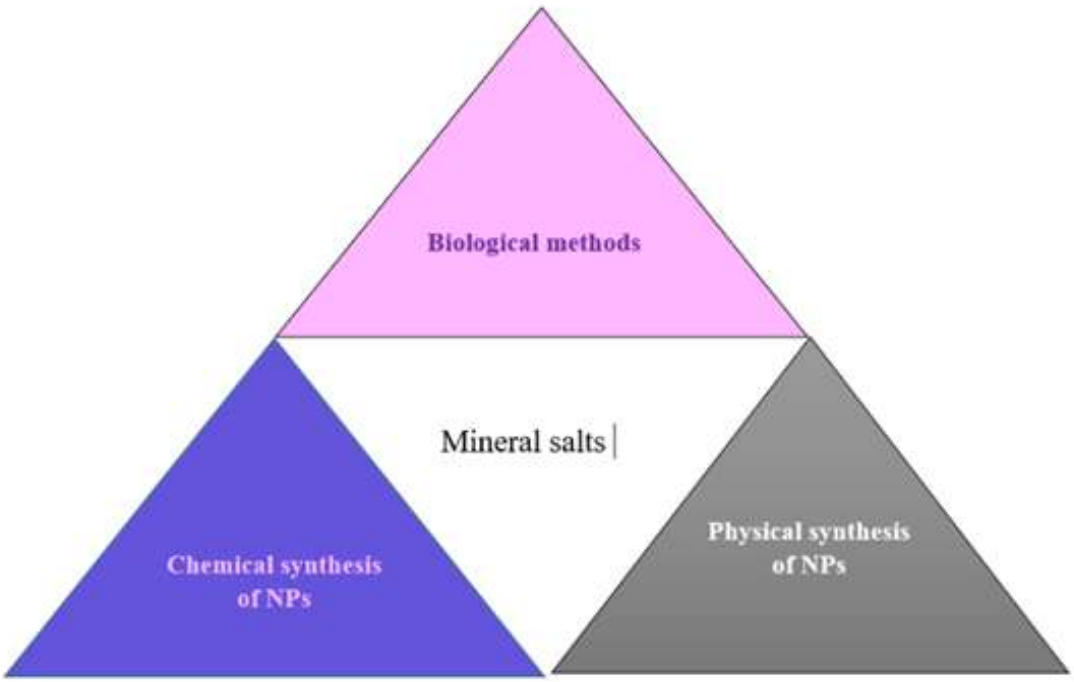


**Fig. 1:** Biological, chemical, and physical methods of nanoparticles synthesis

**Nanotechnology Application in Veterinary Medicine**

In veterinary medicine, a vast scope of nanomaterials is utilized. It comprises carbon nanotubes, polymer nanostructures, liposomes, micelles, nanoparticles (NPs), nanofibers, nano-platelets, and nano-capsules (Danchuk et al., 2023).

**Diagnostics**

Certain nanomaterials (e.g., magnetic, fluorescent, catalytic) can be used as probes for various imaging and diagnostic applications because of their inherent physicochemical qualities and biocompatibility. The successful improvement and employment of bio-chips for the early diagnosis of animal diseases is made feasible by advancements in nanotechnology. The foundation of those chips is a silicon pool that has numerous small recombinant segments of DNA tagged on it. The bio-chips can also be used to screen the different infections in animal feed. Furthermore, that type of probe can function as the main component of nano-diagnostic devices since it can identify particular organic molecules in lymph, blood or any biologic fluids. Different xenobiotics can be identified in the bodies of domestic and farm animals by bioanalytical nanosensors (Han et al., 2019). Antibodies coupled with nanoparticles are much solid, bio-compatible, and susceptible to target-antigens (Lara and Perez-Potti, 2018). In order to identify a variety of animal infectious diseases, including Newcastle disease, PMWS (post-weaning multisystem wasting syndrome), which is brought on by PCV2 (porcine circovirus type 2), and bird flu, efficient bio-analytical kits of ELISAs (enzyme-linked immunosorbent assays) were improved exploitation nanomaterials (Gao et al., 2020; Khodadadi et al., 2020). An excellent non-invasive method of detecting many animal illnesses and diseases is through the use of electronic noses. Food goods like milk and olive oil have successfully had their quality controlled using electronic nose technology (Esfahani et al., 2020). Today, these sensors can be used to diagnose and distinguish between infections of the upper respiratory tract caused by *Pseudomonas aeruginosa, Staphylococcus aureus, Haemophilus influenzae,* and *Streptococcus pneumoniae* pathogens, as well as to detect the Mycobacterium bovis infections of cattle, diarrhea and urinary tract infections (Esfahani et al., 2020; Bernitz et al., 2021).

**Treatment**

Nano-formulations allow for a decrease in drug residues in farm animal husbandry products as well as the amount of antimicrobials given to animals in veterinary medicine. The most effective therapeutic dose-to-effect ratio can be achieved by the use of nanotechnology, which also provides tailored medication delivery into the animal body (Youssef et al., 2019). The drug administration could be carried out through "active targeting" by using certain intermediator which attach to the target cell surface receptors or "inactive targeting" by accumulation of NPs in the target organ according to their specific size) (Yoo et al., 2019). It is well recognized that several metallic nanoparticles, including copper (Cu) (Zhao et al., 2021), silver (Ag) (Chen et al., 2020), gold (Au) (Zuo et al., 2020), calcium silicate (CaSi) (Ding et al., 2021), titanium, and zinc oxide, have antibacterial qualities based on different processes (Mobed et al., 2021). Biofilm induced by bacterial infections are well-known for their antibiotic resistance and therapeutic challenges. Nanotechnology for drug delivery have showed high success in treating biofilm induced by bacteria in different diseases. For example, the activity of tobramycin was noticeably increased in bacterial biofilm by using Liquid crystalline nanoparticles (LCNPs) by enhancing antibiotic entrance into the target sites (Guo et al., 2023).

**Antibiotics and Antibiotic Resistance**

Various international organizations, considering the World Health Organization (WHO) and the United Nations Organization (UNO), have acknowledged antimicrobial resistance as a worldwide concern (Coque et al., 2023). Liposomal antibacterial medication forms have proven to be highly efficient in treating a variety of infectious disorders, including bovine mastitis, infections due to Gram-positive and Gram-negative bacteria, multiple resistant bacterial infections, and Mycobacterium avium disease (Nale and McEwan, 2023). Other nanomaterials as AgNPs (silver nanoparticles) are the best due to their ability to combat antimicrobial drug resistance, also their harmful impact on animals have also been extensively investigated. As a result, nanoparticles of silver have been utilized as an antiviral agent for the African Swine Fever virus and as an antibacterial addition for piglets (Dung et al., 2020; De Silva et al., 2021). These nano-formulations showed excellent antibacterial activity to bacterial infections such as; *Streptococcus uberis, E. coli, Staphylococcus aureus, Candida krusei* and *Candida albicans*. Metal nanoparticles have as well shown bright results toward biofilms and antibiotic-resistant bacteria (Luzala et al., 2022). In aquaculture, up to 80% of antibiotics provided through granulated impregnate feed end up in the aquatic environment. A promising method for removing antibiotics from aquaculture effluents using solar irradiation has been presented, which uses ecologically friendly carbon dot-TiO2 nanocomposites. This method is applied by using light to activate the TiO2-NPs, which in turn show a bactericidal action to many pathogens of fish (*Edwardsiella tard, Streptococcus iniae* and *Photobacterium damselae*), whereas nanoparticles of ZnO were efficient against Vibrio harveyi (Louros et al., 2021).

**Anti-parasitic Properties of Nanoparticles**

Nanotechnology provides various effective options for the therapy of parasite infections in humans as well as animals. Especially, the AgNPs produced from the Duddingtonia flagrans fungus shown an excellent nematicidal effectiveness, as the nanoparticles were capable to enter the larvae cuticle and eventually trigger the ending of zoonotic nematodes

(Barbosa et al., 2019; Bajwa et al., 2022). ZnO and silver nanoparticles were efficient in treating rabbit coccidiosis. Hydrosols containing nanoparticulated bismuth oxides and hydrated antimony have been suggested as novel methods of management of the local therapy of leishmaniasis caused by variety of Leishmania parasites such as; *Leishmania amazonensis, Leishmania guayanensis* and *Leishmania brasiliensis*. Liposome-based anti-parasitic vaccines with high efficacy have been produced. In sheep the injection of encapsulated "plasmid containing MIC3 gene" in liposomes resulted in an efficient immunological defense to Toxoplasma gondii (Nafari et al., 2020; Danchuk et al., 2023).

**Tissue Scaffolds (Electrospun)**

Electrospinning nanofibers enabled the creation of scaffolds for tissue rebuilding and regeneration (Flores-Rojas et al., 2023). One instance of the use of bio-degradable poly (lactide-co-glycolide)/hydroxyapatite (PLGA/HAp) electrospun nanofibers is in the treatment of bone lesions in dogs, where they serve as biomaterials for bone healing (Stevanovic et al., 2022). Therapy by stem-cell have showed promise as potential biomedical treatments for a variety of disorders, particularly in stroke, hind limb ischemia and myocardial infarction. Injecting capsules of bone marrow stem cells implantation with "MPEG-PCL-MPEG hydrogel (alpha-cyclodextrin/poly(ethylene glycol)-b-polycaprolactone-(dodecanedioic acid)-polycaprolactone-poly(ethylene glycol))" into a rabbit with myocardial infarction, improved cell survival and retention, and improved cardiac function compared to bone marrow stromal cells (BMSC) implantation alone (Van Nguyen et al., 2021).

**Administration of Nano-formulations in Neoplasms**

Recently, there has been a significant amount of research dedicated to nanoparticles with the ability to detect and eliminate cancer cells. Dogs and cats with naturally occurring tumors are considered the most reliable animal models for studying human carcinoma. As a result, they have been used in pre-clinical research to enhance the development of new treatments and imaging probes (Onaciu et al., 2020). Several types of malignancies, including osteosarcoma (OSA), mammary gland carcinoma, oral cavity squamous cell carcinoma (SCC), and transitional cell carcinoma, have striking similarities to human cancers and occur naturally in animals. For instance, mammary gland carcinoma in dogs and cats have similar morphological, clinical, epidemiological, and prognostic features as breast carcinoma in human. Cats and dogs are reasoned suitable models for studying human hormone-independent breast cancer. Currently, numerous studies have been conducted on the application of the nanorods of gold in photothermal therapy for breast tumors in cat models (Danchuk et al., 2023; Nosalova et al., 2024). The use of A nanotechnology in formulation of Hyaluronic acid altered with paclitaxel and cisplatin revealed good therapeutic results for mouth sarcoma, anal gland and oral melanoma cancer in dogs (Danchuk et al., 2023).

**Gene Therapy**

Applying nanoscale particles in medicine is a rapidly evolving process, with many practical and functional applications in diagnostic, therapeutic and preventive medicine. One of the recently emerging novel and most promising applications is in gene therapy. Many diseases such as cystic fibrosis, diabetes, cancer, heart disease and HIV/AIDS are difficult to treat without interfering with molecular base mechanisms like nucleic acids (Mendes et al., 2022). Clearly, the majority of studies on gene therapy is directed at curing diseases in human. Nevertheless, animals are commonly employed to investigate the efficacy, pharmacokinetics and pharmacodynamics of nano-formulations. These findings make it possible to apply the most recent advances in gene therapy to veterinary medicine. At present, the most common animal models include guinea pigs, hamsters, mice, rabbits, cows, dogs, cats and pigs. Gene therapy involves nucleic acid delivery for genetic editing and vaccines. It was approved in 2018 and since then many gene therapy products with various applications were introduced; an important example of such an application is the approval of mRNA vaccines to fight the COVID-19 outbreak (Mendes et al., 2022). There are three main ways gene therapy can be applied. One is by using CRISPR-Cas 9 technology to edit mutated genes. Another is by inserting a functional gene copy using molecules like pDNA, mcDNA, synthetic mRNA, circular RNA, or self-amplifying RNA. Lastly, gene therapy can be used to down regulate gene expression using molecules like siRNA, microRNA, antisense oligonucleotides, or short hairpin RNA (Li et al., 2020; Mendes et al., 2022). Currently, veterinary medicine is an essential bridging link between pre-clinical research and human medicine as large animals are used as experimental models in gene therapy. Large animal models offer numerous benefits over small ones (Casal and Haskins, 2006). For instance, both animals and humans exhibit significant anatomical and physiological analogies, corresponding living conditions, and risk factors for the emergence of specific diseases (Danchuk et al., 2023). Various gene therapy techniques were created for the therapy of arthritic, viral, and cardiovascular illnesses in horse and pig (Gopinath et al., 2015). In sheep the gene transfer technologies were used to conduct gene-mark experiments (Kalds et al., 2019). Gene therapy was shown to be effective in treating inherited hypercholesterolemia and cardiac disease in rabbits (Canepari and Cantore, 2023). The function and physiology of feline brain is extremely developed, hence cats are important in the advanced neurological diseases treatment by gene therapy (Gómez et al., 2009). More than half of the inherited disorders in dogs originated by mutations genes that are similar in humans. Also the immune system of dogs and humans are astonishingly similar, hence, promising results were obtained from using gene therapy in treating malignant melanoma,

hemophilia A and B, and mucopolysaccharidosis VII in dog models have been documented (Metcalfe et al., 2013; Almela and Ansón, 2019; Doshi et al., 2024). successfully tested a form of gene therapy for melanoma on horses. The results obtained in large animals show that gene therapy is highly effective and safe, and it was well tolerated. As a result, additional study in this area of veterinary medicine is actively urged.

**Innovative Nanovaccines**

One of the most significant public health implement that is essential in prevention and treatment of infectious diseases are Vaccines. Nanoparticles are integrated within biotechnology, bioinformatics, and cognitive science (NBICS) technologies (Danchuk et al., 2023). The recombinant protein, DNA and RNA molecules elicit cellular and humoral immunity are being developed to what so called "Innovative third-generation nanovaccines", and veterinary medicine is at the forefront of this innovation (Sadr et al., 2023). In veterinary practice, DNA nanovaccines perform noticeably better than traditional vaccines; yet, the protection effectiveness offered by bare plasmids typically ranges from 28% to 90% (Lu et al., 2024). A PLGA-PEI nanocarrier-based IBDV DNA vaccination showed greater effectiveness (up to 80%) in lowering avian morbidity and death (Danchuk et al., 2023). Additionally, liposome-encapsulated plasmid-based nanovaccines toward avian influenza (AIV) in chickens were created (Alqazlan et al., 2022) and ducks Anatid herpesvirus 1 (a liposome-encapsulated plasmid-chitosan) (Sun et al., 2013). Effective vaccinations against the Newcastle disease virus (NDV) of birds are examples of vaccines of chitosan NP carriers that shield DNA from deterioration (Taghizadeh et al., 2024).

**Nanotechnology use in Poultry**

A large amount of minerals is released into the environment in addition to being used by cells, meaning that many animal feeds, particularly those fed to poultry, have minimal amounts of minerals and, even in cases where they are, have extremely low bioavailability. This means that the animal needs more mineral supplements, which raises expenses (Brugger et al., 2022). Fig. 2 shows altered and improved features of mineral nanoparticles with their beneficial aspects for different applications.

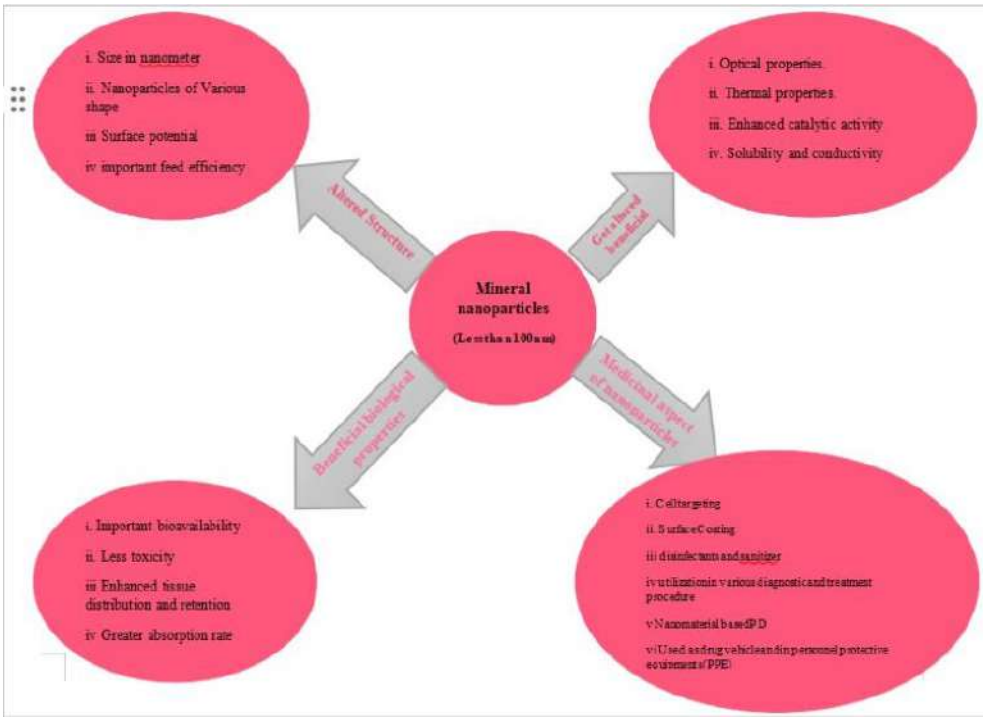


**Fig. 2:** Shows altered and improved features of mineral nanoparticles with their beneficial aspects for different applications

The titanium dioxide, that serves as a feed colorant and a UV protection in feed wrapping, is an example of inorganic nanoparticles that are widely utilized (Ashfaq et al., 2022). Molecules of sugar, fat and protein are categorized as organic nanoparticles. The term "nanocapsule" refers to the ability of organic nanoparticles to encapsulate and transfer nutrients via blood vessels. Nanocapsules are utilized to provide nutrients without changing their flavor or look because of their improved bioavailability (Altemimi et al., 2024). These encapsulated nanoparticles are added to feed as liposomes, as well as serving as an antibacterial agent in stored feed, a shelf-life extender, a biosensor in the feed packaging system, and identifying markers (Ahmad et al., 2022). Another type of organic nanoparticles is called nanoemulsion, and its primary application is as a stabilizing agent to distribute the active ingredients in a continuous phase or at the water/oil interface (Azmi et al., 2019). Many reports are available in the literature about the utilization of nanoparticles in poultry either in feeding, watering, or also through other routes to improve bird's health (Ahmad et al., 2022). Fig. 3 depicts the mode of action of mineral nanoparticles, which enter the cell either directly or indirectly and induce genotoxicity, inhibit the signaling route, or destroy key organic molecules.

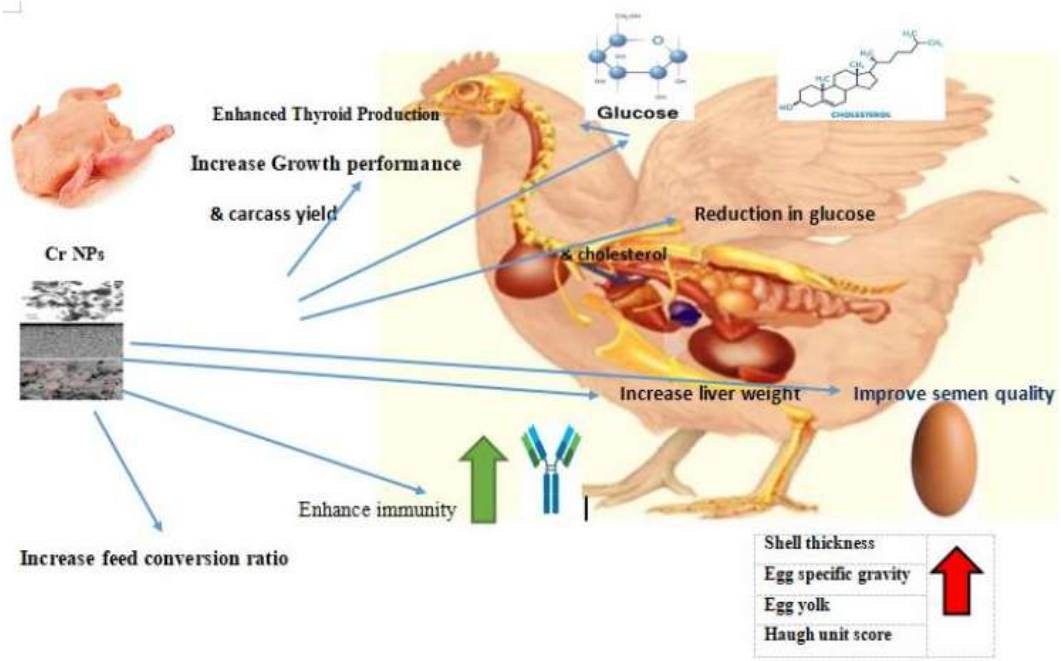


**Fig. 3:** Mode of action of mineral nanoparticles, by entering the cell either directly or indirectly, causes genotoxicity, blocking the signaling pathway, or may cause destruction of major organic compounds.

**Application of Nanoparticles as Meat Packaging Materials**

Materials used to package food should have the right mechanical, thermal, and optical characteristics. As food packaging materials, antimicrobial and barrier qualities against gasses, mist, and scent are also crucial. Because the materials used to package food are currently non-biodegradable, there are environmental problems. However, biopolymer-based environmentally friendly packaging cannot have the best mechanical and barrier qualities. In response to consumer demands for more reasonably priced, high-quality meat products and increased competition, the meat production industry has seen remarkable changes in both the ingredients and the processing system (Weiss et al., 2010; Smaoui et al., 2023). Innovation in the meat product sector has increased as a result of the need for sustainable meat production and the focus on human health and wellness (Young et al., 2013). As a result, there is now greater expectation for the use of functionally enhanced ingredients and additives to improve the quality and reputation of muscle meals (Olmedilla-Alonso et al., 2013). Probably the most normally involved added substances in meat and poultry are cell reinforcements, bioactive materials giving medical advantages are progressively added to food sources to treat or forestall illnesses, ZnO-NPs exhibit strong antimicrobial activity against a variety of bacteria, making them intriguing antibacterial experts in the food industry (Chérif et al., 2022). By limiting the growth of bacteria and slowing down the oxidation of lipids and proteins, NPs improve the quality of preserved meat products as safer nanoparticles. It was surprising to see that dynamic bundling with NPs—whether blended or not—was an environmentally friendly arrangement and potential future alternative for the meat business (Ulloa-Saavedra et al., 2022). After an intravenous (i.v.) dose of NPs, they are distributed foundationally throughout the body, including the kidneys, liver, heart, spleen, and lungs. Nanoparticle attachment causes neurotoxicity, oxidative stress, tissue aggravation, and histopathological abnormalities in adult mice (Ibrahim et al., 2018; Fadia et al., 2022). All in all, we could recommend that there is a squeezing prerequisite for the guideline of nanomaterials before their consolidation into meat handling and bundling.

**Conclusion**

Nanotechnology has the potential to revolutionize various fields, including veterinary medicine, by providing targeted and controlled drug delivery systems, improving animal health, and enhancing therapeutic outcomes. Nanoparticles are superior in terms of bioavailability, bioactivity, stability, and controlled drug release. They offer potential for delivering medicines due to their ability to enter cells, tissues, and organs, surpassing existing drugs' low bioavailability and significant cytotoxicity. Synthesis of nanoparticles is based on the requirements and desired applications of the final product, considering factors such as the active ingredient's reliability, toxicity, release, and potential impact on the living system. Nanotechnology is applied in veterinary medicine, utilizing various nanomaterials such as carbon nanotubes, polymer nanostructures, liposomes, micelles, nanoparticles (NPs), nanofibers, nano-platelets, and nanocapsules. These materials can be used for diagnostics, such as bio-chips for early diagnosis of animal diseases, bioanalytical nanosensors for xenobiotics identification, and electronic noses for non-invasive disease detection. Nanotechnology also allows for a decrease in drug residues in farm animal husbandry products and tailored medication delivery into the animal body. Active targeting of drugs can be achieved through intermediator attached to the nanoparticles.

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Chapter 02

# Silver Nanoparticles: A Revolutionary Approach in Complementary and Alternative Medicine

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## ABSTRACT

In complementary and alternative medicine (CAM), silver nanoparticles (AgNPs) have become a ground-breaking method that offers adaptable remedies with significant therapeutic promise. Silver was well-known for its antibacterial activities and is historically used to treat infections, wounds, and inflammations. AgNPs are attractive candidates for therapeutic interventions due to their unique physicochemical features, which include antibacterial, anti-inflammatory, and wound-healing qualities. Silver nanoparticles work synergistically with chemotherapeutic drugs and increase their cytotoxicity. Silver nanoparticles may synthesized through physical, chemical, and biological methods. They have high surface area to volume ratio, increasing their absorbance into the cells. Their incorporation into CAM procedures emphasizes a comprehensive approach to healthcare, which is consistent with the focus on all-encompassing treatment methods. AgNPs with their transformative potential, opening doors for novel therapeutic approaches and advances in integrative medicine.

**KEYWORDS**
Silver Nanoparticles, Complementary and Alternative Medicine, Clinical Use, Antimicrobial Activity

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## INTRODUCTION

The area of nanotechnology has attracted a lot of interest due to its potential uses in a variety of industries, including the medical industry. Silver nanoparticles (AgNPs), one of the many nanoparticles under investigation for biomedical applications, have shown great promise because of their distinct physicochemical characteristics and diverse range of biological activities (Menichetti et al., 2023). These nanoparticles differ from their bulk counterparts in that they have a high surface area-to-volume ratio, which enhances their efficacy and reactivity in a variety of applications. The unique physicochemical features of AgNPs, such as their antibacterial, anti-inflammatory, antioxidant, and wound-healing capabilities, make them appealing for use in therapeutic interventions in the medical field (Dawadi et al., 2021).

This chapter aimed to give a general overview of AgNPs by tracing their historical background from the conventional uses of silver in medicine to their shift into applications based on nanoparticles, their synthesis, and their mechanism of action. A special emphasis will be placed on the scope and importance of AgNPs in complementary and alternative medicine (CAM).

### Historical Context and Traditional uses in Medicine

Silver has been used medicinally for ages; historical background shows that it has been incorporated into different cultural practices and traditional medical systems across the world (Medic et al., 2019). The Greeks, Romans, Egyptians, and Chinese were among the ancient civilizations that used silver to cure wounds, infections, and other illnesses after realizing its medicinal properties. Silver was valued for its antibacterial potential, which aided in halting the growth of

microorganisms and fostering healing (Knetsch et al., 2011). Over time, a variety of health conditions were treated using silver-based treatments, such as colloidal silver solutions and ointments containing silver, in both conventional medicine and folk medicines (Azevedo et al., 2024). The area of medicine has transformed, thanks to advances in nanotechnology, which have provided new methods for therapeutic interventions, medication administration, and diagnosis. In terms of clinical practice and biomedical research, the transformation from traditional silver-based formulations to nanoparticle-based solutions marks a paradigm change (Bamal et al., 2021). AgNPs and other nanoparticles have several benefits, including enhanced bioavailability, targeted distribution, and customizable physicochemical features that improve their safety and effectiveness profiles. Because of their special qualities and adaptable functions, AgNPs have been used in medicine for wound dressings, antimicrobial coatings, drug delivery systems, and regenerative medicine, among other uses (Ravindran et al., 2013).

The term complementary and alternative medicine (CAM) refers to a wide range of treatment approaches and procedures that are not part of traditional Western medicine. Exploring the potential of AgNPs as natural medicines and supplements for boosting health and wellness is becoming more and more popular within the CAM field. AgNPs' innate antibacterial, anti-inflammatory, and immunomodulatory properties fit very nicely with complementary and alternative medicine's (CAM) emphasis on all-encompassing methods of treatment and disease management (Wei et al., 2021).

**Properties and Synthesis of Silver Nanoparticles**

Silver nanoparticles (AgNPs) have garnered significant importance in various fields, including medicine, due to their unique physicochemical properties and versatile applications (Pryshchepa et al., 2020). Because of their nanoscale size, AgNPs have different physical and chemical characteristics from their bulk counterparts. They have distinctive optical, electrical, and catalytic capabilities as well as increased reactivity due to their high surface area-to-volume ratio at the nanoscale. AgNPs can be used in a variety of applications, such as biosensors, antibacterial agents, drug delivery systems, and imaging agents, thanks to their qualities (Al-Jubbori et al., 2022).

**Physical and Chemical Properties of AgNPs**

AgNPs can vary in size from 1 to 100 nanometers physically, with smaller nanoparticles usually displaying more surface reactivity. They come in a variety of forms, each conveying unique qualities and functions, such as spherical, rod-like, triangular, and cubic (Gibała et al., 2021). AgNPs are chemically stabilized by capping agents, which include biomolecules, polymers, or surfactants, to keep the particles from clumping together and maintain their colloidal dispersion (Tao et al., 2020).

**Synthesis of Silver Nanoparticles**

AgNPs may be synthesized through a variety of techniques, each having unique benefits, restrictions, and effects on the characteristics of the nanoparticles. These techniques may be generally divided into three categories: biological, physical, and chemical synthesis approaches (Ahmed et al., 2016).

**Chemical Synthesis**

To synthesize AgNPs, silver ions in solution are reduced using chemical techniques. Polyol molecules, citrate ions, and sodium borohydride are examples of common chemical-reducing agents. Although these techniques provide exact control over the size, shape, and surface characteristics of nanoparticles, they frequently include the use of dangerous chemicals and produce poisonous byproducts (Ratan et al., 2020).

**Physical Synthesis**

To synthesize AgNPs, physical procedures including sputtering, evaporation-condensation, and laser ablation are used in physical approaches. These techniques often produce very pure, narrowly distributed nanoparticles, but they might also demand energy-intensive procedures and specialized tools (Kaabipour and Hemmati, 2021).

**Biological Synthesis**

Using biological techniques, the biological processes use the reducing and stabilizing powers of living things including fungi, plants, bacteria, and enzymes (Rahuman et al., 2022). By using biocompatible components and moderate reaction conditions, these green synthesis techniques provide economical and environmentally beneficial alternatives to chemical and physical procedures (Vishwanath and Negi, 2021). Furthermore, AgNPs with distinct surface functionalization and improved biocompatibility for use in biomedical applications may be produced by biological synthesis (Chugh et al., 2021).

**Factors Affecting the synthesis Process and Nanoparticle Characterization**

The concentration of the precursor, the temperature of the reaction, the pH level, the duration of the reaction, and the selection of the reducing and stabilizing agents are some of the variables that affect the synthesis and properties of AgNPs (Fig. 1). These factors are essential in establishing the size, shape, stability, surface charge, crystallinity, and biological activity of nanoparticles (Luceri et al., 2023). Furthermore, the yield, repeatability, scalability, and cost-effectiveness of nanoparticles might be impacted by the synthesis process and circumstances chosen, which calls for meticulous optimization and characterization (Yaqoob et al., 2020).

AgNP synthesis methods have come a long way in recent years, with the goals of increasing control over nanoparticle characteristics, increasing scalability, and reducing environmental effects. Innovative techniques that provide quick, effective, and scalable manufacturing of AgNPs with specific features include sonochemical synthesis, microwave-assisted synthesis, and microfluidic synthesis. These techniques have shown great promise as alternatives to conventional procedures. Additionally, the incorporation of machine learning and artificial intelligence (Shume et al., 2020).

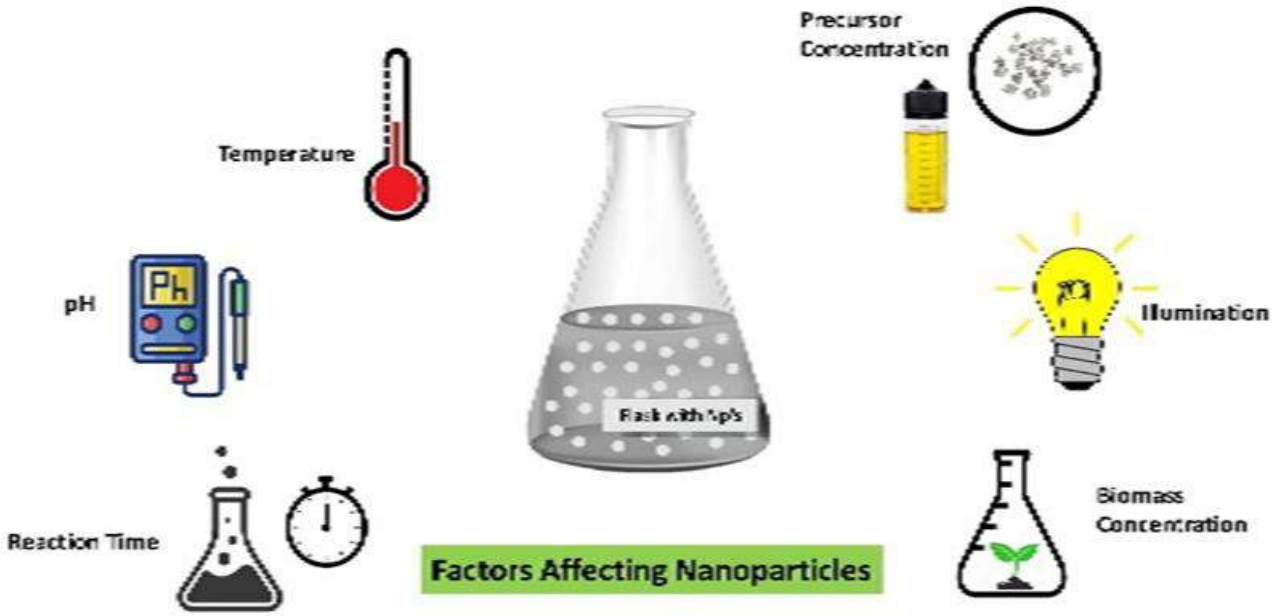


**Fig. 1:** Essential factors in establishing the size, shape, stability, surface charge, crystallinity, and biological activity of nanoparticles.

**Mechanism of Action of Silver Nanoparticles in Medicine**

The medical community has been paying close attention to silver nanoparticles (AgNPs) because of their many therapeutic applications. This chapter explores the many processes that AgNPs use to achieve their advantageous effects. These mechanisms include interactions with microbial cells, antibacterial and antimicrobial capabilities, immunomodulatory effects, and anti-inflammatory and wound-healing qualities (Dakal et al., 2016).

**Antimicrobial and Antibacterial Properties of AgNPs**

Because of its capacity to alter the structures and activities of microbial cells, AgNPs are well known for their strong antibacterial and antimicrobial action (Gong et al., 2023). The processes that underlie AgNPs' antibacterial activities are complex and include (Fig. 2):

**Cell Membrane Integrity Disruption**

AgNPs interact with microbial cell membranes, resulting in increased permeability and structural damage. Because of the compromised membrane integrity caused by this disturbance, cellular contents may leak out and ultimately lead to cell death (Wang et al., 2021). This interference damages the integrity of the membrane, resulting in the processes that underlie AgNPs' antibacterial activities are complex and include:

**Production of Reactive Oxygen Species (ROS)**

AgNPs can produce reactive oxygen species in microbial cells, including superoxide radicals and hydrogen peroxide (Fig.2). ROS causes oxidative stress, which damages lipids, proteins, and nucleic acids, reducing the survival and growth of microorganisms (Singh and Mijakovic, 2022).

**Interference with Cellular Metabolism**

AgNPs obstruct respiration, ATP generation, and enzyme activity, among other vital metabolic functions in microbial cells. AgNPs prevent the development and survival of microorganisms by interfering with these processes. AgNPs are intriguing prospects for fighting bacterial, fungal, and viral infections in a variety of clinical contexts due to their broad-spectrum antimicrobial action (Mazur et al., 2020).

**Mechanisms and Pathways of AgNPs and Microbial Cell Interaction**

The result of nanoparticle exposure is determined by complex molecular pathways that interact with AgNPs in microbial cells. AgNPs attach themselves to the surface of microbial cells and enter the cytoplasm, where they begin to

work as antimicrobial agents. AgNPs' surface charge enables electrostatic interactions with microbial cell membranes, which in turn promotes the adherence and internalization of nanoparticles. Silver ions, or $Ag^{+}$, are released by AgNPs into the environment. These ions can enter microbial cells and cause harm to internal components (Javed et al., 2021).

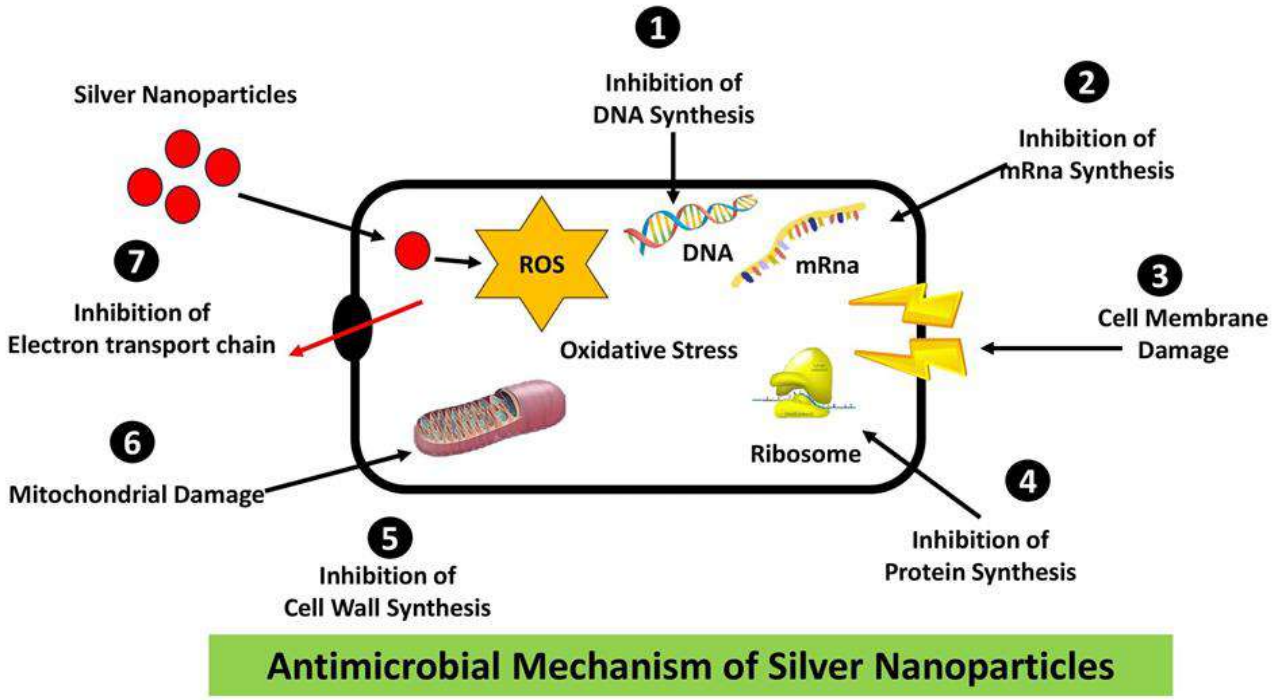


**Fig. 2:** Schematic representation of silver nanoparticles against bacteria ROS dependent pathway, DNA damage, enzyme inactivation, and protein denaturation for antibacterial action of silver nanoparticles.

**Protein Binding**

AgNPs can bind with microbial proteins, changing their structure and function. This causes disruptions to vital cellular functions and ultimately leads to the death of microbial cells. Clarifying the complex interactions between AgNPs and microbial cells is essential to maximizing their therapeutic efficiency and understanding their mechanisms of action (Backx et al., 2021).

**Immunomodulatory Effects of Silver Nanoparticles**

Apart from their direct antibacterial properties, AgNPs demonstrate immunomodulatory actions that impact the immunological response of the host. AgNPs can alter inflammatory signaling pathways, cytokine synthesis, and immune cell activity, which can result in the Activation and proliferation of innate immune cells, such as macrophages and dendritic cells, which are stimulated by AgNPs, improving the innate immune system's capacity to identify and eradicate infections (Vuković et al., 2021).

**Control of Inflammatory Responses**

AgNPs have anti-inflammatory qualities that reduce excessive inflammation and encourage the regeneration and repair of damaged tissue. AgNPs support immunological homeostasis and reduce inflammation by regulating cytokine production and signaling pathways. AgNPs have therapeutic promise in treating inflammatory illnesses, immune-related ailments, and infectious diseases because of their immunomodulatory activities (Tyavambiza et al., 2021).

**Anti-Inflammatory and Wound-Healing Properties of Silver Nanoparticles**

AgNPs have strong anti-inflammatory characteristics that are essential for tissue regeneration and wound repair. AgNPs ease pain, edema, and tissue damage by decreasing inflammation, which speeds up the healing process. AgNPs also encourage angiogenesis, collagen deposition, and wound closure, which speeds up tissue regeneration and healing (Alkhalaf et al., 2020). The following are important processes that underlie AgNPs' anti-inflammatory and wound-healing effects.

**Inhibition of Pro-inflammatory Mediators**

AgNPs prevent the generation of pro-inflammatory cytokines that cause tissue inflammation and injury, such as interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α). AgNPs promote the migration and proliferation of

fibroblasts and keratinocytes, two important cells involved in wound healing, which results in the creation of new tissue and skin regeneration. AgNPs promote the migration and proliferation of fibroblasts and keratinocytes, two important cells in wound healing, which results in the production of new tissue and the preservation of skin integrity (Wasef et al., 2020).

**Effects against Bacteria**

AgNPs' antibacterial activity aids in preventing wound infections and fostering a sterile environment that is favorable to healing. All things considered, AgNPs' diverse modes of action in medicine highlight their promise as adaptable therapeutic agents for treating a variety of medical disorders, from chronic wounds to infectious infections (Yin et al., 2020). Further investigation into the molecular processes behind the biological actions of AgNPs will increase our comprehension and make it possible to create tailored nanoparticle-based therapies that will lead to better health outcomes (Jabir et al., 2021).

**Applications of Silver Nanoparticles in Alternative and Complementary Medicine**

During Recent years, there have seen a considerable increase in interest in silver nanoparticles (AgNPs) because of their special physicochemical characteristics and their uses in several industries, including medicine. AgNPs show potential for a variety of therapeutic uses in the field of complementary and alternative medicine (Verma et al., 2019)

**Use of AgNPs in Traditional Medical Systems**

Ayurveda: The traditional Indian medical system known as Ayurveda acknowledges the medicinal benefits of silver, including its immune-stimulating and antibacterial qualities. AgNPs are used in Ayurvedic formulas because of their supposed rejuvenating qualities and capacity to harmonize the energy of the body. Conventional literature recommends using silver to cure a variety of illnesses and purify water, which is consistent with AgNPs' antibacterial activity (Islam et al., 2021).

Traditionally, silver has been used in Traditional Chinese Medicine (TCM) due to its therapeutic qualities. To increase their effectiveness and advance health and well-being, AgNPs are included in TCM herbal treatments. AgNPs are an important part of traditional medical procedures since silver is thought to balance bodily energy and speed up healing processes (Simon et al., 2022).

**Incorporation of AgNPs to Herbal Formulations and Supplements**

To maximize their therapeutic potential, AgNPs are progressively being added to dietary supplements and herbal formulations. Manufacturers want to build synergistic formulations for a range of health issues by fusing the advantages of herbal extracts with the antibacterial and anti-inflammatory characteristics of AgNPs. These combinations may be used to boost the immune system, promote healthy skin, and achieve other wellness objectives by using the synergistic effects of herbal components and AgNPs (Xu et al., 2020).

**AgNPs in the Wound Care and Management**

AgNPs are effective wound care agents due to their strong antibacterial characteristics. Their efficacy against a wide range of pathogens, such as fungus and bacteria, lowers the likelihood of wound infection. AgNPs aid in the preservation of a sterile wound environment, promoting healing and averting problems brought on by microbial colonization. AgNPs can hasten wound healing by encouraging angiogenesis, tissue regeneration, and cell proliferation. Their ability to penetrate the wound bed efficiently due to their nanoscale size allows them to influence the cellular signaling pathways involved in wound healing (Saratale et al., 2018).

**Potential Role of AgNPs in Cancer Therapy and Treatment**

Targeted drug delivery: AgNPs have potential as vehicles for precisely targeted medication delivery in cancer treatment. Anticancer medications can be encapsulated and delivered to tumor locations effectively thanks to their tiny size and high surface area-to-volume ratio. Targeting ligands, including peptides or antibodies, can be functionalized onto AgNPs to enable targeted delivery of cancer cells with minimal systemic toxicity and maximum therapeutic effectiveness (Ratan et al., 2020).

**Synergistic Effects with Chemotherapeutic Drugs**

AgNPs work in concert with traditional chemotherapeutic drugs in addition to serving as drug transporters. AgNPs increase the cytotoxicity of anticancer medications against cancer cells when used together. AgNPs' capacity to circumvent drug resistance pathways and enhance the therapeutic effects of chemotherapeutic drugs, resulting in better treatment results, is credited with this synergism (Lin et al., 2014).

**Handling Regulatory and Safety Issues while Using Silver Nanoparticles**

**Particle Size and Surface Properties:**

AgNPs' huge surface area and small particle size give them distinctive qualities and give rise to worries about possible toxicity. In contrast to their bulk counterparts, materials at the nanoscale can display unique biological behavior that

affects cellular interactions and biological reactions. The stability and toxicity of AgNPs can be affected by surface coatings and changes, highlighting the significance of comprehending structure-activity interactions (Santos et al., 2014).

**Cellular Uptake and Toxicity**

AgNPs can enter cells by several routes, including endocytosis and direct cell membrane penetration. This leads to cellular uptake and toxicity. AgNPs have the potential to cause cytotoxicity once internalized through a variety of pathways, including DNA damage, disruption of cellular processes, and production of reactive oxygen species (ROS). (Schneider et al., 2017).

**Risk Assessment and Mitigation Strategies**

Following are the various risk assessment and risk mitigation strategies of AgNPs.

**Hazard Identification and Exposure Assessment:**

AgNP risk assessment is determining possible risks related to its use and assessing exposure scenarios in medical environments. This entails evaluating variables like exposure route, dose, duration, and susceptible groups. AgNPs' physicochemical characteristics, which include their size, shape, and surface chemistry, affect their toxicological behavior and need to be taken into account when evaluating risk (Brudusel et al., 2018).

**Risk Mitigation Measures**

Several techniques, such as engineering controls, administrative controls, and personal protective equipment (PPE), can be used to reduce the dangers connected with AgNP exposure. During the manufacturing and handling procedures, engineering controls like ventilation systems and containment measures reduce the amount of AgNPs that are exposed to the air. Standard operating procedures and training programs are examples of administrative controls that guarantee safe practices and regulatory compliance. (Rezić and Meštrović, 2023).

**Overview of Key Clinical Trials and Studies Utilizing AgNPs in CAM: Immunomodulatory Effects**

Clinical trials have looked into the AgNPs' immunomodulatory qualities in CAM, especially immune-related conditions including allergies and autoimmune diseases. The objective of these investigations is to clarify the possible therapeutic advantages of AgNPs in regulating immunological dysregulation by evaluating their effects on immune cell activity, cytokine production, and inflammatory responses (Gherasim et al., 2020).

**Antibacterial Efficacy**

The antibacterial effectiveness of AgNPs against a range of pathogens, such as bacteria, fungi, and viruses, has been assessed in clinical trials. The effectiveness of AgNP-based therapies in treating infections and preventing microbial colonization in wound care, respiratory infections, and other infectious disorders frequently treated in complementary and alternative medicine (CAM) settings is investigated in this research (Samberg et al., 2011).

**Case Studies Demonstrating the Efficacy of AgNPs in Various Medical Conditions: Wound Healing**

Research has shown that AgNPs are effective in controlling chronic wounds, including burns and diabetic ulcers, and in accelerating wound healing. AgNP dressings have been demonstrated to improve clinical results and increase patient satisfaction by hastening wound healing, lowering inflammation, and preventing infection (Railean et al., 2022).

**Dermatological Disorders**

Case studies in dermatology have shown that AgNP-based formulations can effectively treat a range of skin disorders, such as acne, psoriasis, and eczema. AgNPs are useful adjuncts in the management of inflammatory skin illnesses and the promotion of skin regeneration because they possess antibacterial, anti-inflammatory, and wound-healing activities (Papakostas et al., 2011).

**Challenges And Limitations Faced In Clinical Research Using AgNPs**

**Standardization and Quality Control**

The absence of defined procedures for the synthesis, characterization, and dosage of treatments based on nanoparticles is a major obstacle to performing clinical research with AgNPs. The repeatability and comparability of research findings can be impacted by variations in particle size, shape, surface chemistry, and manufacturing procedures (Dos Santos et al., 2014).

**Safety Concerns**

Clinical research is severely hampered by safety concerns about the possible toxicity of AgNPs. AgNPs have been shown to have therapeutic benefits; nevertheless, questions have been raised about their long-term safety, potential for systemic accumulation, and potential negative impacts on human and environmental health. The clinical usage of AgNPs must be guided by a thorough review of safety profiles and risk-benefit analyses (León-Silva et al., 2016).

**Promising Outcomes and Prospects for Further Applications**

AgNPs show potential as carriers for targeted medication delivery in complementary and alternative medicine (CAM), allowing for the exact administration of therapeutic substances to certain tissues or cells. (Fakruddin, Hossain, et al., 2012).

**Personalized Medicine**

With the development of nanotechnology and personalized medicine, customized treatments employing AgNPs in CAM could become possible. Customized nanoparticle-based diagnostics and therapies can maximize therapy efficacy and minimize side effects by taking into account unique patient features such as genetic makeup, disease status, and reaction to treatment. Clarifying the therapeutic potential of silver nanoparticles in complementary and alternative medicine is mostly dependent on case studies and clinical trials. Promising results from clinical research demonstrate the varied applications of AgNPs in treating medical diseases across several disciplines, despite obstacles and limits. It looks promising that further research on standardization, safety evaluation, and customized methods will advance the use of AgNPs in complementary and alternative medicine (CAM) and enhance patient care (Ryu et al., 2014).

**Future Perspective and Challenges: Navigating the Path Forward with Silver Nanoparticles in Complementary and Alternative Medicine:**

Future Perspective and Challenges are required to navigate through the way Forward with AgNPs in Complementary and Alternative Medicine.

**Emerging Innovations and Trends in AgNPs Research**

Emerging trends and breakthroughs that have the potential to advance the use of silver nanoparticles (AgNPs) in complementary and alternative medicine (CAM) are driving the field's ongoing evolution (Ovais et al., 2018). Important areas of attention consist of following points:

**Multifunctional Nanoparticles**

Scientists are working on creating multifunctional AgNPs that may precisely target particular biological pathways or carry therapeutic payloads. Multifunctional AgNPs provide improved efficacy and decreased off-target effects in CAM applications by combining targeting ligands, stimuli-responsive coatings, or therapeutic compounds (Kim et al., 2018).

**Nanoparticle-Enabled Diagnostics**

AgNPs are being used to create biosensors and imaging agents, among other cutting-edge diagnostic instruments. In CAM contexts, these nanoparticle-based technologies facilitate early disease diagnosis and individualized treatment plans by enabling the quick and sensitive identification of biomarkers (Caputo et al., 2020).

**Integration of AgNPs into mainstream of healthcare Practices**

Efforts are in motion to include AgNPs in standard medical procedures as their research advances. To create evidence-based guidelines and protocols for the safe and efficient use of AgNPs in complementary and alternative medicine, researchers, physicians, and regulatory bodies are working together on collaborative efforts. The use of AgNP-based formulations and therapies in clinical settings is further facilitated by healthcare professionals' education and training programs (Kim et al., 2023).

**Addressing Challenges Such as Scalability, Cost-Effectiveness, and Standardization:**

AgNP production and formulation challenges related to scalability, cost-effectiveness, and standardization represent major obstacles to their widespread use in CAM. To overcome these challenges following measures should be adopted.

**Scalability**

To produce AgNPs in large quantities without sacrificing quality or safety, researchers are looking into scalable synthesis techniques and production procedures. The development of continuous flow reactors and green synthesis methods offers hope for getting around scalability issues and cutting production costs (Gottfredson et al., 2015).

**Cost-Effectiveness**

Increasing cost-effectiveness without sacrificing therapeutic efficacy is the goal of optimizing AgNP formulations and production procedures. Cost-effective strategies, like as cheap precursors and effective purification techniques, help lower total production costs and increase patient access to AgNP-based treatments (Singhal et al., 2018).

**Standardization**

To guarantee uniformity and repeatability among various formulations and applications, standardization of AgNP synthesis, characterization, and quality control procedures is crucial. The goal of cooperative projects combining government agencies, business partners, and academic institutions is to provide uniform standards and procedures for the identification and assessment of AgNPs in CAM (McClements et al., 2016).

**Conclusion**

In conclusion, there is no denying the revolutionary potential of silver nanoparticles in medicine. AgNPs, who offer adaptable solutions that combine traditional knowledge with state-of-art-research, represent a paradigm shift in how we approach healthcare as we stand at the nexus of tradition and innovation. We can open up new CAM avenues, enhance patient care, and usher in a new era of integrative medicine where healing is comprehensive, individualized, and enabled by the transformative potential of nanotechnology by utilizing the special qualities of AgNPs and interdisciplinary collaboration. Let us go out on this adventure with hope, curiosity, and dedication to achieving the full potential of silver nanoparticles in transforming the medical field for the benefits of humankind.

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Chapter 03

# Nanoparticles as a Potential Drug Candidate for the Treatment of Leishmaniasis

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**ABSTRACT**

One common parasitic infection that is included in the category of neglected tropical diseases is leishmaniasis. It is to blame for rising rates of sickness and mortality, particularly in low- and middle-income nations. The three most common types of leishmaniasis are visceral, mucocutaneous, and cutaneous. Since the present anti-leishmanial drugs have various drawbacks, such as limited efficacy, toxicity, negative side effects, drug resistance, length of treatment, and cost lines, chemotherapy for leishmaniasis has remained unsatisfactory. Moreover, the absence of efficacious preventive vaccinations impedes the management of the illness. As such, the quest for novel anti-leishmanial compounds is urgently needed. Nanoparticles have been shown in numerous recent studies to be a viable therapeutic agent for the treatment of anti-leishmanial disease. They can also be included with chemical medications to increase their quality, efficiency, and sustainability while also lowering their cost. The goal of this study is to present a thorough analysis of the various nanoparticles that may be employed in the future to treat leishmaniasis.

**KEYWORDS**
leishmaniasis, Chemotherapy, Alternative treatment, Nanoparticles, Anti-leishmanial

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## INTRODUCTION

Leishmania is genus of the protozoan species. It infects humans, carnivores, and rodents that spreads by sandflies bites and Leishmania parasite has 2 forms, amastigotes and promastigotes. Amastigotes are intracellular, without flagellum, spherical, and non-motile forms, which proliferate within the phagolysosomes of phagocytic cells like macrophages of the vertebrate host. The other form, promastigotes, is inside the sandflies, extracellular, spindle-shape, motile, and flagellated, as show in (Fig .1).

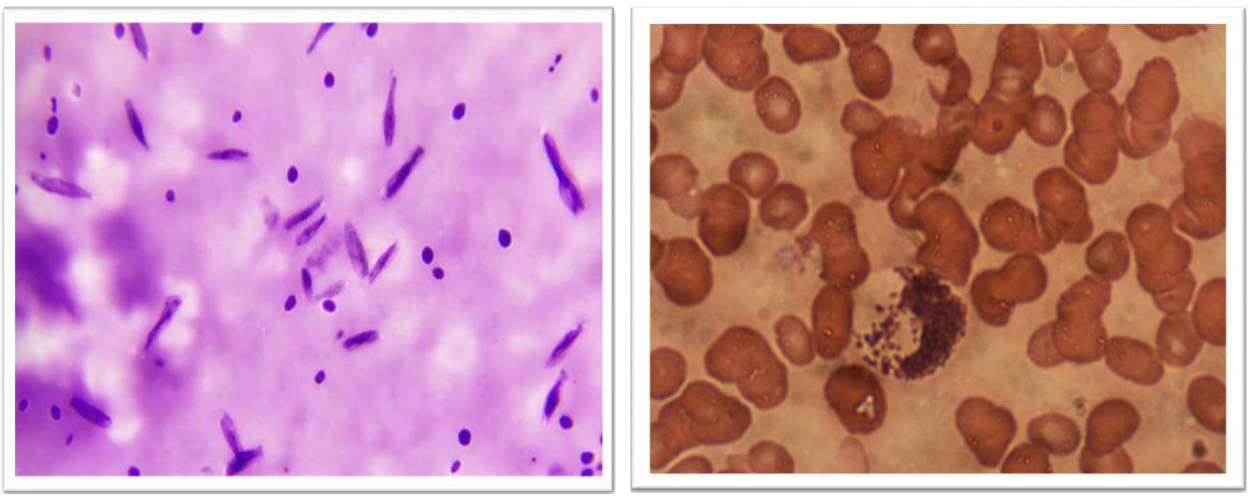

**Fig. 1:** The promastigote and amastigote of the Leishmania parasite (Giemsa stain, 100x)

There are three primary varieties of leishmaniasis that can occur: the most frequent variety, cutaneous leishmaniasis (CL); the deadliest type, visceral leishmaniasis (Kala-azar) (VL); and finally, mucosal leishmaniasis (ML) (Alvar et al., 2012). It occurs more frequently in rural areas than in cities. However, due to deforestation and climate change, leishmanian vectors have colonized metropolitan areas, spreading the illness to formerly uninfected areas (de Barros Dias et al., 2020; Oliveira et al., 2020). It is estimated that 12–15 million individuals globally suffer from this illness, with the majority of cases occurring in impoverished and/or developing nations because of inadequate cleanliness, a lack of preventative measures (such as nets, vector control and curtains), and a lack of health infrastructure (Saleem et al., 2019; Volpedo et al., 2019; Oliveira et al., 2020). Due to its high rates of infection and mortality, leishmaniasis has recently drawn more attention (Vaghela et al., 2017; Nafari et al., 2020; Ahmad et al., 2020). Unfortunately, there is still no effective chemical treatment to fight the disease, nor a vaccination or safe medication to inhibition parasite and other kinds of it (Mahmoudvand et al., 2017).

**Leishmaniasis Treatment**

**Traditional Treatment of Leishmaniasis**

The treatments for leishmaniasis consist of many of anti-leishmanial agents such as (Sodium stibogluconate, Meglumine antimoniate, Amphotericin B deoxycholate, Liposomal amphotericin B, Micellar amphotericin B, Pentamidine, Miltefosine, Paromomycin, Sitamaquine), These treatments are toxic and need a long-term treatment, which results in low patient compliance and drug resistance. Moreover, they usually incur considerable costs and necessitate cure (Akbari et al., 2017; de Souza et al., 2018). Finally, individuals with compromised cellular immune function, such as those infected with the human immunodeficiency virus (HIV), experience treatment failure and higher rates of relapse (Bruni et al., 2017). Moreover, vaccines have not succeeded in reaching clinical trials, and chemotherapy remains the only available treatment option for the time being. Therefore, in order to replace or enhance the current medications, and should develop new, more potent medications with enhanced features.

**New or Alternative Treatment of Leishmaniasis**

A nanoparticle is a small particle with a size (1-100) Nm. NPs is invisible, but it could detect by many of characteristics (physical and chemicals) from their larger material counterparts (Joudeh and Linke, 2022). The vast topic of nanomedicine has surfaced as a potential solution to problems pertaining to the side effects of older medications (Keyhani et al., 2020). This discontent stems from insufficient drug biodistribution, which results in a restricted therapeutic response and a range of adverse effects on healthy organs (Barratt, 2003; Beija et al., 2012). Additional observations point to the significance of nano-dimensionality in enhancing the efficiency of procedures like cellular absorption and biological barrier crossing, which can aid in the development of medications that are efficient in addressing infected tissues (Maeski, 2002; Couvreur and Vauthier, 2006; Huh and Kwon, 2011). In recent years, the use of nanoparticles in medicine, particularly for parasitic illnesses, has gained particular attention. Whereas the costly, energy-intensive, and potentially hazardous physical and chemical synthesis methods were being replaced with more ecologically friendly ones, nanoparticles were being created "biologically" through the use of plant- or microorganism-mediated reduction processes (Bhardwaj et al., 2020). In addition to being less costly and more ecologically friendly than chemically produced compounds, experiments have demonstrated their biological efficacy against diseases and may even be somewhat superior to them. To get rid of parasites, nanoparticles can be applied alone or in conjunction with other materials. Because of their destructive and inhibitory properties, it is therefore advised to utilize nanoparticles to eradicate parasites, improve the safety and efficacy of medications, and create efficient vaccines to prevent and combat parasites, particularly the Leishmania parasite (van Griensven and Diro, 2019). The NPs are believed to use a variety of methods to destroy microorganisms, including microbial membrane disintegration that results in oxidative stress, cell damage caused by ROS that damage DNA and leading into death of the cell by protein and lipid oxidation, and release of the ions that inhibit enzyme activity by displacing metal from metalloenzymes. These mechanisms are thought to prevent pathogens from evolving resistance (Ahmad et al., 2020). These new combinations can decrease side effects and the number of doses at a fair cost, increasing the likelihood of producing effective leishmaniasis treatments, regardless of production costs (Chávez-Fumagalli et al., 2015). Various metal and metal oxide nanoparticles have emerged as potent anti-leishmanial agents thus far such as:

- **Silver Nanoparticle (AgNPs)**

Since silver nanoparticles have a wide range of antibacterial activities, they are very important. As a result, silver nanoparticles have been employed as a substitute therapeutic agent to combat different types of leishmaniasis resistance. *Leishmania spp.* are relatively sensitive to ROS, which is the primary source of silver nanoparticles' antibacterial action (Allahverdiyev et al., 2011). Similar to antimony in its mode of action, silver is inhibitor of trypanothione/trypanothione reductase (Zahir et al., 2015; Baiocco et al., 2011). For promote the NPs accumulation in *L. major*-infected, the delivery of a small amount of silver NPs with electroporation. It causes high toxicity on macrophage and Leishmania (Dolat et al., 2015). Bioactive phytochemicals like flavonoids and alkaloids give biogenic metal nanoparticles (Metal NPs manufactured by use different plants) their extra antibacterial activity (Ahmad et al., 2020), As a result, these NPs may hold promise as anti-leishmanial medications.

Several articles address how biogenic silver nanoparticles can be used to treat leishmaniasis (Bagirova et al., 2020) By significantly lowering the promastigotes' rate of proliferation and the amastigotes' metabolic activity, they were

demonstrated that both chemically and biosynthesized (using cumin seed extract) silver NPs significantly inhibited *L. tropica* promastigotes and intracellular amastigotes, In addition, Bio-AgNPs induced the release of NO by macrophages, which killed Leishmania parasites. As a result, Bio-AgNPs were discovered to be more efficient than AgNPs against both kinds of Leishmania parasites, the results showed that green nanoparticles have a strong antileishmanial potential against *L. tropica* parasites, which are the cause of Cutaneous Leishmaniasis. Similarly, when evaluated in vitro, silver nanoparticles which using a *Mentha longifolia* (L.) (Aqueous Extract). The leaves can cause death of 66% of Leishmania (Javed et al., 2020). In order to produce silver nanoparticles, (Awad et al., 2021) used myrrh (*Commiphora molmol*). The antiparasitic efficacy of the MSNPs was evaluated on *L. major*, and both in vivo and in vitro experiments showed that MSNPs were superior to pentostam and CNPs. Thus, MSNPs may be excellent choices for a range of nanomedicine applications. The effect of Ag-NPs that generated from ginger rhizome extract on *L. major* promastigotes and amastigotes was to be effective, and it exhibited an inverse relationship with concentration, Ag-NPs caused programed cell death in *L. major* promastigotes and showed 60.18% of apoptosis (Mohammadi et al., 2021). Additionally, it was discovered that silver nanoparticles phytosynthesized with *Sechium edule* were effective in killing *L. donovani* that their activity was dosage dependent (Baranwal et al., 2018). Silver NPs showed dose-dependent cytotoxic activity against *L. tropica* by use of *Flammulina velutipes* (Faisal et al., 2021).

There are many studies on the biosynthesis of AgNPs from other microorganisms (fungi) and their anti-leishmanial effect of which study the effects of *Fusarium graminarum* AgNPs on *L. tropica* , where found that these particles have a significant impact on number of parasites; the number of parasites decreased after 24, 48, and 72 hours compared to pentostam and the control( Mohammed et al., 2019).In a second study, the same fungus was used to synthesize AgNP and study their effect on *Leishmania donovani*, where results recorded a significant gradual decrease in viability of the parasite within infected macrophage cells with the concentrations and the time (Ghadi et al., 2018). In another study, silver NPs biosynthesized with *Fusarium oxysporium* fungus killed *L. amazonensis* promastigotes and amastigotes in vitro due to increased ROS formation, loss of mitochondrial integrity, destruction of membrane, reduction of infected macrophages, and decrease of intracellular amastigotes. Another research used corn-extracted nanoxylan-containing silver NPs. Compared to free nanoxylan, NPs reduced *L. amazonensis* promastigotes' vitality (Viana et al., 2020). Additionally, (Jebali and Kazemi, 2013) showed that visible light, IR ray, and UV increased the anti-leishmanial activity of many inorganic NPs. UV light suppressed *L. tropica* promastigotes' growth and metabolic activity, a leishmanicidal effect of silver NPS (Allahverdiyev et al., 2011).

- **Gold nanoparticels (AuNPS)**

AuNPs have the potential to treat a number of illnesses (Dykman, 2020). AuNPs can be quite effective against different pathogenic parasites important for public health, among them there are protistias (Benellei, 2018). One experiment spotted in the area of skin injuries, AuNP-integrated into natural rubber membranes targeted leishmania promastigotes. The experiments had an outcome consisting of a retardation in the proliferation rate, changed behavior and reduction in lifespan of the organisms (Barboza-Filho et al., 2013). Effect of gold NPs treatment simulated with microwave at (2450 MHz) on *L. major* promastigotes and intracellular amastigotes as opposed for thermotherapy revealed that gold NPs alone significantly reduced the parasite survival rate of promastigotes after 48 hours of incubation and those of intra- J744 cell amastigotes after 24 hours The investigation conducted in conjunction with the other concerned the interactions of the Gold NPs prepared utilizing the plant species *Rhazya stricta decne* with the intra cellular amastigot cells of *L. tropica*. After two days of incubation, these Gold NPs were found to be inhibiting the intracellular amastigotes growth while being non-cytotoxic to the THP-1 cells (Uniform sized gold NPs were initially expected with use of polyphenolic substances. This inhibits growth of *L. donovani* in all forms, wild type as well as both sodium stibogluconate and paromomycin resistant. The NPs were shown to be low toxic and macrophages carry them in less than one hour. Furthermore, their effectiveness against drug-resistant strains (SSG, paromomycin) was demonstrated, and they demonstrated a high selectivity index (Das et al., 2013).

- **Selenium nanoparticles (SeNPs)**

In the recent studies,some protozoa including Trypanosoma and Leishmania, have been found to require trace levels of selenium ions as potential drugs, with encouraging results (Lobanov et al., 2006). According to Beheshti et al. (2013), biogenic selenium nanoparticles may be employed as a potential therapeutic agent to treat the local lesions associated with cutaneous leishmaniasis, In vitro and in animal models, the growth of *L. major* amastigote and promastigote forms is inhibited by selenium nanoparticles. Furthermore, Se NPS can prevent *L. tropica's* promastigote and amastigote growth (Mahmoudvand et al., 2014).

- **Metal Oxide Nanoparticles**

**Zinc Oxide Nanoparticles (ZnONPs)**

GRAS (generally regarded as safe) substance zinc oxide being ZnO NPs bring along the use of zinc oxide nanoparticles (ZnO NPs) in lipstick, moisturizers, sunscreen creams and deodorants. However, many researchers have been involved in creating considerable volumes of ZnO NPs and using them in different biomedical applications (Hameed et al., 2019). ZnO rod-shape NPs of L. ledebourii tuber was helpful in mitigation of *L. major* growth in in vitro; similar to other previous

studies on plant extract the effect also depended on the dose (Khatami et al., 2020). The in vitro culture medium also contained different levels of ZnONPs (0.18 µg/mL, 0.37 µg/mL and 0.75 µg/mL and 1.5 µg/mL) where the treatment was done on the *L. donovani* amastigote forms. Using the colorimetric assay to assess the cell viability data, it was revealed that the ZnONPs treated amastigote cells had a cytotoxic effect, and impeded their proliferation whose activity also got suppressed by *L. donovani*. As given in the study, ZnONPs would generate a unique and lightweight formation for anti-leishmanial medications (Delavari et al., 2014). The plants (*Verbena officinalis* and *Verbena tenuisecta*) leaf extracts were produced by Sumaira et al. (2018) to obtain ZnONPs , and the evaluation of their superb anti-Leishmanias efficiency demonstrated that the ZnONPs derived from the leaf extract of the *V. officinalis* was a better option because of its higher phenolic amount as In addition, a synergistic effect was observed when the ZnO NPs were decorated with *Geranium wallichianum* leaf extracts increasing the leishmanicidal activity of the nanozymes, (Abbasi et al., 2020). In further the like manner, the *Sageretia thea* water-based extract derived ZnO NPs were proven to be effective against *L. tropica* both promastigote and amastigote stage (Khalil et al., 2017). Zn NPs mixed in carbon or nitrogen to examine ability to kill *L. tropica* promastigote, it have ability to limitation of the growth by ROS generation. It has anti leishmanial effects (Nazir et al., 2019). Zn NPs biosynthetic in water and some solutions showed anti leishmanial effects (Ali et al., 2017).

**Titanium Dioxide (TiO2)**

The material with a semiconductor property, namely titanium dioxide (TiO2), induces antibacterial and photocatalytic features under UV radiation (Lopera et al., 2018). Moreover, the nanoparticles show enhanced the toxic effects of the particles when IR and UV light will be used together. It is by the production of the heat and the reactive oxygen species. The next option for CL is the application of photodynamic therapy where TiO2 NPs combined with UV light causes killing of the viruses. In this regard, prophylactic treatment should use different concentrations of TiO2 NPs (Rutile, Anatase) together with UVA and UVB to attain photodynamic treatment; the performance should be measured by variation in the death of promastigotes as the result of the various levels of TiO2 NPs and the types of UV light (Dolat et al. 2020). Through the strategic association between them, Lopera and associates came up with TiO2 nanostructure doped with multiple metals such as Zn, Fe, and Pt to induce ROS upon exposing light to visible light spectrum. When macropthages that were engaged with morpholonnate *L. amazonensis* amastigotes were treated by using different TiO2 respect to palladium and zinc, and then exposed to visible light, both doped Pt and Zn TiO2 showed a strong antiamastigote potential that was accompanied by elevation in ROS production (Lopera et al., 2018). Adding to that, findings of Jebali and Kazemi (2013) showed that TiO2 NPs change significantly features of *L. major* promastigotes as well as possessing antileishmanial activity. While blue light is a good source of producer of the Saharan haze, the effect of UV and IR rays on this issue is also noticeable.

**Iron Oxide Nanoparticles (Fe3O4)**

Iron oxides engineered nanoparticles (NPs) that have a wide utility in biomedical applications have even obtained FDA and EU approval as iron replacements or as contrast agents (Bobo et al., 2016). In this type of applications, hydrophilic decorating agents such as ligands are typically used to improve the stability of the nanoparticles in aqueous media (Moise et al., 2017). Fe3O4 NPs that is functionalized by citric acid for the elimination of *L. Mexicana* amastigotes in the cell culture medium were achieved, and magnetic hyperthermia evaluation was done using nanoparticles. The results revealed the potential therapeutic effect of this form of treatment in the obliteration of intracellular amastigotes in a heat-dependent manner. Furthermore, it elaborated a theoretical framework of the potential mechanism through which amastigotes respond to the host system challenged by heat (Berry et al., 2019). Where kind, Rosmarinus officinalis based NPs were synthesized in the presence of Fe3O4 and tested for antileishmanial efficacy (the efficacy of the newly synthesized nanoparticles against *L. major* promastigotes), the antileishmanial activity was dose dependent (Khatami et al., 2017). Finally, Trigonella foenum-graecum-synthesised ferromagnetic iron oxide nanorods (FIONs) demonstrated considerably greater leishmanicidal capabilities against *L. tropica* forms when exposed to LED light than when co-administered with AmB in the dark. ROS production was shown to cause growth impedance (Islam et al., 2020).

**Magnesium Oxide Nanoparticles (MgONPs)**

MgONPs are a desirable substitute for heavy metal-based NPS like ZnO because they are metabolized easily. Furthermore, renal function is normal, the body can effectively remove the products of Mg2+ and OH- ions, allaying worries about excessive metal accumulation (Nguyen et al., 2018). So Bafghi et al. (2015) investigated the impact of glucose-coated MgONPs) on *L.major* by the gene analysis of (GP63, CPB). It was demonstrated that longer incubation times and higher NP concentrations reduced cell viability while increased concentration also resulted in a decrease in gene expression, but not incubation time. Surprisingly, gene silencing was accomplished using NPs at non-toxic quantities. Also, in vitro research was also done on *L. major* and the impact of lectin-coated MgO NPs, The results showed that the functionalized NPs had high levels of macrophage activation and leishmanicidal action (Jebali et al., 2014). As for Tavakoli and his co-workers they have demonstrated that Mn2O3 NPs are advantageous effect to *L. major* promastigotes both in vitro and in vivo, and they may be a good option for treating this illness. According to flow cytometry studies, Mn2O3 NPs in vitro caused apoptosis in almost 57% of the promastigotes. In vivo investigations, the

mice's survival rate was higher than that of the control group and the ulcers' size was dramatically decreased (Tavakoli et al., 2019).

**Nickel Oxide Nanoparticles (NiO NPs)**

NiO NPs are widely used in biomedical applications due to their excellent qualities, tiny size, and biocompatibility. The greatest thing is the fact that they have a very good outcome in fighting the bacteria and they post the hope that they will one day be selected as the drugs against leishmaniasis. A recent work involving the antileishmanial activity of NiO NPs synthesised bio-chemically at *Sageretia thea* aqueous leaf extract level was evaluated and compared against *L. tropica* amastigote and promastigote cultures. A mastigotes and promastigotes were all found to be dose-dependently decreased (Khalil et al., 2018). Nevertheless, there is another evidence which is very informative where (NiONPs) biosynthesised with floral extracts from *Callistemon viminalis* persistently prevent the growth and even act on the kill *L. tropica* promastigotes (Sani et al., 2020). Artificial tulips of Iqbal and his team along with *Rhamnus virgata* extraction can lead to NiO NPs, this results in antileishmanial potential with the values of amastigotes and promastigotes of *L. tropica* being 10.62 µg/mL and 27.58 µg/mL, respectively (Iqbal et al., 2019).

**Chromium Oxide Nanoparticles ($Cr_2O_3$NPs)**

$Cr_2O_3$Nps are useful for treatment many of medical conditions, such as leishmanial (Thakur et al., 2023). $Cr_2O_3$-NPs were formed from *Rhamnus virgata* leaf extract and when *L. tropica* promastigotes and amastigotes were treated with NPs, the higher dose is leading into increased cytotoxicity (Iqbal et al., 2020).

**Conclusions**

The misuse and contraindications of leishmaniosis chemotherapeutic treatments have restricted their usage; hence parasitologists have researched NPs to control the parasite. NPs' non-toxicity and parasite resistance make them a better therapy than others. To create safe parasite treatments, additional study is required to understand nanoparticle modes of action. NPs produced in various ways may kill or limit parasite development. Nanoparticles may revolutionize Leishmania parasite therapy and management.

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Chapter 04

# Review on Plant based Green Synthesis of Silver and Gold Nanoparticles

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## ABSTRACT

The green synthesis of nanoparticles (NPs) becomes more popular due to their wide range of applications. In this study the recent development of plant based green synthesis of Gold (Au) and Silver (Ag) nanoparticles and their applications were evaluated that how plants and their extracts can be used to synthesized nanoparticles. NPs can be synthesized by various chemical and physical methods, which carry toxicity to environment. To overcome these environmental threats a green approach was implemented. Several studies on the reduction of nanoparticles by plants have been conducted, the phytochemical or metabolites present in plant acts as a capping and reducing agents which is responsible for the synthesis of NPs, clearly demonstrating the validity of green approach. It is a simple, environmentally friendly, and cost-effective method due to availability of plants.

**KEYWORDS**
Green synthesis, Silver (Ag) and Gold (Au) Nanoparticles, Plant extracts, Application

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## INTRODUCTION

Nanotechnology is receiving a lot of attention as a new field of research dealing with the fabrication of nanomaterials and nanoparticles (NPs) for use in a variety of industries, including biomedicine, food technology, catalytic processes, drugs, sensors, electrochemistry, beauty care products, and so on (Vanlalveni et al., 2021). Green nanotechnology offers tools for transforming biological systems into ecological friendly methods to synthesize nanomaterial while avoiding any related toxicity (Nasrollahzadeh et al., 2019). "Nano" is a Greek word which mean dwarf or tiny (Zafar et al., 2019).

Nanomaterials have exclusive properties because of their small size (1–100 nm). They have significant electrical features, excellent mechanical as well as thermal stability, large surface areas, and strong optical and magnetic fields (Yaqoob et al., 2020). Metallic nanoparticles (NPs) have fascinated researchers for more than 100 years and have since been widely utilized in the fields of engineering and medicine. These are the topic of interest, due to their tremendous potential in the field of nanoscience (Nadaf et al., 2022).

### Green Synthesis of Nanoparticles

The three most important parameters involved in the synthesis or production of nanoparticles are: (i) the determination of a green or environmental friendly solvent; (ii) an effective reducing agent; and (iii) a safe stabilizing material (Jadoun et al., 2021). There are various large-scale approaches for synthesizing MNPs that are either chemical (e.g., the sol-gel method, polyol production, chemically based reduction, and precipitation) or physical (such as microwave-assisted combustion, pulsed laser depositing, as well as laser evaporation) (Paiva-Santos et al., 2021). Despite of their widespread and ease of use, they have several limitations. The yields of nanoparticles using laser evaporation processes are low, and they require the use of expensive, specific instruments and careful handling. In the chemical reduction process, metal nanoparticles are created with toxic chemical species adsorbing to the metallic surface, which may affect their biocompatibility (Dhayalan et al., 2018). However, the green fabrication of nanoparticles provided a more economical and cost-effective solution to many environmental pollution issues (Chan et al., 2022). The key benefits of plants, among other biological resources, include their low cultivation cost, being safe to handle, great variety, being readily available, quick production, non-toxic and the presence of various metabolites that may help in reduction (Eisa et al., 2019; Ouassil et al., 2021; Saratale et al., 2018). The use of metal nanoparticles (such as Ag, Cu, Au, Pt, Zn, Mg, etc.) and metal oxide nanoparticles (such as $TiO_2$, $Ag_2O$, ZnO, and so on) have been clearly demonstrated to have therapeutic effects in the field

of medicine (Yaqoob et al., 2020). Moreover, metallic nanoparticles have also been employed for diagnostic imaging, biosensors, drug delivery, and labeling applications (Katas et al., 2018).

Notably, metal NPs, particularly silver (Ag) and gold (Au), are currently the topic of significant research due to their unique properties, such as their size and large surface area (Nadaf et al., 2022). Generally, as shown in Fig. 1, Au and Ag nanoparticles can be manufactured using top-down and bottom-up approaches. The top-down methods that focus on breaking down bulk materials into smaller particles, such as physical methods, have the advantage of producing large amounts of nanoparticles, but this consumes a lot of energy and requires expensive equipment investments (Jiang et al., 2022). While the bottom-up approaches use chemical agents to create nanoparticles by assembling atoms into "seed" nuclei that will later develop into nanoscale clusters and small particles, like chemical and biological processes. The chemical method is distinguished by its low cost, ease of use, and robust scalability. Still, additional purification steps are required in order to get rid of excessive surfactants and residual reactants before manufacturing the functional nanomaterial. Biological methods are known for synthesizing nanoparticles that are highly stable and biocompatible, but it is difficult to control their size, shape, and crystallinity (Jiang et al., 2022).

Using plants to synthesize nanoparticles may be advantageous in comparison to other environmentally friendly biological methods because it eliminates the laborious process of maintaining cell cultures and may be scaled up appropriately for large-scale synthesis (Mohammadi et al., 2019). Hence, in this review, we give an overview of recent developments and their applications that have been recently published about the synthesis of silver and gold nanoparticles from plant materials.

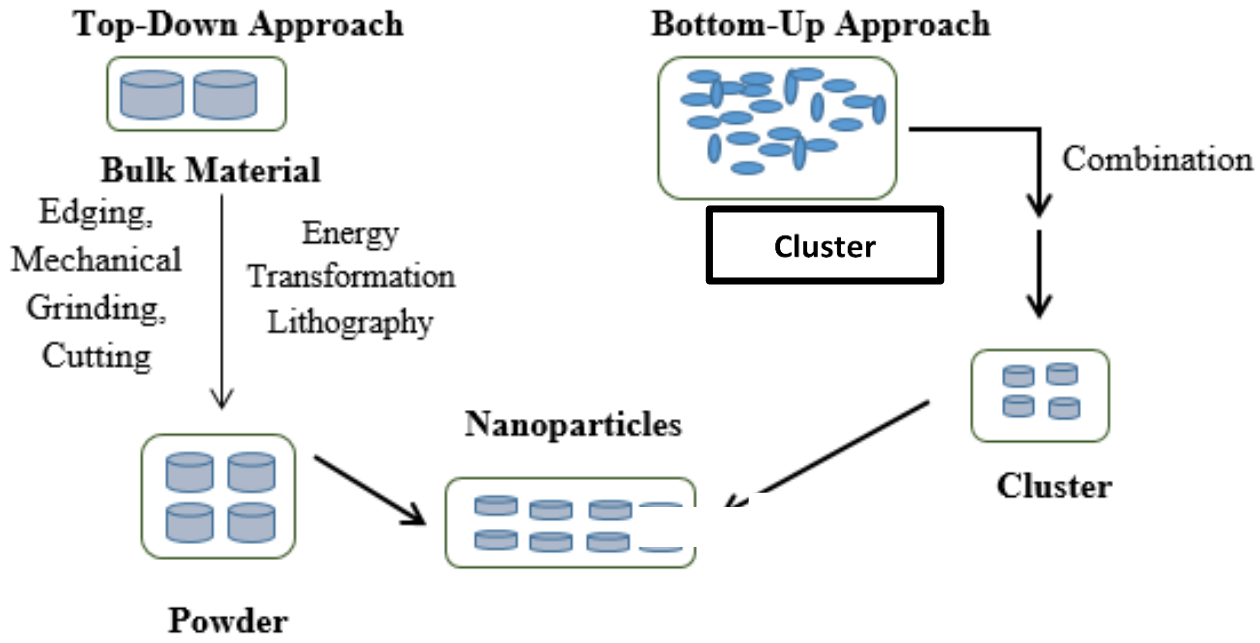


**Fig. 1:** Top-down and bottom-up approaches for the synthesis of Nanoparticles.

**Extracellular Synthesis of Metal NPs Utilizing Plant Extracts**

The presence of different secondary metabolites, catalysts, and proteins, as well as other reducing agents and electron-shuttling compounds, is generally associated with the synthesis of metal NPs by plant parts (EL-Moslamy et al., 2017). In order to recover these nanoparticles from plant tissues, which is a laborious and expensive process, and requires enzymes to break down the cellulosic materials surrounding the NPs (Nadaf et al., 2022). By utilizing plant tissue culture techniques as well as optimizing the downstream procedures, it is also possible to produce appropriate and stable metal NPs with a particular size on an industrial scale (Ramrakhiani and Ghosh, 2018). The bio-reduction of metal NPs utilizing extracts from plants is partitioned into three fundamental steps. (i) The first is the activation phase, which involves the reduction as well as the nucleation of metal ions. (ii) The second phase, known as the growth phase, is when the small adjacent NPs combine to form larger particles, which is followed by an increase in the NPs' thermodynamic stability. (iii) The termination phase is the last step in which the NPs are formed. in Fig. 2 (El-Seedi et al., 2019). Plant extracts have reducing and stabilizing properties that can be employed in the biosynthesis of nanoparticles (Hernández-Díaz et al., 2021).

**Surface Plasmon Resonance (SPR)**

SPR phenomena occur when free electrons in metals exhibit collective oscillation in resonance with particular wavelengths after being affected by an electromagnetic field. The SPR band is dependent on the size, shape, type, and surrounding environment of the NP (Karthika et al., 2017). When the electron cloud is displaced in relation to the nuclei, a restoring force from Coulomb's attraction between electrons and nuclei emerges, which causes an oscillation in the

electron cloud (Kumar, 2021). At the boundary or surface between metal and dielectric layers, SPR were aggregate oscillations of free-electron gas (Rizal et al., 2019). The resonance in silver and gold nanoparticles occurs in the visible part of the electromagnetic spectrum. This is demonstrated by the bright colors that particles immersed in transparent media (like glass or an aqueous solution) exhibit in both transmitted as well as reflected light (Kumar, 2021).

**Nanoparticles Synthesis from Plant Metabolites**

Plant metabolites such as terpenoids (eugenol), amino acids (tryptophan and tyrosine), flavonoids (luteolin and quercetin), alkaloids, phenolic acids, sugars, proteins, and energy or electrons released during the process of glycolysis (Fig. 2) have the greatest potential for reducing nanoparticles and may also act as NP stabilizers by preventing NP aggregation (Raafat et al., 2021; Yousaf and Saleh, 2018).

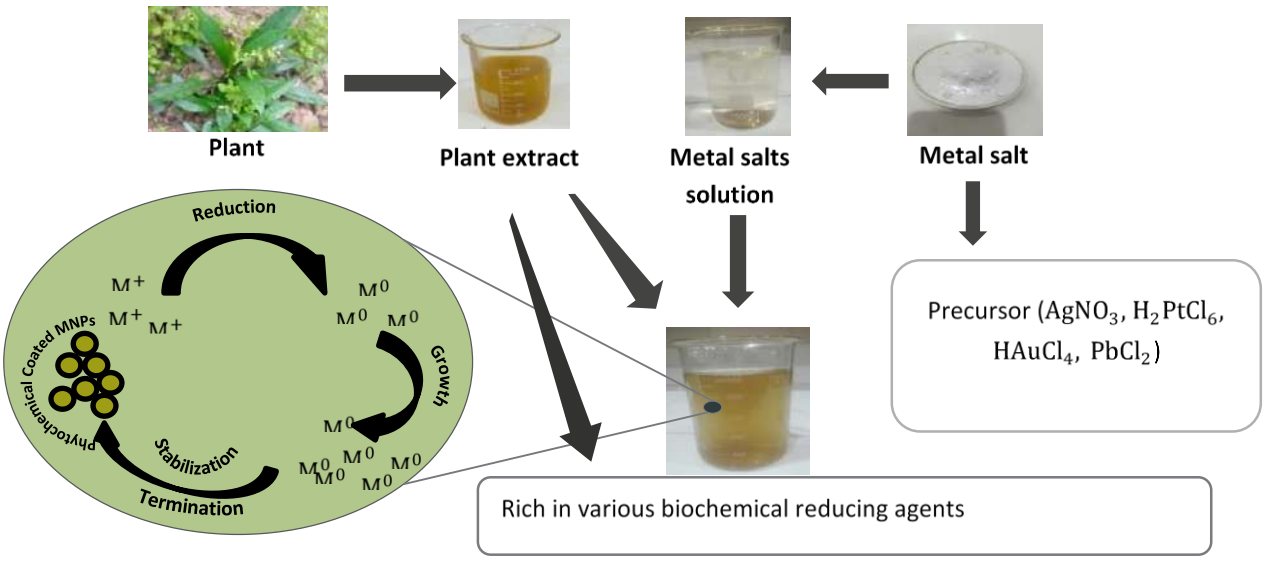


**Fig. 2:** Plant metabolites accountable for the Synthesis of metallic nanoparticles

The primary plant metabolites used to synthesize metal nanoparticles are: phytochemicals (polyphenols, carboxyl, glutathiones, amine, metallothioneins, ascorbates, as well as water-soluble carbohydrates), (Nadaf et al., 2022) Ascorbic acid, (Chen et al., 2020) saponins, (Kumar et al., 2017) triterpenes, (Ghiulai et al., 2020) plant-based surfactants, (Sur et al., 2018) sterols, (Mohanta et al., 2020) catechins, (Gottimukkala et al., 2017) heterocyclic compounds, (Sila et al., 2019) and reducing sugars [aldoses and ketones] (Odongo et al., 2022). Fig. 3 labels the main plant metabolites accountable for the reduction of metal particle into metallic nanoparticles(Nadaf et al., 2022).

**Constituents Liable for the Reduction**

NPs are reduced and stabilized by combining biomolecules such as proteins, sugars, amino acids, tannins, enzymes, vitamins, polysaccharides, alkaloids, terpenoids, saponins, and phenols found in naturally occurring plant extracts with therapeutic benefits (Pagar et al., 2019).

**Silver Nanoparticles (AgNPs)**

Silver nanoparticles (AgNPs) are the most popular and might be one of the most significant nanomaterials. AgNPs are a highly desirable material in a number of industries due to their distinctive chemical and physical qualities, such as shape and size dependence on optical, electrical, and antimicrobial potentials (antibacterial, antifungal, antiviral, etc.), which differ greatly from bulk materials (Barkat et al., 2018; Moosa et al., 2019), Apart from applications in the food and textile sectors, nanotechnology finds utility in various fields such as water disinfection systems, orthopedics, drug delivery, biosensors, and medical diagnostics (Zivic et al., 2018). According to Inshakova and Inshakov, AgNPs are the most widely used engineered nanomaterials (ENMs) and make up more than half of all consumer products containing nanomaterials worldwide (Inshakova and Inshakov, 2017). Considering the advantages of AgNPs, numerous researchers have thought about synthesizing AgNPs using various techniques and determining their potential in various fields (Alharbi et al., 2022).

The green or plant-mediated synthesis method has drawn a lot of attention as an environmental friendly way to create silver nanoparticles (AgNPs) because it avoids toxicity. (Tamilarasi and Meena, 2020). The antimicrobial, anti-inflammatory, antiviral, anti-diabetic, anticancer, and anti-angiogenesis properties of biosynthesized AgNPs have also been reported (Sumitha et al., 2018). The synthesis of AgNPs has been successfully carried out using a variety of plant parts, including leaves, roots, fruits, flowers, rhizomes, etc. (Ahmad et al., 2017; Sumitha et al., 2018; Vanaraj et al., 2017). In this section, we aim to discuss the various plant parts extracts, as shown in Table 1, that mediate the biosynthesis of

silver nanoparticles (AgNPs) and their application. As a metal precursor, various concentrations of silver salt ($AgNO_3$) are used (Poudel et al., 2022).

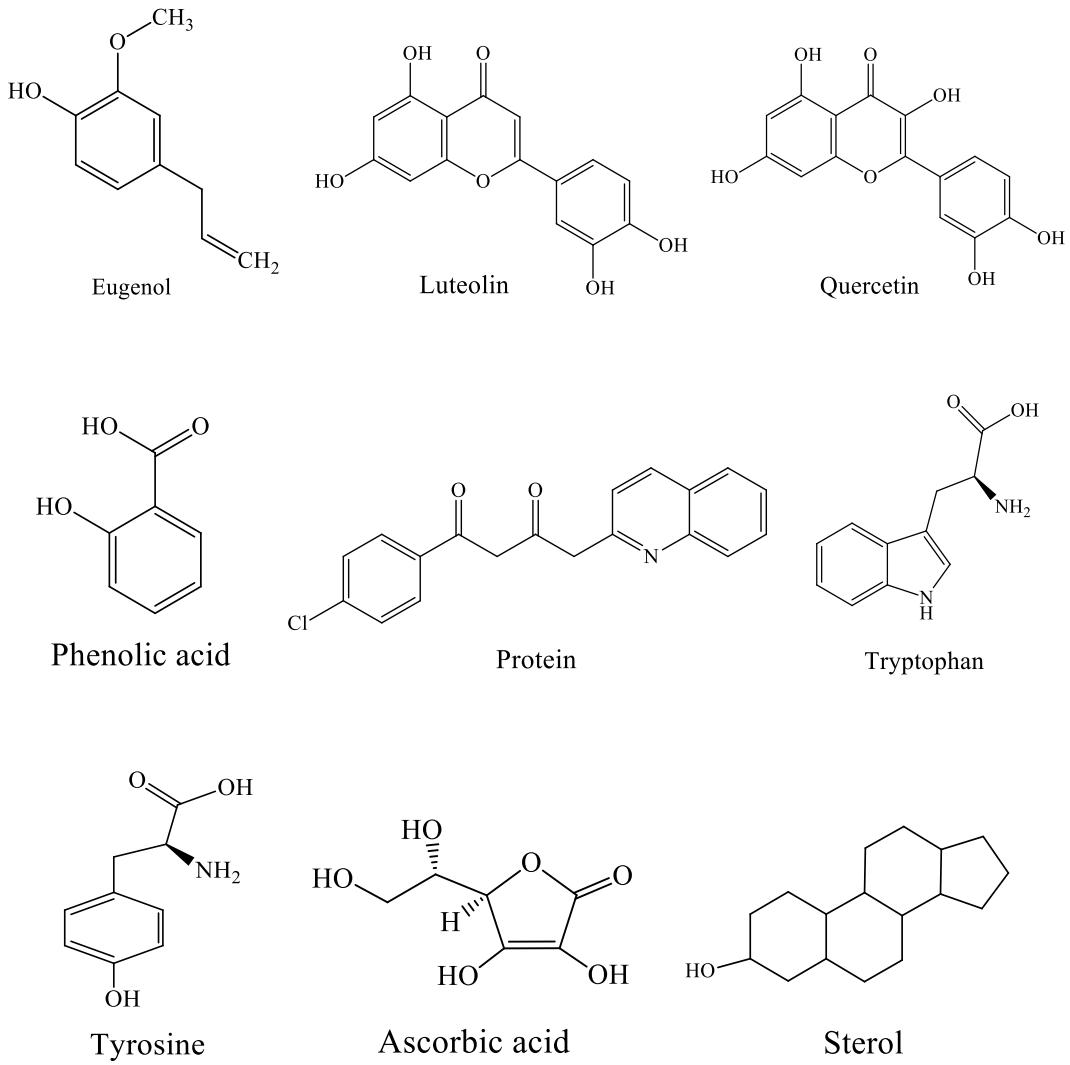


**Fig. 3:** Plant metabolites accountable for the reduction of metal particle into metallic nanoparticles

It has been reported that *Arbutus unedo* fresh leaf extract (LEA) can produce AgNPs in two different sizes. Both AgNP variations were tested for their antibacterial effectiveness against the gram-positive species (*Bacillus subtilis* and *Staphylococcus epidermidis*), as well as the gram-negative species (*Escherichia coli* and *Pseudomonas aeruginosa*) (Skandalis et al., 2017). According to Rajkumar et al. (2021), the silver nanoparticles synthesized from *Chlorella vulgaris* extract worked catalytically to degrade the pollutant methylene blue. After 180 minutes, photocatalytic degradation revealed that 96.51% of the methylene blue had been broken down (Rajkumar et al., 2021). In another study, the green synthesis of AgNPs in the presence of jasmine flower extract was reported by Aravind et al. (2021). They demonstrated that green synthetic silver nanoparticles had photocatalytic activity for the degradation of methylene blue contaminant (78% degradation efficiency after 120 min).(Aravind et al., 2021). The plant *Prunus persica* leaf was used to biosynthesize AgNPs, which have varying degrees of antibacterial and catalytic activity against the bacterial strains *E. coli* and *V. cholera* as well as heavy metals like cadmium (Cd) and palladium (Pd). *E. coli* is more effectively inhibited, and Cd removal efficiency is greater than Pd (Hemaiswarya et al., 2008). Another report mentioned the environmental friendly synthesis of spherical AgNPs using the latex extract of *Ipomea carnea* in the $AgNO_3$ solution. At roughly 413 nm, AgNPs' absorption peak can be observed. Both Gram-positive and Gram-negative bacteria were significantly inhibited by the green-fabricated AgNPs (Chandrakar et al., 2022). Several plant extracts, including *Anthemis atropatana, Azadirachta indica, Bauhinia variegate* flower, *Stigmaphyllon ovatum* leaf, *Cotyledon orbiculata, Diospyros lotus, Caesalpinia pulcherrima* flower, *Melissa officinalis* leaf, and *Prosopis juliflora* bark, have been successfully reported for the biosynthesis of AgNPs (Arya et al., 2018; Chinnasamy et al., 2021; de Jesús Ruíz-Baltazar et al., 2017; Dehghanizade et al., 2018; Elemike et al., 2019; Johnson et al., 2018; Moteriya and Chanda, 2017; Tyavambiza et al., 2021; Yasmin et al., 2020). In one of the studies, spherical AgNPs with sizes ranging from 24 to 49 nm were produced using flower extracts of *Tagetes erecta* (marigold flowers), which serve as a reducing agent. Silver NPs are produced when $AgNO_3$ is completely reduced during the reaction. XRD, FTIR, UV-Vis, SEM, and energy-dispersive X-ray (EDAX) spectroscopy were used to characterize the synthesized AgNPs. The induced surface plasmon resonance (SPR) is

responsible for the absorption peak at 420 nm, which was demonstrated by UV-Vis spectroscopy analysis. Finally, the photocatalytic activity of silver nanoparticles was demonstrated by the photodegradation study of rhodamine B dye (Katta and Dubey, 2021).

Silver nanoparticles were synthesized by Ahmadi et al. using an extract from summer savory. The results showed that the biologically produced AgNPs had antibacterial activity against two gram-positive and two gram-negative bacteria, particularly against *E. coli*, as well as anticancer effects against the human cancer cell lines K-562 and MCF-7, with IC50 values of 50 and 200 mg/ml, respectively (Ahmadi et al., 2020). *Allium sativum* (garlic), *Zingiber officinale* (ginger), and *Capsicum frutescens* (cayenne pepper), among others, are significant spices with well-known medical applications. These plants are used to make nanoparticles that are used in pharmaceutical products. Various biological activities, such as antimicrobial and antioxidant properties, were reported to be present in these spices. These spices were used to prepare silver nanoparticles that had broad-spectrum antibacterial properties (Otunola et al., 2017). *Galinsoga formosa* leaf and flower extract was used to prepare Ag-NPs, which demonstrated antibacterial activity against both gram-positive (*S. aureus, S. mutans*, and *S. epidermidis*) and gram-negative (*K. pneumoniae* and *P. aeruginosa*) bacteria. In particular, *P. aeruginosa* showed the highest zone of inhibition(13.33±0.58 mm), and *S. epidermidis* showed the lowest zone of inhibition (6.33±0.58 mm) (Mahmod et al., 2021).

Apart from antibacterial properties, AgNPs produced by *Mangifera indica* (Mango leaves) are also utilized in dental restoration (Sundeep et al., 2017). An aqueous extract of *Ficus hispida* leaf was used to synthesize silver nanoparticles (Ag NPs), which demonstrated strong catalytic activity in the conversion of 4-nitrophenol to 4-aminophenol as well as improved antioxidant and antibacterial activity against both *Bacillus subtilis* and *E. coli* (Ramesh et al., 2018). It has been reported that *Brassica nigra, Lavandula angustifolia, Origanum vulgare*, and *Capsella bursa-pastoris* are used in the synthesis of AgNPs (Salayová et al., 2021). AgNPs synthesized from *Aconitum violaceum* leaf extract were used as colorimetric probes to detect $Pb^{2+}$. In the existence of $Pb^{2+}$, the biosynthesized AgNPs displayed a color change from yellow to red, which was easily identified by UV-Vis spectroscopy (Khan et al., 2018). Behravan, M., et al. synthesized AgNPs using aqueous extract of *Berberis vulgaris* leaf and root. By using the disk diffusion test and the Minimum Inhibitory Concentration (MIC) test, the antibacterial effects of these nanoparticles were examined against *Escherichia coli* and *Staphylococcus aureus* bacteria.(Behravan et al., 2019). The usage of medicinal plants root extracts like *Rumex dantatus, Rumex hastatus, Bergenia stracheyi* and *Bergenia ciliata* for the synthesis of AgNPs has also been reported, used in therapeutic and homeopathic industries to treat human diseases (Rashid et al., 2019).

Cakić, M., et al., Synthesized silver nanoparticles using plant extract of Aerial part of *Fumaria officinalis L.* (earth smoke), (AgNPs-E). It exhibited strong antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as antifungal activity at a much lower level against *Candida albicans* (Cakić et al., 2018). In another study, fruit and tuber aqueous extracts of *Momordica cymbalaria* were used in the bioreduction process to synthesize AgNPs and their antibacterial activity against pathogenic organisms was evaluated. In vitro cytotoxicity, AgNPs prepared from tuber extract exhibit lower cytotoxicity toward prostate and breast adenocarcinoma cells than AgNPs prepared from fruit extract (KPJ et al., 2021). Sk et al., (2019) synthesized AgNPs by fruits aqueous extract of *phyllanthus acidus*. It has been discovered that the synthesized AgNPs exhibit a reversible response towards mercury ions, and again, these nanoparticles exhibit detectable antibacterial activity against A. hydrophila (Sk et al., 2019). The green synthesized AgNPs from powder of *Zingiber officinale* (Ginger) show excellent catalytic activity in the oxidation of 4-nitrophenol and methylene blue dye. According to evidence from FTIR and X-ray photoelectron spectroscopy, *Zingiber officinale* powder has hydroxyl and carbonyl functionalities, which are responsible for reducing $Ag^{+1}$ ions to silver nanoparticles. Additionally, a free-standing film made of polyvinyl alcohol (PVA) and *Zingiber officinale*-AgNPs is assembled as a useful way to repair and reuse the nanocatalyst for a number of cycles, and after the reaction is finished, the end result is metal-free (Eisa et al., 2019).

Biosynthesized AgNPs using the aqueous extract of some plants leaves and stems, such as *Trachomitum venetum, Teucrium polium, Ferula latisecta,* and *Ferula gumosa*, which function as reducing and stabilizing agents. The biosynthesized AgNPs displayed great antibacterial activity against *Staphylococcus aureus* and *E. coli* (Mohammadi et al., 2019). In another study, an aqueous extract of *Gomphrena globosa* (Globe Amaranth) fresh leaves was used to synthesize AgNPs. The active phytochemicals found in the leaves lead to a rapid reduction of $Ag^{+}$ to metallic AgNPs. The synthesized silver nanoparticles display outstanding antibacterial activity against both gram-positive bacteria and gram-negative bacteria (Tamilarasi and Meena, 2020). Although biologically formed AgNPs using methanolic extract of the plant *Catharanthus roseus* (C. AgNPs) effective against diabetes and extremely play important role in the annihilation of cancer (Jamil et al., 2022). Spherical and well-dispersed, 45 to 110 nm AgNPs were synthesized by Kambale, E.K., et al. utilizing leaf extract of three different Congolese plant species, that is *Senna siamea, Brillantaisia patula* and *Crossopteryx febrifuga*. The synthesized AgNPs displayed greater antibacterial activity, in comparison to three bacterial skin pathogens (Kambale et al., 2020).

*Clinacanthus nutans* plant leaf and stem extract has been utilized to synthesize silver nanoparticles (AgNPs). The synthesize AgNPs effectively inhibited bacteria, but not fungi. The inhibition results demonstrated that the *Staphylococcus aureus* has the highest activity while *Escherichia coli* exhibited the lowest inhibition in the disc diffusion method (Chiu et al., 2021). The flavonoids and terpenoids in *Azadirachta indica* (Neem) leaf extract are responsible for the reduction of silver ions or green synthesis of AgNPs. *Escherichia coli* was more resistant to the synthesized AgNPs, compared to Gram-

positive bacteria (Roy et al., 2017). Lubis, F.A., et al. synthesized AgNPs from *Persicaria odorata* leaf extract (PO-AgNPs). Through FESEM and HR-TEM images, PO-AgNPs were found to be nanospheres with a diameter of 11 ± 3 nm. PO-AgNPs have antibacterial and wound healing properties that are beneficial in biomedical applications (Lubis et al., 2022).

**Table 1:** Recently published research work on green synthesis of AgNPs using various part of plant.

| S. No | Plant Name | Plant part | Solvent | Shape and Size | Metabolites | Application | Year and Refs. |
|---|---|---|---|---|---|---|---|
| 1. | *Eriobotrya japonica* | Leaf | Deionized water | Spherical, 20 nm | Flavonoids, glycosides, sesquiterpenes triterpenic acids, proteins, polysaccharides and so on | Antibacterial properties against *Escherichia coli* and *Staphylococcus aureus* | (Rao and Tang, 2017) |
| 2. | *Parkia Speciosa* | Leaves | Distilled water | Spherical, 26-39nm (31nm) | Polyphenols | Antimicrobial, photocatalytic, and antioxidant activities | (Ravichandran et al., 2019) |
| 3. | *Terminalia arjuna* | Leaf | Boiled deionized water | Spherical, 10-50 nm | Leucoanthocyanidins, hydrolyzable tannins and arjunetin | Catalytic degradation of hazardous or organic dyes (MO, MB, CR and 4-NP) | (Raj et al., 2020) |
| 4. | *Piper betle* | Leaf | Double distilled water (DDW) | Spherical, 6–14 nm | Phenolic biomolecule(s) | Significant fungicidal efficacy against plant pathogenic fungi. | (Khan et al., 2020) |
| 5. | *Melia azedarach* | Leaf | Boiling distilled water | Spherical, 18-30 nm (23 nm) | Hydrolyzable tannic acid | Antifungal against Verticillium dahliae (in eggplants) | (Jebril et al., 2020) |
| 6. | *Dodonaea viscosa* (L.) | Leaves | Deionized water | Spherical, 60.22 nm | Phenolic, protein or glycosides | Dose-dependent anti-tumor effect on human lung and ovarian cancer. | (Al-Musawi and Al-Saadi, 2021) |
| 7. | *Mangifera indica* (Mango) | Leaves | Double distilled water (DDW) | Pseudo-spherical, 18.2 ± 0.12 nm | Alkaloids, flavonoids polyphenols and so on | Displayed enhanced photocatalytic potential against Methylene Blue (MB), and $IC_{50}$ 83.85 ± 9.63 µg/mL against cervical cancer cells (HeLa), | (Panwar et al., 2022) |
| 8. | *Panax ginseng* | Root | - | Spherical, 5 – 15 nm | Amino acid or protein residues, ginsenosides and alkyl halides | Displayed dose-dependent cytotoxicity against HeLa cells and virucidal activity against the influenza A virus | (Sreekanth et al., 2018) |
| 9. | *Nigella sativa* | Seeds | Deionized water | Spherical, 10-20 nm | Alkaloids, terpenoids, flavonoids, tannin and polyphenols | Potent cytotoxic effect against human hepatocellular carcinoma cells | (Usmani et al., 2019) |
| 10. | *Borago officinalis* | Flower | Deionized water | Spherical, 30 nm | Linolenic acid, polyphenols, and flavonoids | Harmful to testicles and negative effect on the male reproductive system by altering testicular functions, blood levels of the testosterone hormone and elevations in serum levels of malondialdehyde | (Jasem and Abas, 2022) |
| 11. | *Euphorbia tirucalli* | Latex | Deionized water | Spherical, 20 – 30 nm | Ether, methyl, secondary amines, ketone and C=C groups | Toxicity against Meloidogyne incognita | (Kalaiselvi et al., 2019) |
| 12. | *Calotropis procera* | Latex | Ultra-pure water | Spherical, 22.14 ± 0.42 nm | Alkane's amide and carboxylic acid groups | Used for the photocatalytic degradation of MO dye as well as anti-biofilm agents | (Chandhru et al., 2022) |
| 13. | *Emblica ofcinalis Gaertn* | Fruits | Distilled water | Spherical, 5-30 nm | Phenolics and flavonoids | cytotoxicity against breast cancer (MCF-7) and human colorectal carcinoma (HCT-116) cells and antioxidant activity | (Abdel Bar et al., 2021) |

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| 14. | *Ficus religiosa* | Bark | Distilled water | Spherical | _ | Used to remove chromium from synthetic wastewater (removal efficacy is above 74.8%) | (Riaz et al., 2022) |
| 15. | *Acacia nilotica (*Kikar) | Bark | Water | Variable shapes, 20 – 50 nm | Gallic acid, rutin, leucocianidolum, m-catechol, eleagic acid, apigenin-6, glucopyranoside, isoquercetin, 7-diglucoside, and kaempferol 3, 8-bisC-glucopyranoside | Anticancer, antioxidant and antidiabetic potential | (Zubair et al., 2022) |

**Table 2:** Recently published research work on green synthesis of AuNPs using various part of plant.

| S. No | Plant name | Plant part | Solvent | Shape and Size | Metabolites | Application | Year and Refs. |
|---|---|---|---|---|---|---|---|
| 1. | *Sasa borealis* | Leaf | Triple distilled water | Oval and spherical, 10 – 30 nm | Alcohol, phenol, 1° amines, aromatic compounds and aliphatic amines | Toxic effect on HEK293 cells and Anticancer activity on AGS cells by WST-1 | 2018 / (Patil et al., 2018) |
| 2. | *Corchorus olitorius* (Malvaceae) | Leaves | Deionized water | Quasi-spherical, 37 – 50 nm | 5-caffeoylquinic acid, 3,5-dicaffeoylquinic acid, ascorbic acid, quercetin 3-galactoside derivatives and α-tocopherol | Anti-inflammatory, anti-proliferative activities and Cytotoxicity against breast MCF-7, carcinoma HepG-2 and colon carcinoma HCT-116 | 2018 / (Ismail et al., 2018) |
| 3. | *Citrus limonum* | Leaf | Sterile distilled water | Spherical,40 – 90 nm | Flavonoids, alkaloids, terpenoids and polyphenols | Detection of organophosphate pesticide, and application in forensic chemistry and toxicology | 2020 / (Bhagat et al., 2020) |
| 4. | *Parsley leaf* extract | Leaves | De-ionized water | Spherical, semi rod or flower shape, 80 nm | Protein and polyphenols. | Anticancer towards (HCT116), Catalytic activity in degradation of MB and antibacterial against two gram-negative pathogenic. | 2020 / (El-Borady et al., 2020) |
| 5. | *Lawsonia inermis* | Leaf | Distilled water | Spherical, ~20 nm | Flavonoids, alkaloids, quinones, saponins, phenylpropanoids, fatty acids, terpenoids, carbohydrates, phenolic, proteins, tannins, xanthones, coumarin, triterpenoids, sterols, etc. | Degradation of 4-NP, BPB, and MR using NaBH4, Catalyst for reduction of anthropogenic pollutants present in the wastewater. | 2020 / (Kumari and Meena, 2020) |
| 6. | *Verbascum speciosum* | Leaves | Deionized water | Spherical, polygonal 118 ± 72 nm | Flavonoids, glycosides, saponins, carotenoids | Cytotoxicity assets to HepG2 cancer cell line | 2021/ (Mousavi-Kouhi et al., 2022) |
| 7. | *Clerodendrum infortunatum* | Leaves | Ethanol | Spherical, 33 ± 5 nm | Clerodin | Cytotoxicity to leukemia monocyte cells, antimicrobial activity | 2022 / (Nagaraj et al., 2022) |

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| 8. | Green tea | Leaves | Deionized water | 30 – 50 nm | Polyphenols, flavonoids, carbohydrates, amino acids and proteins | Biosensor for CD44 cancer biomarker detection | 2023 / (Ashikbayeva et al., 2023) |
| 9. | *Lonicera japonica* | Flower | Tripled distilled water | Spherical, hexagonal and triangular, 10 – 40 nm | Alkaloids, phenolic, polyphenols, amino acids and vitamin | Cytotoxic effect on normal embryonic kidney cells (HEK293) and cervix cancer (HeLa) cells | 2019 / (Patil et al., 2019) |
| 10. | *Rosa canina* | Fruits | Aqueous extract | _ | Polyphenols, ascorbic acid, flavonoids and tannins | Catalyst in reductive degradation of toxic water pollutant organic dyes | 2022 / (Alikhani et al., 2022) |
| 11. | *Peganum harmala L.* | Leaf and seed | Distilled water | Spherical, 43.44 nm and 52.04 nm | β-carboline and quinazoline, Harmaline, harmine and other alkaloids | Antibacterial activity, used in cancer drug delivery, thermotherapy, and cellular imaging | 2019 / (Moustafa and Alomari, 2019) |
| 12. | *Hygrophila spinosa* | Plant materials | Ethanol | Spherical, polygonal, rod and triangular, 68.44 ± 0.30 nm | Proteins, flavonoids, phenolics, carbohydrates and terpenoids | Cytotoxicity against various tested cancer cell lines (breast, ovarian, multi-drug resistant and brain) | 2020 / (Satpathy et al., 2020) |
| 13. | *Chondrus crispus* | Plant extract | KOH solution, distilled water | Quasi-spherical 8.4nm | Phenolics and protein | Formation of printable hydrogels | 2022/ (Álvarez-Viñas et al., 2022) |
| 14. | *Ephedra* | Plant extract | Distilled water and methanol | Spherical and triangle, 1.3 – 15.6 nm | Flavonoids, terpenoids, glycosides, tannins and ephedrine derivatives | Anti-asthma and antipyretic agent and Antibacterial activity | 2023 / (Al-Radadi, 2023) |
| 15. | *licorice* root, olive oil and coconut oil | Roots and oil | Sterilized water, de-ionized water | Spherical 3.4, 5.7, 2.9 and 10 nm | Amino acids, phenols, flavonoids, fatty acids and minerals | Active against H. pylori and ulcer | 2022 / (Al-Radadi, 2022) |

**Gold Nanoparticles (Au NPs)**

Now a days, nanotechnology and nano science are all the time joined to create nanostructured materials and, additionally, decide how much the control of issue on a nuclear, atomic, and supramolecular level might influence the ideal nanomaterial properties (Ielo et al., 2021). Among metallic nanoparticles, gold nanoparticles (AuNPs) are overall generally utilized in the human contact regions, for example, recognition, microscopic organisms, microorganism and disease treatment, which was because of their tunable size and shape, surface plasma reverberation, and great biocompatibility (Zhang et al., 2020). At a fundamental level, AuNPs exhibit low toxicity and possess numerous intriguing chemical, biological, and physical properties, including optical, electrochemical, and biocompatible characteristics, as well as potential catalytic activity.(Castillo-Henríquez et al., 2020). By and large, they have been utilized for different applications, for example, sensors(i), catalysis(ii) , anticancer medications (iii), cell reinforcements (iv), larvicides (v), antimicrobials (vi), nanofluids (vii), farming (viii) and drug conveyance (Balalakshmi et al., 2017). In the majority of the cases, plants parts are utilized for the combination of AuNPs because of the accessibility of phytochemicals in leaves, natural products, natural products strip, roots, and seeds. Besides, the plant separates are more ideal for the combination of NPs on account of the chance of huge scope creation, variety of size, and state of nanoparticles. In this cycle, the radically change of temperature and tension are not needed (Khan et al., 2022). For the synthesis of AuNPs, the principal study was acted in 2003 by Shankar and his gathering by utilizing the geranium leaf separate for diminishing and covering specialist. This response was completed for 48 hr by utilizing the terpenoids present in leaf extricate which was liable for the decrease of gold particles to gold nanoparticles (Jadoun et al., 2021). Biosynthesized AuNPs were obtained using *Cibotium barometz* root extract which is consider to be the non-toxic therapeutic delivery agent to the cancer diagnostic (Wang et al., 2017). From the dried fruit extract of *Amomum Villusom,* AuNPs were synthesized having size of 5-10 nm which show antibacterial properties toward bacterial pathogen and also act as a cytotoxic agent toward breast cancer cells and catalytic activity

toward 2,2- diphenyl-1-picrylhydrzyl and methylene blue (Soshnikova et al., 2018). Leaves extracts of *Elaeis guineensis, Malva Verticillata,* and *Camellia sinensis* have also been reported as the reducing agent for the green synthesis of AuNPs (Ahmad et al., 2019; Ahmeda et al., 2020; Sk et al., 2020). Gold NPs, 34.2 ± 3.1 nm in size, have been synthesized using leaves extract of *Tragopogon dubius*. The synthesized gold nanoparticles exhibited bactericidal activity in vitro and in vivo (Layeghi-Ghalehsoukhteh et al., 2018). The biosynthesized AuNPs from the extract of *Massaendra Glabrata* having size 10.59nm exhibit catalytic activity toward the reduction of 4-nitrophenpl and also remove Rhoda mine B and methyl orange from waste water and marine environment (Francis et al., 2017).AuNPs were also synthesis from methanolic bark extract *Terminalia arjuna* which is Cooperative in the handling of neurodegenerative illnesses like Alzheimer's disease (Suganthy et al., 2018).The biosynthesize AuNPs from leaf extract of *Sasa borealis* exhibit Anticancer movement in the molecular level, Toxic effect on HEK293 cells (Patil et al., 2018). AuNPs synthesize from Aqueous extract of *Hibiscus sabdariffa* having size 15-45 nm Used as a chemotherapeutical medication for the action of acute myeloid (Zangeneh and Zangeneh, 2020). In one of the study, Au and Au@Ag core-shell nanoparticles were synthesized using leaves extract of *Brassica oleracea var*. gongylodes showing Catalytic and antibacterial activity (Rani et al., 2023).

**Conclusion**

The present review presented the information about an eco-friendly plant based green synthesis of Au and Ag nanoparticles and their applications. The biosynthesize metal nanoparticles such as, AuNP's and AgNP's become a topic of interest due to their applications in different fields. Nanoparticles can be synthesized by various physical and chemical methods which carry toxicity to environment as compared to this green synthesis which provide clean, nontoxic, and environmental friendly approach. A variety of plant materials show a potential ability for the synthesis of nanoparticles. The secondary metabolites present in plants act as a reducing and capping agent which is responsible for the synthesis of nanoparticles. Furthermore, these metal nanoparticles have a wide range of applications like water dealing, catalysis, dye deprivation, drugs, sensor, microchip technology, biomedical field and imaging etc.

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Chapter 05

# Nanoparticles-the Future of Fish Medicine

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**ABSTRACT**

The unique properties of nanoparticles have led to the development of nanotechnology as a vast field of study, opening the door to new medical applications such as diagnosis, immunization, medication, and gene delivery. A remarkable rise in the uses of nanoparticles is due to their specific characteristics (specifically particle, size, surface reactivity, surface area, surface charge, and shape) compared to their bulk forms. Moreover, their unique properties have raised concerns due to their physiological reactions to biological structures after rapid interactions with surrounding constituents. Different nanotechnological tools like nanosensors, nanomaterials, DNA nanovaccines, drug delivery, gene delivery, DNA structure probing, and pathogens bio-detection, have the role in resolving many problems of animal health and treatment of diseases. Nanoparticles have also shown promise as sensitive and precise diagnostic tools for bacterial, fungal, and viral illnesses in aquaculture. This chapter discusses different sources, synthesis, types of nanoparticles, their role in diagnosing and treating fish diseases. However, further investigation is needed to explore the potential uses of nanotechnology in fish medicine.

**KEYWORDS**
Nanomedicines, Nanotechnology, Diseases, Pathogens, Medications

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## INTRODUCTION

The most promising field of the twenty-first century is nanotechnology, which physicist Richard Feynman first described in 1959. Since then, scientists have examined how it could affect various fields, including medical research (Roco 2003; Gonzalez et al., 2013). Particles with special characteristics that set them apart from their bulk forms are called nanoparticles. Numerous fields, including electronics, transportation, telecommunication, imaging, environmental remediation, chemical and biological sensors, and medication and gene delivery systems, are potential applications of nanoparticles (Fig. 1). Nanoparticles can produced artificially for various industrial and biological uses, or they can released from natural sources like volcanic eruptions (Aziz and Abdullah, 2021). Many NPs naturally dissolve in aquatic environments or grow larger following particle aggregation in the medium. The manufactured NPs, on the other hand, are more persistent than the natural ones because they are stabilized by priming and capping components (Handy et al., 2008). The bioavailability, persistence, toxicity, reactivity, and chemical and physical structure of metal nanoparticles (MNPs) could be used to assess their fate in the environment. Nanomaterials' nature is influenced by several variables, including bonded surface species, shape, molecular size, surface area, solubility, oxidation status, surface coating, and degree of agglomeration (Nel et al., 2006). Nanoparticles (NPs) significantly interact with natural organic matter (NOM) in aquatic ecosystems, altering their physicochemical properties and behavior. Functional groups in NOM, such as quinones and carboxyl groups, complex with NPs, affecting their dissolution and bioavailability (Philippe and Schaumann, 2014). Small molecular weight NOM components, like citric acid from aquatic plants, further enhance NP dissolution (Mudunkotuwa et al., 2012). These interactions allow NPs to penetrate subcellular structures, raising concerns about their environmental and biological impacts.

### Need of Nanotechnology

In the medical field, the fastest-growing food production sector is aquaculture, while infectious disease creates significant challenges. There are very few vaccines available, and viral diseases have a significant negative impact on the health and well-being of fish as well as significant financial losses. Vaccination is an important part of aquaculture. A

vaccine is a biologically based product that aims to enhance immunity against specific diseases or combinations of diseases. Vaccination is an effective therapeutic strategy for the prevention of a wide variety of bacterial and viral diseases. Vaccines are biological agents that trigger an immune response to a specific antigen acquired from an infectious pathogen causing disease. Vaccination against infectious diseases has been a practice for many years. Fish vaccines can be classified into two main types: modified live vaccines and killed fish vaccines. Killed fish vaccines contain formalin- killed or heat-killed vaccines. The term "killed fish vaccines" refers to immunizations that have been heat- or formalin-killed. Duff's 1942 report was the first to discuss the use of killed vaccination against *Aeromonas salmonicida* (Ma et al. 2019). They conducted oral immunization of *Oncorhynchus clarkia*. Generally, oral injection and immersion are not the only methods used to administer immunizations to fish (Adams et al. 2008). The most likely method of administration is determined by considering a variety of parameters, including the pathogen, the host fish's life phase, immunological memory level, vaccination manufacturing processes, labor costs, and so forth (Yanong and Erlacher-Reid, 2012). Even though the application of nanomaterials is still in its early stages, researchers working on aquaculture vaccinations are currently paying close attention to nanoparticles. An oral DNA vaccine against *Vibrio angulliarum* in Asian sea bass (*Laters calcarifer*) was studied utilizing chitosan/triploy and chitosan phosphate nanoparticles; the results demonstrated that the vaccine-induced immunological responses (Vimal et al. 2014). In a separate trial published by Shaalan et al. (2016), a nanoparticle-based vaccine against the virus ISAV was administered. The results demonstrated great promise for fish, showing that 77% of Atlantic salmon were protected from ISAV. (Rivas-Aravena et al. 2015). Recently, a study was conducted on the use of various organic materials, such as polylactic acid gel (PLGA), nanopoliplexes, chitosan, and virus-like particles, for the development of nanovaccines to control pathogenic fish diseases. The study showcased the potential of oral nanovaccines in fish aquaculture. Another study discovered that selenium nanoparticles were a new tool for controlling bacterial and viral fish diseases (Nasr-Eldahan et al. 2021).

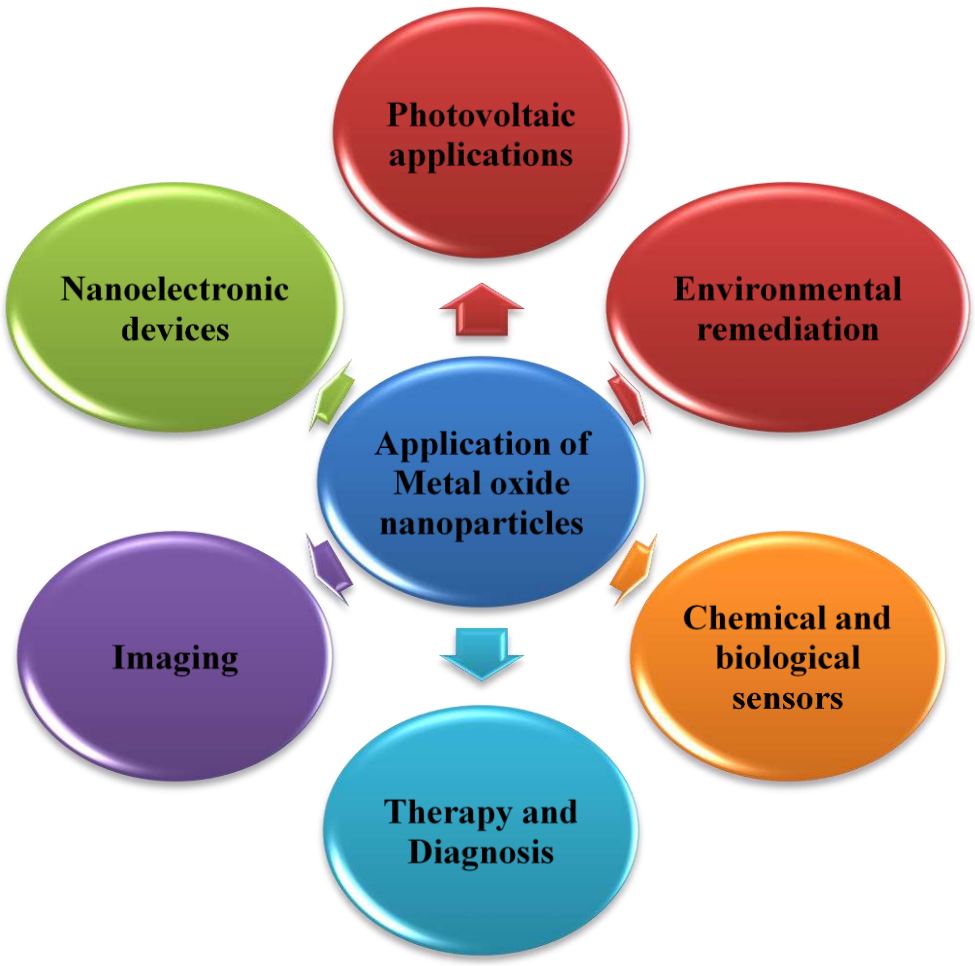


**Fig. 1:** Applications of nanoparticles.

**Synthesis of Nanoparticles**

Nanoparticles are typically synthesized by two main methods: top-down or bottom-up. In the top-down method, nanoscale particles are obtained by mechanically grinding bulk metal. Stabilizing agents, such as colloidal protecting agents, are then added to prevent oxidation or reassembly of the nanoparticles back to the microscale. While in Bottom-up methods formation of nanoparticles involves different physical and chemical methods, viz. electrochemical reduction of metals, etc., (Thirumalai Arasu et al., 2010). There are many different techniques are being developed for nanoparticle synthesis, which can be categorized into physical, chemical, and biological methods.

**Physical and Chemical Synthesis**

In organic solvents, nanoparticles are synthesized by thermal decomposition method. The cryochemical method synthesizes metal nanoparticles ranging from 5 to 80 nm in diameter (Sergeev et al., 1999) For silver nanoparticles, the microwave synthesis method has been adopted, which results in physically reducing silver using different microwave radiation frequencies (Jiang et al., 2006). This method was quicker and resulted in a higher concentration of silver nanoparticles compared to thermal method, under the same temperature and exposure conditions. Jiang et al (2006) also observed that large particles could be obtained by using a higher concentrations of silver nitrate, longer reaction times and higher temperatures. High concentration of polyvinyl pyrrolidone (PVP) (used for nanoparticle coating) resulted in smaller silver particle sizes, between 15 and 25 nm. Various methods for synthesizing metal nanoparticles include pulsed laser ablation, electro-reduction of $AgNO_3$ in an aqueous solution with the presence of polyethylene glycol, spark discharge, micro-emulsion for the preparation of Ag-$Fe_3O_2$ nanoparticles (Gong et al., 2007), and chemical reduction of silver nitrate using trisodium citrate along with sodium borohydride as a reducing agent to synthesize PVP-coated silver nanoparticles (El Mahdy et al., 2015). Synthesis of non-metallic nanoparticles e.g. polymeric chitosan nanoparticles comprises different methods. Such as ionotropic gelation method which involves dissolving chitosan in acetic acid and the addition of polyanion tripolyphosphate (TPP). This method relies on electrostatic interaction between the chitosan amine group and groups of polyanion polymer (Rajesh Kumar et al., 2008). The polyelectrolyte complex method is based on electrostatic interaction between cationic groups in chitosan and DNA, neutralizing the charges and formation of nanoparticles. The microemulsion method uses surfactants but has drawbacks such as using organic solvents, long preparation time, and the complexity of the washing processes. The most common method for synthesizing Poly D, L-lactide-co-glycolic acid (PLGA) nanoparticles is the precipitation method along with the double emulsion solvent evaporation method (Tinsley-Bown et al., 2000).

**Biological Synthesis (Green synthesis)**

Developing eco-friendly and economical methods to synthesize nanoparticles is a very significant approach (Kalishwaralal et al., 2008). The biologically synthesized nanoparticles are originate from three main groups of organisms: bacteria, fungi, and plants. Biosynthesis of nanoparticles is a bottom-up method that mainly involves reduction/oxidation reactions (Prabhu and Poulose, 2012). Different phytochemicals of plants or enzymes with reducing or antioxidant properties play role as a precursor compounds to synthesize the required nanoparticles. There are three main components in a green synthesis system: an eco-friendly reducing agent, a solvent medium for synthesis, and a harmless stabilizing agent. Biologically synthesized silver and gold nanoparticles from cashew nut shell liquid exhibited bactericidal activity against many fish pathogens (Velmurugan et al., 2014). Moreover, in juvenile *Feneropenaeus indicus*, silver nanoparticles synthesized from tea leaf extract (*Camellia sinensis*) also showed antibacterial activity against *Vibrio harveyi* at high doses (Vaseeharan et al., 2010). Biologically synthesized zinc oxide nanoparticles (ZnO-NPs) using Aloe leaf extract indicated higher antibacterial activity than nanoparticles by standard chemistry (Gunalan et al., 2012). This process is environmentally friendly and economically achievable.

**Types and their Modes of Action**

Nanoparticles are frequently utilized in medicines in various forms (Fig. 2) (De Jong and Borm, 2008). They come in different shapes, sizes, structures, and compositions. The basic classification include carbon-based, metal-based, and polymeric nanoparticles (Prakash et al., 2024). There are several types and their modes of action are given below;

**Nanospheres**

Nanospheres are spherical nanoparticles that enable easy drug coating due to their large surface area. They also contribute to tissue regeneration (Shaalan et al., 2016).

**Nanocapsules**

Nanocapsule's structure contains an oil or water-based core and a shell. The drugs are placed within the core, while the shell protects the drug against hydrolysis and degeneration (Torchilin, 2006). Liposomes are examples of nano capsules that are lipid bi-layers in the form of nano-sized spheres. They can deliver both hydrophilic and lipophilic medicines because their structure is similar to eukaryotic cell membranes.

**Carbon Nanotubes (CNT)**

Carbon nanotubes are nano-sized tubes of cylindrical shape formed by graphene sheets roll. Due to their unique structure, multiple antigens can attach to them simultaneously. They can efficiently penetrate any kind of cell, including dendritic cells of immune system. Properties of carbon nanotubes include inertness, non-toxicity, and non-immunogenicity. They also serve as a scaffold for antigen targets, making them a promising option for fish vaccine development (Torchilin, 2006).

**Dendrimers**

Dendrimers are nanscale molecules with three-dimensional structures. They have a central core from which low molecular weight, multifunctional branchlike units extend. These branches act as antimicrobial agents and serve as a delivery system for genes, medicines, vaccines, etc. Additionally, dendrimers form the basis for tissue regeneration (Wu et al., 2015).

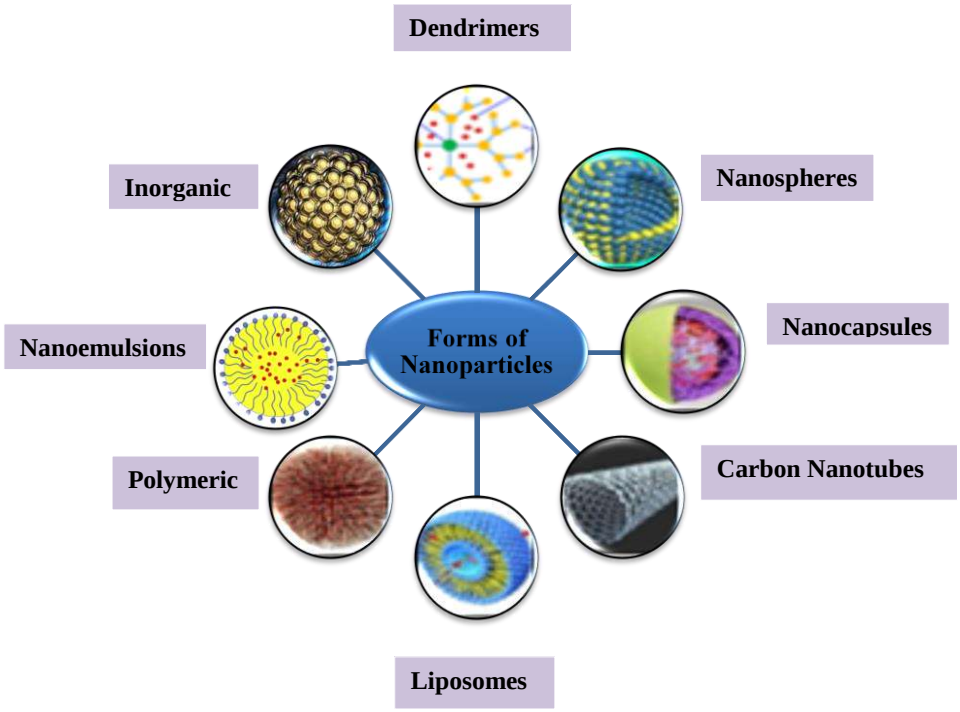


**Fig. 2:** Forms of nanoparticles used in nanomedicine

**Nanoliposomes**

Nanoliposomes are lipid-based nanoparticles that have a hydrophilic core and lipophilic outer part. This unique feature facilitates in encapsulation of both hydrophilic and hydrophobic antigens. The capsule helps in protection against enzyme reactions when the vaccine is delivered. These physiochemical characteristics of structure, size and fluidity have enabled nanoliposomes to maximize immune responses. Similarly, larger liposomes of diameter 2mm and more promoted interleukin-10 production effectively, whereas smaller liposomes of 500nm diameter influenced splenocytes by promoting a higher level of interferon production. Thus, nanoliposomes could be manipulated for targeted immune cell activation, antigen production, and maximizing the immune system potential. Their biocompatibility ensures safe interactions with fish immune cells and tissues, making them a safer choice for vaccine delivery (Wani and Ahmad, 2013).

**Polymeric Nanoparticles**

Polymeric nanoparticles are another type of nano-scaled particle having a core and a shell. Core has dissolved drugs, and shell controls their release (Zielińska et al., 2020). Their biocompatibility makes them a versatile platform for encapsulating antigens of fish. Their size, composition and structure affect antigen uptake by antigen-presenting cells and immune responses in fish. Polymeric nanoparticles can carry drugs and release antigens, which helps provide long-lasting protection and reduces the need for frequent booster vaccines. Natural polymeric nanoparticles such as chitosan, hyaluronic acid, and alginate can be found in nature. Chitosan, for example, is a commonly used biodegradable polymeric nanoparticle that has been used to improve mucosal immunity through oral vaccination in fish. In human vaccinations, synthetic polymers like poly-lactic acid (PLA) and poly-lactic-co-glycolic acid (PLGA) are used to deliver synthetic proteins, nucleic acids, and peptides (Wani and Ahmad, 2013). These polymers have also been tested for use in oral vaccinations for fish.

**Nanoemulsions**

Nanoemulsions are solid spheres of nanometer size, with an amorphous surface. These are stable emulsions containing water and oil-based phases with a negatively charged lipophilic surface to facilitate encapsulating drugs (Jasiwal et al., 2015). In the oil phase, hydrophobic antigens can be effectively incorporated, while the hydrophilic antigens can be enclosed within the water phase. Due to their adaptability, the antigens are more stable and soluble, maintaining their integrity throughout storage and vaccination administration. Nanoemulsions, due to their small droplet size, make controlled and sustained antigen release possible. This sustained antigen exposure stimulates the immune system in fish, which is required for the development of a robust and long-lasting immune response to infections (Wani and Ahmad, 2013).

**Inorganic Nanoparticles**

These nanoparticles have a high surface area-to-volume ratio, and good physicochemical features for vaccine formulation. This allows for efficient antigen adsorption and modification, resulting in increased stability and controlled release. Metal oxides and metallic nanoparticles are medicinal devices that deliver drugs (Mody et al., 2010). They are ideal for administering a diverse range of fish vaccine antigens. Furthermore, inorganic nanoparticles can act as additives

boosting the immune response and greatly increasing the efficiency of fish vaccines against various fish diseases (Wani and Ahmad 2013). Non-organic nanomaterials include gold, carbon, calcium phosphate, nickel, cobalt, and quantum dots.

**Role of Nanoparticles in Diagnosis and Treatment of Fish Diseases**

In fish, nanotechnology is used for the synthesis of nanoparticles, which have numerous applications for diagnosis. These include the use of gold nanorods, and the application of carbon nanotubes, for quick and affordable disease detection, making illness identification feasible at an early stage. Nanoparticles are also crucial for sensitive and specific diagnosis of viral, bacterial, and fungal diseases in aquaculture and fish, and they can even cross the blood-brain barrier in fish. Instruments of sub-micrometer sizes are used for diagnosis, prevention, and better cure of illness as well as to improve the overall life quality of patients. Nanotechnology is used to accelerate the development of regenerative drugs, allowing for the generation of bone, cartilage, prosthetic skin, and other tissues to treat serious injuries or organ insufficiency in patients. The use of this technology changes cellular functions more effectively to emulate the effects of organs and natural tissues (Hobson 2016; Haleem et al., 2023).

In recent years, medical imaging has become increasingly important for diagnosing a wide range of diseases. The advancement and progress in computer tomography and MRI technology have been remarkable, providing highly sensitive and accurate diagnostic tools for both in vivo and in vitro applications that surpass the capabilities of modern equipment. The progress in magnetic resonance and computer tomography is impressive. However, nanotechnology offers instruments for in vitro and in vivo diagnostics that are sensitive and very accurate, well beyond modern equipment's capabilities. The ultimate goal of these advancements is to facilitate early disease diagnosis, with nanotechnology potentially revolutionizing cellular and sub-cellular diagnostics.

Different types of nanoparticles can be used in fish cells to deliver medication effectively. Once they reach the target area, nanoparticles start working at the molecular and cellular levels. Gills in fish are responsible for exchanging gases between their internal and external water environments. Various substances, including organic compounds and metal nanoparticles, can interact with fish gills and eventually enter their bloodstream (Polizu et al., 2008; Huang et al., 2018; Zhang et al., 2021). Nanoparticles have been found to reduce the death rate in fish by influencing cell behavior. Nano-medicines are expected to bring significant benefits to drug delivery and treatment. Physicians can use these particles to target the source of illness more effectively, reducing side effects and increasing treatment efficacy. They also provide new methods for controlling drug release. Due to the various types of nano-drugs available, as well as their applications in drug delivery and diagnostics, nano-medicines offer substantial benefits in the fields of instrumentation and pharmaceutical manufacturing.

The production of nanoparticles and treatments based on nanotechnology is being developed as a research field. In the recent decade, by using imaging, therapies, and diagnostics, manufacture of nano-drugs has significantly improved. Nano-drug systems mainly focus on upgrading the bioavailability of tissue delivery, half-life, and orally giving therapeutic items. Nano-drugs are controlled at smaller levels with significant developments in their medicinal effects and minimize adverse health effects (Haleem et al., 2023). In pharmacology, nanoparticles play a significant role in the nutrient supply of fish, such as in the synthesis of vaccines. These nanoparticles have broad applicability due to their numerous advantages, such as their ability to protect antigens from destruction by enzymes.

When biological materials combine with nanotechnology, there is a significant increase in innovative nanoparticles that are synthesized effectively at a suitable cost. Nanoparticle synthesis is also a niche for researchers, but it is a fast-growing and impacting key economic field, providing new nano-labeled products with novel and unique functions. Nanoparticles improve newly developed nano-labeled products, which are important factors for the success of the nanotechnology industry (Munawar, 2021). By utilizing the intrinsic qualities of different types of nanoparticles, the increasing science of nano-medicine shows numerous opportunities to improve fish health. Nano-silver and zinc oxide have been employed by prior research as antibacterial and therapeutic nanomaterials to decrease infections in aquaculture systems. In short, the development of nano-medicine has vast, and non-specific use in the field of animal health (Kakakhel, et al., 2021).

Researchers are investigating the antibacterial properties of nanoparticles, which could be beneficial as nano-medicines for fish species (Table 1). Graphene has emerged as a viable, low-cost commercial nanomaterial. The oxidized graphene is easily processed and soluble in water (Brisebois and Siaj, 2019). Graphene oxide has been found to inhibit important waterborne pathogens such as *P. aeruginosa*, *S. aureus*, *E. coli*, and *V. harveyi*. When graphene oxide interacts with pathogens, it causes cell membrane impairment, lysis, and mechanical enfolding (Kumar et al., 2019). The application of metal oxide nanoparticles in aquaculture disease management has been extensively studied, and it has been found that nanoparticles such as ZnO-NPs, CuO-NPs, Au-NPs, Ag-NPs, and TiO2-NPs possess the ability to fight against various disease-causing pathogens.

ZnO-NPs are reported to inhibit the growth of various pathogenic bacteria viz. *Staphylococcus aureus, Flavobacteriam branchiophilum, Aeromonas hydrophila, Vibrio species, Pseudomonas aeruginosa, Edward seillatarda, Bacillus cereus and Citrobacter spp* (Swain et al. 2014), whereas CuO-NPs are effective against Saprolegnia sp. from the host white fish and also used as strong antifungal agent. Au-NPs have been tested against bacterial pathogens of fish and observed to possess antibacterial properties. Ag-NPs are used as an effective antibacterial agent as it release Ag+ ions which bind with the proteins cell membrane of the bacterial cells and damage bacterial cell membranes. Also, Ag-NPs are found to be effective against multidrug resistant bacteria among them, methicillin-resistant *Staphylococcus aureus* is one.

**Table 1:** Applications of nanotechnology in fish disease management

| Nanoparticle | Test Organism | Pathogen | Nanomedicines / Nano-vaccines | References |
|---|---|---|---|---|
| PLGA | *Salmo salar* | IPNV | PLGA Nanoparticle-TA | Munang'andu et al. (2012) |
| Chitosan | *Danio rerio* | VHSV | NPrgpG, pICrgpG | Kavaliauskis et al. (2016) |
| PLGA | *Labeo rohita* | *Aeromonas hydrophila* | Np-rOmpW | Dubey et al. (2016) |
| Alginate | *Oncorhynchus mykiss* | *Ichthyophthirius multifiliis* | OCMCS-HA/aerA-NPs | Heidarieh et al. (2015) |
| Chitosan | *Saltator maximus* | TRBIV | pDNA-CS-NPs | Zheng et al. (2016) |
| Chitosan/TPP | *Lates calcarifer* | Nodavirus | pFNCPE42-CS/TPP | Vimal et al. (2014) |
| Calcium phosphate | *Labeo rohita* | *Aeromonas hydrophila* | SP-CaNP | Behera and Swain (2011) |
| PMMMA-PLGA | *Oreochromis niloticus* | *Streptococcus agalactiae* | PTRBL/Trx-SIP | Zhang et al. (2015) |
| PLGA | *Panaeolus olivaceus* | LCDV | pEGFP-N2-MCP | Tian and Yu (2011) |
| Chitosan | *Acanthopagrus schlegelii* | *Vibrio parahaemolyticus* | pEGFP-N2-OMPK | Li et al. (2013) |
| PLGA | *Oncorhynchus mykiss* | IHNV | PLGA-pCDNA-G 11<br>PLGA-pCDNA-G 22 | Adomako et al. (2012) |
| Liposome | *Epinephelus bruneus* | *Vibrio harveyi* | Liposome-V. harveyi | Harikrishnan et al. (2012) |
| Carbon nanotubes | *Ctenopharyngodon idella* | GCRV | SWCNTs- pcDNA | Zhu et al. (2015) |
| OCMCS-hyaluronic acid | *Cyprinus carpio* | *Aeromonas hydrophila* | OCMCS/aerA-NPs | Liu et al. (2016) |

(Latif et al., 2022)

Various herbal and phyto-extracts are used to treat fish diseases as potential drugs. Different nanoparticles are prepared under optimized hydrodynamic conditions by using medicinal plant/herbal extracts, and a complex of the phyto-nanoformulation is then administered as a medicine with synergistic effects of both. Phyto-nanoformulation of plant extract and Ag-NPs composite has been observed to work as an antibacterial against *Aeromonas hydrophila*, causing motile *Aeromonas septicemia* in fish.

**Future Scope**

Nanomedicine is expected to play a vital role in the future of personalized medicine, covering everything from prediction to monitoring. Nanoscale materials are propelling the development of highly sensitive sensors and biomarkers, allowing for the early and precise detection of multiple diseases simultaneously. Nanomedicine enables precise mapping of diseases with enhanced targeting and chemical sensitivity, resulting in more effective treatment with reduced side effects and harm to healthy cells. While several nanomedicine products, such as nano-encapsulated doxorubicin, are already in use, future challenges lie in optimizing drug loading and release and further harnessing the diagnostic and therapeutic potential of metallic nanoparticles. As with any advanced technology, nanomedicine must balance its promising potential against potential risks and challenges. Nanomedicine, like any medical device or treatment, must undergo rigorous testing and evaluation before being used on patients. This includes assessing its full potential and toxicity and conducting multistage clinical studies. In the future, nanotechnology may enable the direct detection of health issues rather than relying on external sensors, medical expertise, and probabilistic diagnostic algorithms. Additionally, nanotechnology could be used by athletes to identify muscles with excellent circulation and lower lactic acid production, allowing them to adjust their training frequency and maximize their potential in less effective muscles, thereby enhancing overall efficiency.

**Limitations**

Several significant scientific and technological challenges hinder the advancement of nanotechnology, including ensuring the reproducibility and quality control of nanomaterials, achieving scalability and efficient production, and addressing unwanted by-products of nano-engineering. Furthermore, the high cost and unknown health and environmental implications of nanoparticles pose significant barriers. These structural hurdles deter investors, making pharmaceutical companies reluctant to invest in nanotherapeutics. While nanotechnology offers numerous potential applications, its use in the biological sciences is limited by several obstacles that need to be addressed. The in vivo application of nanotechnology raises concerns about its short-term and long-term impact on the body, as the small particles can evade the immune system, accumulate in specific tissues, and potentially cause harm.

**Conclusion**

The demand for fish is increasing, but global fish stocks are declining, presenting challenges for food security in many developing countries. This chapter discusses how nanotechnology can support sustainable aquaculture and improve the

aquatic environment essential for fish farming. It highlights how nanomaterials can revolutionize the fisheries and aquaculture sector by offering innovative solutions for disease detection, drug absorption, and water treatment. The chapter emphasizes the need for further research to optimize the use of nanoparticles in fish disease diagnostics and treatment to meet the increasing demands of aquatic animal health.

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Chapter 06

# Revolutionizing Aquatic Environments with Nanoparticles for Fish Health

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**ABSTRACT**

Aquaculture, an essential field in worldwide food supply, faces several issues including epidemics of diseases, environmental deterioration, and the need for environment friendly methods. The nanotechnology is a possible path for solving these challenges by transforming aquatic ecosystems through nanoparticles to improve fish health. This chapter investigates the complex role of nanoparticles in aquaculture, with a focus on their use in water purification, preventing illness, and nutritional enrichment. Nanoparticles have unique physicochemical features that allow for targeted administration of antibacterial medicines, immuno-stimulants, and nutrients to aquatic species. Furthermore, nanoparticles are extremely efficient at removing pollutants, which improves water quality and reduces environmental stressors. However, widespread adoption of nanoparticle-based aquaculture technologies requires a detailed understanding of their potential ecological consequences and regulatory frameworks. This chapter emphasizes the importance of interdisciplinary research efforts targeted at leveraging nanoparticles' revolutionary potential to increase sustainable fish production while protecting the aquatic ecosystems.

**KEYWORDS**
Antimicrobial, Disease, Aquaculture, Ecosystems, Nutrition

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## INTRODUCTION

The worldwide need for fish is rising as a result of an evolution toward intensive aquaculture systems and artificial feeding, which heavily relies on fish (Abdel-Ghany and Salem, 2020; Maclean, 2003). This is particularly significant in developing nations where fish are a key source of animals' protein. About 15% of the 2.9 billion people who consume animal protein on a global average come from aquaculture and fisheries, and this percentage is still rising. About 520 million people indirectly depend on aquaculture and fisheries, whereas 43.5 million people are actively employed by them (Asche et al., 2015). In order to sustain the growth of aquaculture, novel and non-traditional fish meals that enhance the physiological and biological functions of cultivated fish must be created. The delivery of food, vaccinations, and other biological components to diverse fish systems is greatly aided by the nanotechnology. The medical applications for nanoparticles are numerous and include drug and gene delivery, vaccination, and diagnostics. Many nanoparticles are less damaging to the different immune system cells in fish. The nanoparticles transmit biological materials at a lower cost than other materials (Minetto et al., 2016). Aquaculture makes extensive use of:

- ✧ Traditional disinfectants (like hydrogen peroxide and malachite green)
- ✧ Antibiotics (like tetracyclines and sulfonamides)
- ✧ Anthelmintic agents (like avermectins and pyrethroid insecticides)

However, these methods have a number of disadvantages, such as high chemical drug costs, adverse effects on non-target organisms, and increased pathogen resistance. The growth of the nanotechnology sector has been greatly aided by the newly designed nanoenabled items that are boosted by nanoparticles (NPs). The NPs have unique physicochemical properties that differentiate them from bulk materials. For example, their greater surface area to volume ratio leads to enhanced reactivity. The NPs range in size from 1 to 100 nm in at least one dimension (Aiken et al., 2006).

**Nanoparticles for Fish Health**

The nanoparticles are gaining widespread attention as a sensitive and precise approach for identifying viral, bacterial and fungal diseases in aquaculture (Oberdörster et al., 2005a). When it comes to drug and nutrient delivery in fish, these nanoparticles are less toxic than their biological counterparts; however, metal toxicity in fish can be assessed more successfully in order to clarify the effects of nonmetals on the different fish cell types (Kreyling et al., 2006; Borm et al., 2006).

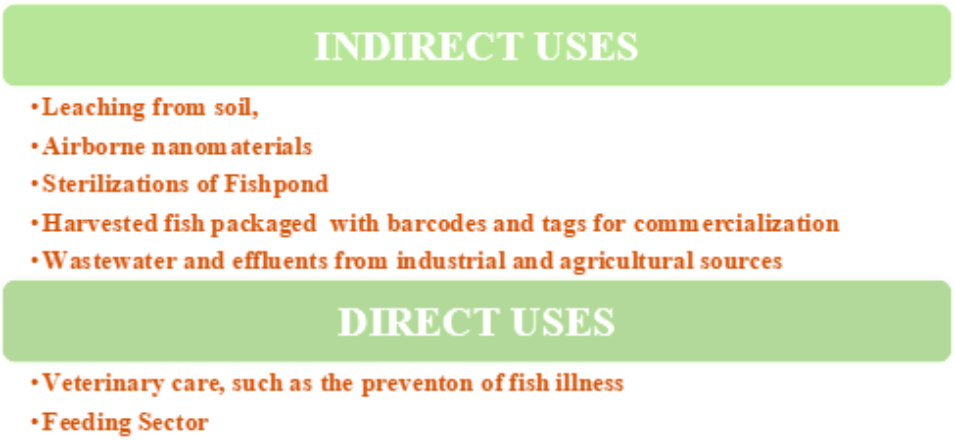


**Fig. 1:** Nanoparticles are utilized for a variety of direct and indirect purposes.

**Table 1:** Types of Nanoparticles

| Types of Nanoparticles | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **1.** | Ag NPs | **2.** | $TiO_2$ NPs | **3.** | ZnO NPs | **4.** | Nanocomposite films | **5.** | Se NPs |
| **6.** | CNPs | **7.** | FeNPs | **8.** | Nanodevices | **9.** | CSNPs | **10.** | $La_2O_3$ NPs |

**Silver Nanoparticles**

The widespread usage of silver nanoproducts may enhance the release of these particles into the aquatic environment (Taju et al. 2014; Benn and Westerhoff, 2008). It exits in four oxidation states: Ag+3, Ag+2, Ag+, and Ag in the aquatic environment. The silver nanomaterial can be found in the aquatic media as colloidal particles. There are several ways it could be discharged into the air and water, including:

- ✧ Rock weathering
- ✧ Fossil fuel combustion
- ✧ The silver in the water and ground reservoirs is released by rain (Wijnhoven et al., 2009)
- ✧ Biocidal agents will cause an approximate 68% increase in the nano silver burden in waste water (Blaser et al., 2008)
- ✧ Ore processing
- ✧ Cement production

The silver (Ag) nanoparticles (nAg) are the most well-characterized multiple mechanism nano-based antibacterial agent. The fish illness causing pathogens *Aliivibrio salmonicida* is resistant to the antibacterial qualities of chitosan-Ag nanocomposites (CAgNCs). The inhibition of A. salmonicida growth by CAgNCs demonstrated minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of 50 and 100 mg/L, respectively. The CAgNCs were utilized as a feed ingredient, there were no adverse effects on *Oplegnathus fasciatus* testis cells up to 50 mg/L or Danio rerio at 12.5 mg/kg of body weight/day (BW/day). This implies that fish pathogenic bacteria can be inhibited by CAgNCs acting as an antibacterial agent (Dananjaya et al. 2016).

The AgNPs can be used in aquaculture facilities' water treatment systems to enhance water quality and reduce microbiological contamination in recirculating aquaculture systems (RAS). This contributes to keeping aquatic life in a healthy habitat. The AgNPs have demonstrated promise in aquaculture for accelerating the fish wound healing. They can be administered topically to heal wounds and lesions, accelerating healing and lowering the chance of recurrent infections. The AgNPs have been investigated as a way to improve growth performance in fish feed formulations (Søiland, 2018).

During the production process, Ag-NPs are coated with various organic chemicals that might alter the particles' stability, toxicity, and destiny in biological and aqueous media. Fish are more resilient to xenobiotic exposure that contains silver waste since they are aquatic species. The Ag-NPs enter fish bodies primarily through the gills, where histological changes happen quickly (Sayed et al., 2020).

**Titanium Dioxide**

The paints used on facades have the potential to discharge titanium dioxide nanoparticles into aquatic life (Kaegi et al., 2017). Most of the nanomaterials are not as accessible to living things because of their tendency to aggregate and their rather poor solubility in water (Brant et al., 2005; Maynard et al., 2004). The fish bowls can be treated with water by coating ceramic or stone with nanoparticles of titanium dioxide (TiO2). The moss and germs in fish tanks can be removed with stones or ceramics covered in TiO2 nanoparticles. The technique can be applied to commercial aquariums and fish ponds

to reduce the cost of water treatment. One potential use of nanotechnology in the seafood industry is the implementation of various conservation and packaging methods that prevent enzymatic and microbiological decomposition, hence ensuring food safety. The Ag and TiO2 both lessened the bacterial accumulation in estuary water. The Ti photoelectrolysis has been applied to environmental applications such as disinfection and sterilization (Mühling et al. 2009). To protect farmed fish and shrimps from UV rays, the TiO2 NPs are added to aqua feed compositions. In aquatic creatures, the UV radiation from sunshine can lead to stress, damage DNA, and increase susceptibility to illness. By physically obstructing harmful UV rays, TiO2 NPs lower the risk of sunburn and related health problems. The TiO2 NPs can be used to coat tanks, nets, and pipelines used in aquaculture to give them antibacterial qualities (Vibhute et al., 2023). The aquaculture systems can remain hygienic because of the photocatalytic activity of TiO2 NPs, which prevents the formation of pathogenic bacteria and biofilms on surfaces and allows for continual cleaning (Yesilay et al., 2023).

**Carbon Nanoparticles**

The carbon nanoparticles can be ingested by aquatic invertebrates such as; for copepods (*Amphiascus tenuiremis*), amoebae (*Entamoebahistolytica*), infusoria (*Stylonychia mytilus* and *Tetrahymena pyriformis*) and cladocerans (*Daphnia magna*) (Oberdorster et al., 2006). The internal organs are permeable to nanoparticles.

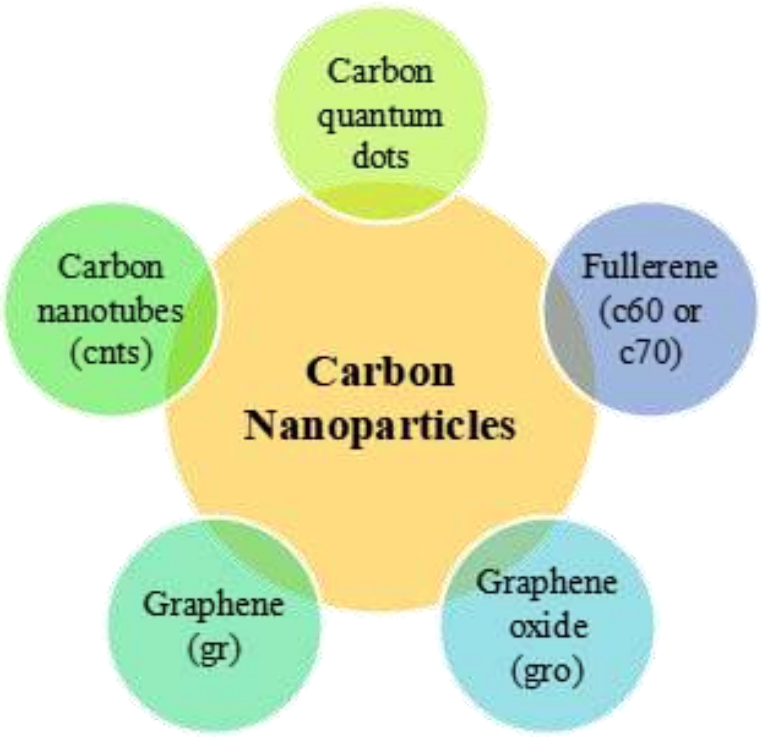


**Fig. 2:** Different CNPs for water purification and remediation in aquaculture systems.

Because of the adsorption qualities of these nanoparticles, organic pollutants, heavy metals, and other impurities can be removed from water, enhancing its quality and lowering pollution levels in the environment. In aquaculture, carbon nanoparticles can be used as medicine delivery vehicles (Templeton et al., 2006). Functionalized CNPs have the ability to encapsulate and transport medicinal substances, including vaccines, antifungal agents, and antibiotics, to specific locations in aquatic species. This tailored delivery method minimizes negative effects on non-target organisms while increasing the efficacy of therapies. The creation of sensors and biosensors for measuring water quality indicators in aquaculture facilities, like pH, temperature, dissolved oxygen, and pollutant levels, has made use of carbon nanoparticles (Zhu et al., 2009; Roberts et al., 2007; Zhu et al., 2006). These sensors give off real-time data on the state of the environment, allowing for prompt management and intervention measures to keep the conditions ideal for aquatic life. By improving digestion and nutrient absorption in the gastrointestinal tract of aquatic organisms, the carbon nanoparticles may increase the feed efficiency and nutrient utilization (Elias et al., 2007).

**Nanoparticles of Iron**

The date palm, or *Phoenix dactylifera*, is used to produce iron nanoparticles (FeNPs), which exhibit outstanding antibacterial properties. When given FeNPs, sturgeon and young carp showed a higher development rate 24% and 30%, respectively.

The aquaculture systems have made use of iron nanoparticles for water purification. They can be used to rid water of impurities such organic pollutants, heavy metals, and microorganisms. The FeNPs can help pollutants precipitate or aggregate due to their adsorption qualities, which enhances water quality and guarantees a healthy environment for aquatic life (Asad et al., 2023). The aquaculture can benefit from the addition of iron through the use of iron nanoparticles.

For aquatic creatures, iron is a vital micronutrient that is needed for many physiological functions, including as oxygen transport, metabolism, and enzyme function. The FeNPs can be added to aqua feed formulations to improve nutrient uptake and encourage shrimp and fish farming's growth and development.

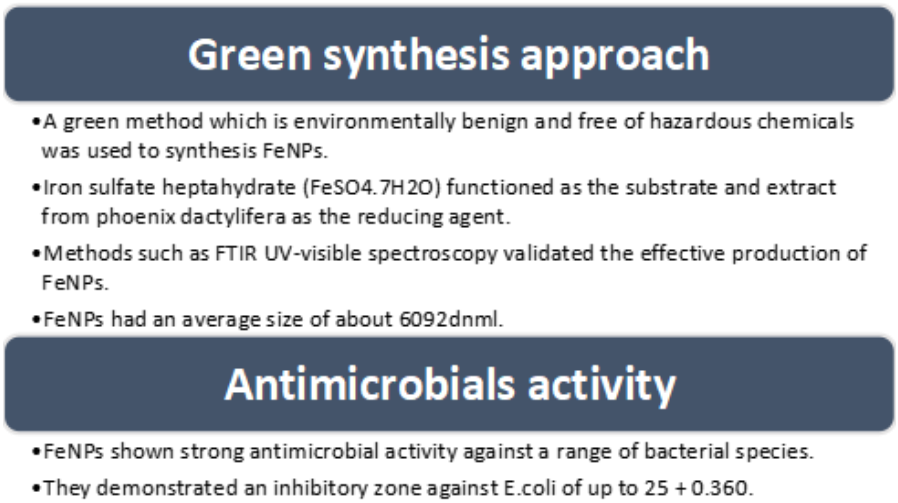


**Fig. 3:** Green synthesis approach and antimicrobials activity of nanoparticles of iron

The potential of iron nanoparticles in aquaculture bioremediation applications has been studied. In bioremediation procedures, they can function as electron donors or acceptors, enabling microorganisms to break down or change organic pollutants and toxins (Brahmchari et al., 2023). The FeNPs have the potential to improve the efficacy of bioremediation methods for treating aquaculture effluents and wastewater, such as artificial wetlands and microbial fuel cells. The aquatic ecosystem habitat restoration efforts can make use of iron nanoparticles. They can be used to improve nutrient cycling and ecosystem productivity, repair damaged habitats, and replenish aquatic vegetation. The FeNPs may promote the development of macrophytes and other helpful microbes, which would restore a variety of healthy aquatic environments for fish and other aquatic species (Brahmchari et al., 2023).

**Nanocomposite Films**

Proteins, lipids, and polisaccharides are the examples of natural biopolymers that can be used to create nanocomposite films. These sources are recognized as a good alternative to petrochemical-based plastics for packaging because they are safe for the environment, edible, and anti-cancerogenic. They also use reverse osmosis and nano filtration to reduce the salinity of drilling water, which is used to wash and process seafood (Dananjaya et al., 2017). The nanocomposite films can be used in aquaculture equipment as coatings or wound dressings to help repair wounded fish and shellfish faster and avoid infection. According to Dar et al. (2020), these films can act as a barrier against waterborne pathogens, lowering the chance of secondary infections and enhancing the general wellbeing and health of aquatic species. The food packaging materials for marine goods in aquaculture can be made using nanocomposite films that possess antibacterial characteristics. According to Laorenza et al. (2022), these films have the ability to prolong the shelf life of seafood by preventing the growth of germs and microorganisms that cause deterioration. This ensures food safety and quality throughout storage and transportation. The aquaculture facilities' water treatment systems can make use of nanocomposite films that have been integrated with nanoparticles like titanium dioxide, copper, or silver. These films have the ability to efficiently remove pollutants, pathogens, and toxins from water, enhancing its quality and fostering an environment that is favorable to aquatic life (Azari et al., 2020).

**Selenium (Se)**

It has lately been taken into consideration in numerous case studies for animal nutrition as a trace element that is necessary for life. Selenium is a part of the glutathione peroxidase enzymes (GSH-Px), which use glutathione reduction to shield the cell membrane (Sabbioni et al. 2015). The dietary supplements containing Se, can be obtained (Fotedar and Munilkumar 2016). The Se-NPs are becoming more and more popular because of their bioavailability and antioxidant defense qualities (Sonkusre et al. 2014). When crucian carp (*Carassius auratus gibelio*) were fed enriched diets, the ultimate weight, muscle protein content, GSH-Px activity in liver and blood plasma, and FCR all decreased (Zhu et al. 2009).

The Se enhanced LDH, cellular protein levels, Na+/K+ -ATPase, SOD, and GSH-Px in crucian carp (C. auratus gibelio); these effects were size- and dose-dependent. Nile tilapia (Oreochromis niloticus) showed considerable growth influence with moderate (0.5 mg/kg) and high (2.5 mg/kg) doses of Se NPs provided via spiked feed; the fish showed a weight gain rate of 86.3 ± 4.7 g (Deng and Cheng, 2003). The selenium is an essential component for aquatic life since it plays a role in numerous physiological functions such antioxidant defense, thyroid hormone metabolism, and immune system function. To address the nutritional needs of farmed fish and shrimp, aqua feed formulations can incorporate the SeNPs as a source of selenium supplementation. When compared to conventional selenium sources like selenite or selenate, selenium nanoparticles have higher bioavailability and efficacy, which benefits aquatic creatures' growth performance, disease

resistance, and general health. In aquaculture facilities, selenium nanoparticles can be utilized to control the quality of the water, especially in intensive production systems and recirculating aquaculture systems (RAS). By creating stable complexes or precipitates, the SeNPs can reduce the bioavailability and toxicity of hazardous compounds to aquatic species, hence aiding in their detoxification. These substances include heavy metals, pesticides, and pollution. Additionally, fish development and productivity can be promoted by the addition of sulfur nanoparticles to water by increasing oxygenation and clarity (Deng and Cheng, 2003).

**Zinc (Zn)**

This crucial micronutrient is engaged in multiple metabolic pathways and plays a crucial role in, Vit A metabolism, protein synthesis, lipid metabolism and regulating energy consumption (Muralisankar et al. 2014). In grass carp (*Ctenopharyngodon idella*), the nZnO as a dietary Zn source showed enhanced development and immunological response (Faiz et al. 2015). The freshwater shrimp (*Macrobrachium rosenbergii*) has shown a considerable increase in protein content, antioxidant enzyme activity, and greater weight following ninety days of feeding with feed improved with the nZnO (Muralisankar et al. 2014). The TiO2 will enhance rainbow trout (Oncorhynchus mykiss) growth performance.

**Toxicity Reduction**

Water bodies can have their level of toxicity reduced by zinc nanoparticles. They support a better aquatic environment by reducing dangerous pollutants.

**Bacterial Load Reduction**

By lowering the bacterial burden in water, these nanoparticles improve the quality of the water.

**Wastewater Treatment**

When treating wastewater, zinc nanoparticles are utilized to help remove impurities and pollutants.

**Wound Healing**

In aquaculture, zinc nanoparticles can speed up the healing of wounds in wounded fish and shellfish (Ramsden et al. 2009). The zinc nanoparticles (ZnNPs) have regenerative qualities and can promote tissue regeneration, collagen formation, and cell proliferation to speed up the healing process. The aquatic species can heal more quickly and have higher survival rates when ZnNPs are applied topically as wound coatings or dressings. This is because ZnNPs can improve wound closure and lower the risk of secondary infections.

**Chitosan NPs**

The chitosan (CS) and chitosan nanoparticles (CSNP), which are natural, safe cationic biopolymers, improve the immunological response and growth of fish (El-Naggar, 2020). The deacetylation of chitin results in the production of CS. Chitin is an essential component of the exoskeletons of both terrestrial and aquatic crustaceans, such as those of shrimp, crabs, and crayfish, as well as the cell walls of some microorganisms (Xu et al., 2020).

**Growth Enhancement**

As feed additives, chitosan and chitosan nanoparticles improve the growth efficiency of aquatic organisms. In fish species such as *Misgurnus anguillicaudatus* and *Cyprinus carpio*, their inclusion in diets has been demonstrated to improve weight increase and lipid metabolism (Abdel-Ghany and Salem, 2020 and Zaki et al*.,* 2015).

**Immune Stimulation**

These substances are essential for increasing immunological function. They improve fish health by stimulating the immune system. The chitosan nanoparticles are very useful in inducing immunological responses in species such as Oreochromis niloticus (Nile tilapia), due to their enormous fast transport to target areas and surface area. It was shown that CS reduced the death rate by inducing non-specific immunity (Abdel-Ghany and Salem, 2020 and Zaki et al*.,* 2015). Supplementing *Oncorhyncus mykiss* with 2.5 g of CS per kg of feed has been shown to dramatically increase *O. mykiss* survival rates under stressful settings (Abdel-Ghany and Salem 2020).

**Antioxidant Activity**

The aquatic creatures are shielded from oxidative stress by the antioxidant qualities of chitosan and its nanoparticles. This advantage helps fish populations remain resilient and generally healthy. The chitosan nanoparticles are capable of promoting tissue regeneration and repair in wounded fish and shellfish in aquaculture due to their wound-healing characteristics. For topical application to wounds or injuries, CSNPs can be synthesized into hydrogels, films, or dressings that can hasten the healing process and lower the chance of secondary infections. The chitosan nanoparticles promote angiogenesis, collagen synthesis, and cell proliferation in aquatic creatures, which speeds up wound healing and increases survival rates (Abdel-Ghany and Salem 2020).

**Water Treatment**

Applications beyond aquaculture involve the use of chitosan and its nanoparticles in water treatment. They are useful instruments for preserving water quality because of their biodegradability and capacity to bind to contaminants (Zaki et al., 2015).

**Nanodevices**

The nanodevices are a wonderful approach to improve the water quality in shrimp farming since they increase the survival and yield rates of shrimp while decreasing the rate of water exchange. Another important problem in the aquaculture is managing biofouling. The bacterial biofilm promotes the attachment of macrofoulers, which in the case of mariculture cages can result in serious problems such corrosion, weight growth, surface alteration, and disarray of submerged structures (Champ 2003). Antifoulings are sprayed directly to kill the fouling organisms, however they can unintentionally harm non-target species, such TBT (Lofrano et al., 2016), the nanoparticles based antifoulings such as nZnO, nCuO and nSi seem to be viable choices because of their high surface-to-volume ratio, which offers a more effective barrier to fouling agents (i.e., at equal or lower concentrations). The cage nets that show a notable decrease in fouling 90 days after treatment will be treated with nCuO. Aquatic habitats, such as water bodies, sediments, and ecosystems, can be monitored and evaluated using nanodevices for their integrity (Rather et al. 2011). Changes in water chemistry, microbial populations, and biodiversity can be detected using nanoprobes and nanosensors, which can reveal important information about the dynamics and operation of ecosystems. The nanodevices make it possible to continuously monitor and survey aquatic environments, which helps aquaculture make educated decisions and manage its ecosystem (Ashraf and Edwin, 2016).

**La oxides NPs**

The phosphate removal from aquatic environments is one application for lanthanum oxide nanoparticles in water treatment. High phosphorus concentrations can cause eutrophication and toxic algal blooms. The phosphate ions may be successfully adsorbed by $La_2O_3$ NPs, which can help to minimize these environmental problems. Antimicrobial capabilities are demonstrated using the La2O3 nanoparticles. This has the potential to improve water quality and lower the risk of waterborne illnesses in aquatic areas by limiting the growth of harmful bacteria and algae (Khan et al., 2022). Because of their catalytic qualities, lanthanum oxide nanoparticles may be useful in encouraging specific metabolic reactions in aquatic species. For example, they could be used in aquaculture systems to speed up the decomposition of organic matter or improve the uptake of nutrients by aquatic plants.

The La2O3 nanoparticle-based sensors can be made to detect several kinds of toxins or pollutants in water. With the help of these sensors, water quality measurements may be monitored in real-time, allowing for the quick mitigation of any negative impacts on aquatic life (Khan et al., 2022).

It has been discovered that lanthanum oxide nanoparticles are comparatively biocompatible, particularly when they are correctly functionalized or stabilized. This indicates that, in comparison to some other forms of nanoparticles, they are less likely to harm aquatic creatures. If medications are utilized to treat illnesses or infections affecting aquatic life, La2O3 nanoparticles as drug delivery vehicles may help aquatic organisms inadvertently.

These nanoparticles can lessen the necessary dosage and minimize any possible negative effects by assisting in the targeted distribution of therapeutic substances to particular areas within the organism (Khan et al., 2022). The nanoparticles of lanthanum oxide have demonstrated potential in managing harmful algal blooms (HABs) inside aquaculture systems. Fish kills and environmental damage can result from HABs' production of chemicals that are toxic to people and aquatic life. The La2O3 NPs have the ability to attach to algal cells and stop their growth, which stops HABs from forming and spreading in aquaculture ponds and other bodies of water. The productivity and well-being of aquaculture systems are protected by this application (Almukhlafi et al., 2021).

**Conclusion**

In conclusion, the use of different nanoparticles holds great promise for transforming aquatic ecosystems and improving fish health. The nanoparticles provide a broad approach to improving the well-being of aquatic organisms, ranging from water quality enhancement to disease prevention. However, further research is needed into the long-term effects and environmental impact of nanoparticle use in aquatic ecosystems. By further investigating their potential and ensuring ethical implementation, we may work toward long-term solutions that benefit both fish populations and the ecosystem.

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Chapter 07

# Use of Nanoparticles to Compete Drugs Resistant Against Emerging Disease Agents

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**ABSTRACT**

Recent research in nanotechnology has proved that nanoparticles (NPs) attain countless potential in veterinary medicine fields. NPs offer a more advantageous tool for the target-specific and controlled release of micro- and macromolecules in disease therapy due to their ability to form stable bonds with ligands, change size, and shape, exhibit fast activity, and bind both hydrophobic and hydrophilic elements. Advanced therapies must be adopted to combat resistance to antibiotics and developing disease agents, such as bacterial infections brought on by biofilm formation or by established resistance. Nanomaterial-based treatments provide promising methods to prevent hard-to-treat bacterial infections by circumventing established processes linked to acquire antibiotic resistance. In addition, because of their extraordinary size and physical characteristics, NPs can target biofilms and cure incurable illnesses. We observed the general methods of using NPs to target bacterial illnesses linked to biofilms and the emergence of antibiotic resistance in this chapter. We emphasized the characteristics and design components of NPs that can be bioengineered to improve their efficacy. We concluded by outlining the latest developments and new obstacles to the broad therapeutic application of NPs as an antibacterial therapy and their suitability for the current wave of animal health problems.

**KEYWORDS**
Nanoparticles, Disease, Immunomodulation, Drug resistance

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## INTRODUCTION

The utilization of NPs has been a familiar research concept since the previous century. A well-known 1959 lecture ("*There's Plenty of Room at the Bottom*", 2023) presented by Richard P. Feynman, a Nobel laureate, represents the concept of nanoparticle usage was introduced, and various revolutionary developments in the field of nanotechnology have been witnessed. NPs facilitate the manufacture of innovative materials and technologies that help to improve the production and well-being of animals (Bai et al., 2018). Materials with NPs in animal science have incorporated several applications, including improving disease diagnosis, animal nutrition, reproduction, breeding, drug delivery, treatment, and ensuring the safety of food for human beings and animal health (Hassan et al., 2020). NPs usage additionally includes benefits such as the detection of biological molecules, sensor development, MRI contrast enhancement, tissue engineering, tumor vaccines, tumor detection, and biomedicine. Nanotechnology generates a wide range of materials at the nanoscale. NPs make a broad classification of materials encompassing particles that are less than 100 nm in at least one dimension. The size of these ultrafine units is expressed in nanometers (nm), where 1 nm is equivalent to 10−9 meters. In addition to being produced by human activity, NPs exist naturally in the environment. Due to their submicroscopic dimensions, NPs have unique material properties, and specially designed NPs may be used in various industries across various domains, including environmental remediation, catalysis, engineering, and medicine. The International Organization for Standardization (ISO) established a definition for a nano-particle in 2008. According to this definition, A distinct nano-object with all three Cartesian dimensions less than 100 nm is called a nanoparticle (Britannica, 2024). Two-dimensional nano-objects are similarly defined by the ISO standard (i.e., nanoplates and nanodiscs) and nano-objects having one-dimensional (i.e., nanotubes and nanofibers). In 2011, a more technical but broader definition was endorsed by the Commission of the

European Union. NPs are complicated substances and consequently made of three levels i.e. (1) The surface layer, which enables altered using a variety of polymers, surfactants, metal ions, and small molecules, (2) The shell layer, which is a chemically distinct substance from the core in all areas and (3) The core, Mostly, this is the NP's central region and generally referred towards the NPs directly (khan et al., 2019).

Nanomedicine resistance improvements are a developing technique for the deployment of nanotechnological systems in illness diagnostics and treatment (Yetisgin et al., 2020). This discipline of nanotechnology may be categorized into two primary groups: nano-devices and nano-materials. Small devices at the nanoscale, including microarrays, are included in nanodevices (Chandrasekhar et al., 2013; Rabl et al., 2010), and some artificial intelligence-operated machines like respirocytes (Shabnashmi et al., 2016). NPs are becoming a more useful technique in combating drug-resistant emerging disease agents due of their distinctive features and breadth of uses. Here's how they can be utilized. Tuning the sizes and morphologies of nanomaterials can result in obtaining the following key properties among a range of unique characteristics. The following are aspects under which we can improve antibiotic resistance.

**Targeted Drug Delivery**

Drug release chemistry and encapsulation: efficient drug encapsulation within the carrier matrix prevents the emergence of disease-fighting resistance by ensuring medication stability in circulation and regulated release chemistry in response to stimulation at the target location through hydrophobic or electrostatic interactions. Chemical adjustments can be made to this encapsulation to maximize drug loading and release kinetics. NPs can be created to deliver medications directly to the site of infection, bypassing biological barriers and minimizing systemic side effects. Surface modifications of NPs can enable specific targeting of drug-resistant pathogens, enhancing drug efficacy. In the field of cancer therapy, targeted drug administration is a new frontier, aimed at boosting therapy effectiveness while minimizing undesired adverse effects. A potential technique involves the utilization of Drug carriers can be accurately guided to cells using cell membrane receptors like the folate receptor (FR). Targeted nanocarriers and small molecule-drug conjugates have been produced using the cellular folate receptor, which is a characteristic of cancer cells, incorporating various biochemical and mechanical aspects with unique benefits as well as challenges. Systemic drug delivery and controlled release are enabled by these novel folic acid-conjugated stimuli-responsive drug transporters, allowing for lower doses, overcoming drug resistance, and reducing adverse effects. As the design of drug carriers based on the structure becomes increasingly relevant for precision treatment and diagnosis, current methods to delivering diagnostics or therapeutic drug resistance problems using folic acid-conjugated. The related chemistry and metabolic processes are sought to be understood. Focusing on brain and mammalian infectious diseases as examples, this study took into account current developments in conjugated nanocarriers and small molecule drug conjugates from 2017 to 2023. Researchers are investigating chemical and biological issues to bridge the gap between chemical and biomedical perspectives to improve drug resistance among field-accessible diseases. This will ultimately guide future research for NPs in targeting immunotherapeutic and diagnostics.

**Multidrug Delivery**

NPs can carry multiple therapeutic agents simultaneously, allowing for combination therapy to combat drug resistance. This approach can target different pathways within the pathogen or employ synergistic effects to overcome resistance mechanisms. In addition, specific drugs can be delivered by drug transporters to inhibit efflux transporters such as P-glycoprotein can cause medication resistance to many drugs for treating brain tumors (Dal Corso et al., 2019) In pharmacology, researchers consider NPs as an optimal medication delivery method that promotes wound healing, reduces pain, and protects animals from bacterial or viral infections. Furthermore, these innovative materials transport medication drugs to specific organs and tissues. These systems can influence the speed of drug absorption, distribution, metabolism, and elimination in the body and make it possible to track the dynamics of drugs. They achieve healing effects, secure drug stability, availability and extend the duration of action, reducing the frequency of required doses to maintain therapeutic responses and minimizing toxicity.

**Enhanced Drug Stability and Disabling Efflux Pump Mechanisms**

NPs are used in many ways as drugs or remedies in animal production which includes; they are used in drugs, modified vitamin structures, probiotics, and nutritional supplements, likewise, NPs used in therapeutics and removal of causes of infection without surgery for enhancement of animal health status, in contrary to this due to their efficacy NPs reduce he antibiotic residue after treatment in animal production. NPs can protect drugs from degradation, thereby improving their stability and prolonging their therapeutic effects (Gurvic and Zachariae, 2024). This is particularly advantageous for combating drug-resistant pathogens, where maintaining drug potency is crucial for treatment success (Oake et al., 2019). Drug delivery methods have made it possible to design a wide range of pharmaceutical treatments that improve patient health by maximizing therapeutic administration to their target location, avoiding off-target accumulation, and encouraging patient compliance. Drug delivery technology changed to meet new obstacles as treatment modalities grew beyond small molecules to encompass antibodies, proteins, peptides, and nucleic acids. This review article discusses the fundamental strategies that resulted in the creation of effective medicinal products incorporating both tiny and large molecules. There are 3 models for the delivery of drugs that underpin contemporary drug delivery are identified, and their

contributions to each treatment class's early clinical success are described. Additionally, how these paradigms will support delivering live-cell treatments is outlined (Manzari et al., 2021). Many drug-resistant pathogens employ efflux pump mechanisms to expel drugs from within the cell, reducing their effectiveness. NPs can encapsulate drugs and prevent their recognition by efflux pumps, thereby overcoming this resistance mechanism (Hassan et al., 2020).

**Immunomodulation and Diagnostic Tools**

Some NPs possess inherent immunomodulatory characteristics that might strengthen the host's defenses against infections. By stimulating immune cells or modulating cytokine production, NPs can help overcome drug resistance by bolstering the body's natural defence mechanisms. NPs can also be employed in the development of diagnostic tools for rapid and accurate detection of drug-resistant pathogens. They can be functionalized by targeting ligands or biomolecules for specific recognition of pathogens, facilitating early diagnosis and appropriate treatment.

**Gene Silencing and Therapy**

NPs can deliver therapies based on nucleic acids, such as short interfering RNA (siRNA) or antisense oligonucleotides, to prevent genes from being expressed that are associated with drug resistance against bacteria and other pathogens. This approach can effectively suppress resistance mechanisms and restore susceptibility to conventional drugs (Lin et al., 2021).

**Biofilm Disruption**

NPs can disrupt the formation and integrity of microbial biofilms, which often contribute to drug resistance by providing a protective environment for pathogens. By destabilizing biofilms, NPs have the potential to the efficacy of antimicrobial drugs against drug-resistant strains. Overall, the unique physicochemical properties of NPs provide an adaptable foundation for creating innovative methods to combat drug-resistant emerging disease agents, potentially revolutionizing the field of infectious disease management (Huang et al., 2022).

**Surface Area and Magnetism**

Control of size, shape, and surface charge: adjusting the nanocarrier's size, shape, and surface charge chemically affects not only its circulation and mobility characteristics but also how it interacts with biological components. Improved biodistribution is frequently observed in well-dispersed carriers. In order to completely realize the clinical viability of resistance happening through mediated drug delivery, it will be essential for chemists, material scientists, bio-sciences researchers, and clinicians to continue their research efforts in joint synergy. Nanomaterials generally display notably greater surface areas in comparison to their bulk counterparts, and all nanomaterials have this type of attribute (Shreffler et al., 2019; Tomar et al., 2020). An element's magnetic characteristics can change at the nanoscale. At the nanoscale, a non-magnetic element may develop magnetic characteristics (Geoffrion and Gibsier, 2020), hence the purpose of their application can meet the present need and be modified for uplifting antibiotic resistivity.

**Quantum Effects**

Quantum effects become increasingly prominent as we go down to the nanoscale. However, the scale at which these effects become relevant is primarily dictated by the characteristics of the semiconductor material (Geoffrion and Gibsier, 2020). In the meantime, when seen in various physiological circumstances relevant to fertility, quantum dots (QDs) improve our understanding of the motions and interactions of mammalian spermatozoon and oocytes (Hill and Li 2017). Moreover, Nanocomposites have the potential to produce products and applications that yield antibiotic-effective therapy and diagnosis outcomes. Additionally, drug-nanomaterial composites have enhanced drug solubility and facilitated drug delivery to specific affected regions via the bloodstream.

**Mechanical Properties and Support for Catalysts**

Excellent mechanical properties are exhibited by nanomaterials that are absent in their macroscopic counterparts (Mekuye et al., 2023). The potential for excellent dispersion of NPs of active catalysts has been provided by 2D sheets of various nanomaterials, significantly enhancing catalytic performance (Zhou et al., 2020). To enhance the performance of antibiotics catalysts have recently been distributed atomically on 2D nanomaterial sheets (Fernandez et al., 2017). Further modifications are being done by researchers to counter with the present era.

**Antimicrobial Activity**

Microbial infections present numerous health risks to both animals and human beings. Even with great improvements in diagnostic and treatment technology, microbial spoilage continues to have a substantial financial impact on the world's food supply which is still a major cause of illness epidemics in both humans and animals. In the continuous battle over the emergence of antibiotic resistance and the creation of novel antimicrobial medications, bacteria seem to be the focus of various research efforts (Hassan et al, 2017; Jamaran et al., 2016). Antiviral, antibacterial, and antifungal activities are possessed by some nanomaterials, which possess a great capacity to manage illnesses caused by pathogens (Castro et al, 2017; Makvandi et al, 2020). Overall, nanoscale materials' attributes have rendered them advantageous for an extensive array of uses, significantly enhancing the performance of different tools and substances across many fields. Below, we will

discuss details about distinct nanomaterials, their characteristics and their uses in several industries (Ernsting and Murakami , 2013).

**Hybrid Nanomaterials**

Hybrid nanomaterials combine nanometric organometallic and organic/inorganic components chemically to maximize the release and distribution of drugs by using the unique qualities of each substance. Specifically, the development of FA-conjugated drug-loaded multifunctional nanohybrids advances targeted multimodal imaging and treatment of cancer (Choi et al., 2021; Tian et al., 2022) For example, the strong absorption of Au(gold) NPs, which converts near-infrared (NIR) light to heat through the LSPR effect for localized photo-thermal therapy (Yang et al., 2021; Jahangiri-Manesh et al, 2022; Kozlovskaya et al, 2023). Goyal et al., performed photothermal ablation under NIR irradiation using AuNPs coated on GO sheets and covered with FA to deliver DOX to MCF-7 and HeLa cells (Chauhan et al., 2017). Fluid colloidal stability was achieved in this design by conjugating FA to the hydrophilic sulfonated GO surfaces. The greater release of DOX and ionic Au was caused by NIR laser irradiation at λ = 808 nm. G0/G1 cell-cycle arrest was induced, in response to NIR stimulation, increasing DNA intercalation and premature apoptosis and spreading infection among surrounding cells of infection. At pH 5.3, DOX and AuNPs were released in vitro at a rate that was almost greater at pH 7.4. The silica GO hybrid system's outstanding drug absorption efficiency was attained via GO's π-π stacking interaction and the mesoporous silica NPs' absorption. CUR was delivered to MCF-7 and A549 cells via immobilized FA-conjugated AuNPs on amine-modified dendritic silica-coated decreased GO nanosheets, which were also used for cell imaging (Malekmohammadi et al., 2018). The oil-water biphasic stratification procedure used to synthesize the nanosheets is comparable to the one used by Zhao and coworkers to immobilize AuNPs on silica-coated reduced GO. FA-conjugated NPs are around 1 μm in size, with nanosheet thickness ranging from 5-25 nm . After 1 hour of irradiation with an 808 nm laser, the cumulative releasing of CUR at pH 5.7 was almost three times greater than before. FR(+) MCF-7 cells showed increased absorption of FA-conjugated nanohybrid, cytotoxicity, and death in comparison to FR(−) cells, highlighting the importance of FR-targeting for drug internalization. Zhao et al., 2019 developed a same bi-functional GO/silica hybrid that can deliver cancer cells when stimulated with pH changes and NIR irradiation (Farran et al., 2020). To increase blood circulation time, a PEGylated gold/iron oxide core-shell nanohybrid coupled with an FA molecule was developed for targeted photothermal therapy (Ghaznavi et al., 2018). Iron oxide MNPs were synthesized from a mixture of FeCl3/FeCl2 and 3-aminopropyltrimethoxysilane, which AuNPs were used to immobilize. In this work, PEG-FA was conjugated to the gold core-shell surface using cysteamine. The authors stated that because of the nanohybrid's biocompatible PEGylated surface, it did not significantly harm KB and MCF-7 cells. Apart from the aforementioned accomplishments, Selvaraj et al., developed a diagnostic tool, a nanohybrid composed of Au nanorods coupled with FA and centered in mesoporous silica (Prasad et al., 2018). Captured For a whole day, tumors treated with a modest dosage of 10 mg/kg body wt. of Au nanorods in FA-conjugated silica showed preferential in vivo bio-distribution. Remarkably, the effect of surface modification on hemolysis caused by nanohybrids (red blood cell destruction) was investigated by the researchers (Huang et al., 2020). When 3-aminopropyl triethoxysilane was used as a linker, the hemolytic effect was only about 5%. Nanohybrids without surface functionalization, on the other hand, demonstrated substantially greater levels of hemolysis, topping 75%. In this technique, DOX's natural red fluorescence was used to track the in vitro administration of drugs to 4T1 and NIH-3T3 cells. Increased cytoplasmic fluorescence suggested efficient FA-FR axis internalization. In vivo, toxicity and renal elimination within an hour of the injection revealed a high CT contrast (produced by Au nanorods) showing dosage problems in the kidneys, liver, spleen, and heart, in comparison to pre-injected animals. The healthy kidney suggests that the nanohybrid construct was successfully really cleared, demonstrating its nontoxicity.

Smart lipid-polymer nanohybrids represent a promising new approach owing to their core-shell nanostructures, which effectively encapsulate and release pharmaceuticals upon stimulation by combining bio-degradable PNPs with biomimetic lipid-based NPs. Another example is the creation of solid lipid NPs coated in chitosan and conjugated with FA to deliver the steroid-mimetic letrozole (LTZ) to MCF-7 and PC-12 cells (Hemati et al., 2017). A 2:3 mixture of tripalmitin glyceride, stearic acid, 5 mg LTZ, and 20 mg chitosan was homogenized using an oil-in-water procedure. The resulting particle measured 148 nm and exhibited a +ve electrostatic-potential of around +6 mV. Minimizing repulsion between NPs with reduced electrostatic-potential (almost zero) reduces the risk of NP aggregation. Other factors influencing aggregation include Particle charge, which refers to interaction of heavily charged NPs interact with physiological fluids and proteins. Cytotoxicity investigations employing FA-conjugated LTZ-loaded nanohybrids for MCF-7 cells showed an IC50 value of 79 nM, demonstrating the efficacy of FR to free LTZ, which did not approach the IC50 value after 24 hours. Elzoghby et al., created a PEGylated phytosomal phospholipid bilayer that encapsulated casein-loaded micelles decorated with FA to deliver resveratrol (RSV) and fungus-derived Moascus yellow pigments (MYPs) to MCF-7 cells in a precise way (El-Far et al., 2018). Nanocomplexes of 137 and 272 nm were highly colloidal stable. FA and PEGylated micelles dramatically decreased VEGF, aromatase, CD1, and NF-κB activity relative to free medicines. Furthermore, caspase-3 activity was observed to be higher relative to the groups under authority. The arginine-glycine-aspartate (Arg-Gly-Asp) RGD tripeptide sequence and FA were added to produce PTX-loaded mesoporous silica NPs (Gao et al., 2020). It expresses integrin αvβ3 and FR on the surface of cancer with high affinity MCF-7 cells (Yang et al., 2021). This technique is based that integrin expression is greater in metastatic breast cancer cells compared to healthy cells, such as HeLa or non-malignant MCF-10A cells. Conjugation of NHS-PEG-FA and NHS-PEG-RGD onto the NP's surface enables active tumor-targeting treatment via FR and integrin αvβ3 (Dewhirst et al., 2017). The NPs' stability in vivo is improved by the lengthy PEG. The association between

the tripeptide sequence and the –NH2 groups on the surface is responsible for the positive zeta potential value, whereas MSNs-NPs have a negative zeta potential. After 48 hours, the IC50 (Half maximal inhibitory concentration) values of free PTX and PTX-loaded NPs on MCF-7 cells were 35 and 22 ng mL−1. This indicates that PTX-loaded NPs had 1.6 times higher inhibitory efficacy (antitumor activity) than free PTX.

NPs have emerged as promising therapeutic agents, thanks to recent advancements in biotechnology. A few examples of the many uses for nanocomposites include the use of alumino-silicate NPs to control bleeding, carbon NPs for drug delivery and sensing, gold NPs (Au NPs) for diagnosis, silver NPs (Ag NPs) as antimicrobial agents, and iron oxide NPs for enhanced MRI imaging (Hassan et al., 2017). Additionally, addressing various challenges in disease detection and management can be achieved through additional tools such as bioanalytical nanosensors, NPs, and microfluidics (Meena et al., 2018). This chapter aims to highlight the latest developments in nanotechnology and their applications in veterinary care. However, the effective delivery of therapeutic agents to target locations remains a major hurdle in treating various diseases. Conventional therapeutic drugs often face limitations due to their non-selectivity, adverse side effects, low efficacy, and inadequate distribution in the body (Kadam et al., 2012). Therefore, the primary goal of current research efforts is to create versatile and well-controlled delivery systems. One potential approach for delivering a variety of molecules to specific body sites is by combining therapeutic drugs with NPs that exhibit unique physicochemical and biological properties and customizing their routes for precise targeting (Jahan et al., 2017). This targeted strategy increases the concentration of medicinal agents in cells, allowing for the use of smaller doses, especially when there is a discrepancy between therapeutic efficacy and adverse effects. By enhancing efficacy and resistance capacity in biological systems, increasing the concentration of therapeutic substances at the target site also improves their therapeutic index. Furthermore, water-insoluble medicinal drugs can be combined with NPs to enhance absorption, reduce antibiotic resistance, and protect them from physiological barriers. Additionally, coupling medicinal NPs with various agents provides a means of monitoring their trajectory and imaging their distribution within in vivo systems (Villaseñor et al., 2019)

**Antimicrobial NPs in Veterinary Practice**

According to (Turos et al., 2007), It was discovered that treating MRSA benefited from the covalent bonding of a penicillin polyacrylate nanoparticle, which was around 100 nm in size, by preventing the activity of bacterial β-lactamases. Ceftiofur-loaded PHBV Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) has been demonstrated to enhance the treatment of infectious diseases in cattle (Vilos et al., 2012). It was discovered that giving tilmicosin-loaded lipid NPs orally to broiler chickens increased their bioavailability and may be an effective way to administer the drug (Rassouli et al, 2016). Methicillin-resistant *Staphylococcus aureus* was more effectively inhibited by streptomycin-coated chitosan-magnetic NPs that were included using an accelerated release strategy that slowed down with time (Hussein-Al-Ali et al., 2014).

Fluorfenicol, a fluorinated derivative of thiamphenicol, is a widely used and broad-spectrum antibiotic that is affordable and effective against germs that cause both gram-negative and gram-positive illnesses (Tao et al., 2014). Since 1999, To prevent and cure respiratory infections in pigs and hens, it has been widely used in the People's Republic of China, vibriosis in fish, and other bacterial infections (Miranda et al., 2007). Florfenicol works through a mechanism similar to that of chloramphenicol and thiamphenicol (Gutierrez et al., 2023). However, due to its modified chemical structure, fluorfenicol eliminates the risk of aplastic anemia associated with chloramphenicol and reduces the potential for bacterial resistance mediated by acetyltransferase (Jiang et al., 2006). Compared to its structural counterparts, fluorfenicol demonstrates enhanced activity at lower dosages against various bacteria that are resistant to thiamphenicol or chloramphenicol (Anadón et al., 2008).

The inability of florfenicol to dissolve well in water is one of its drawbacks, which requires the use of organic solvents in clinical fluid formulations. In certain animals, less than three hours is the half-life of florfenicol elimination (Ali et al., 2003; Wang et al., 2015). Moreover, frequent dosing is necessary to achieve optimal efficacy in clinical applications. However, frequent administration can increase labor costs and induce stress. The development of new florfenicol formulations presents promising and practical options.

**Silica NPs as Carriers for Controlled Release of Antibiotic**

In an aqueous solution, antibiotic was loaded onto silica NPs as a carrier. Drug was able to bind to silica NPs in an aqueous solution by naturally cooling from high temperature to room temperature. Thermal gravimetric analysis, FTIR (Fourier transform infrared), Zeta(Z) sizer laser particle size analyzer, and transmission electron microscopy were used for characterization. The adsorption was completed without any deterioration, according to the results. Compared to the quick release of antibiotics by natural procedure, developed NPs exhibited a delayed sustained release of antibiotics (Song et al., 2009). Antibiotics classified as broad-spectrum, poorly absorbed bactericidal aminoglycosides are recommended for the management and prevention of bacterial infection in animals, which is caused by specific bacteria. Ribosomal function is the site of its activity. Merely a small portion of oral administration is absorbed systemically have proved to be beneficial. Nephrotoxicity and ototoxicity are two drawbacks of neomycin (Youssef et al., 2019).

**Assessment of Nanoparticle Formulations**

The antibacterial activity of NPs loaded with selected antibiotics can be evaluated for study purposes. The resulting formulation exhibited a smooth surface and round shape under scanning electron microscopy, with increased entrapment

efficiency (%EE). It was found that the in-vitro release profile maintained for a specific interval, indicating sustained release. Additionally, compared to pure medications, the drug release was improved (Supriya et al., 2018). A comparison of several commercialized neomycin nano bio composite ointments was considered. Scientists hypothesized that the delivery of silver NPs to the cell may be the reason for this synergistic effect. Targeted therapeutic NPs offer numerous benefits across various medical specialties. This section highlights the physicochemical characteristics of NPs that make them essential tools in nanomedicine. It also provides an overview of the development of therapeutic NPs over the past decade, focusing on targeted delivery and how antibiotic resistance has impacted their use in treating various illnesses, including cancer and neurodegenerative diseases.

**Current Limitation and Safety of NPs**

A formulation of metallic NPs coated with neomycin was found to have a synergistic impact on bacterial strains causing diseases. Scientists hypothesized that the drug delivery of metallic NPs to the cell may be the reason for this synergistic effect. The presence of hydrophobic groups in cell membranes is widely recognized and while metallic NPs are hydrophobic, gentamicin and neomycin are hydrophilic. In the twentieth century the discovery of antibiotic along with NPs coating somehow controlled morbidity and mortality and considered as great innovation there arise the problem of antimicrobial resistance which needs to be improved, in chronic infection organisms may survive from months to years, Some factors like shipping and miscarriage of samples may spread and cause microorganism to spread in an uncontrollable way which becomes a problem for researchers and pharmaceutical production labs which them carrier and create a huge issue like antibiotic resistivity. To compete with present-era researchers, have to speed up the innovation of antibacterial capsule production and drug delivery improvement to defend drug-resistant against antibiotics (Patel et al., 2017). Because of this, NPs may more readily pass through cellular membranes and efficiently enter the target cell compared to antibiotics like gentamicin and neomycin (Jamaran et al., 2016). While most NPs are harmless, some may have harmful effects. For instance, workers in pharmaceutical companies may experience reproductive issues if exposed to carbon nanotubes for prolonged periods (Johansson et al., 2017). Additionally, the unstable interaction between medication and particles may result in the delivery of medication in solid tissues rather than the target tissues, leading to toxicity to healthy tissue and delivery of doses below the recommended therapeutic level. Their ability to penetrate various biological barriers such as the blood-brain barrier (BBB) has significant environmental implications, increasing the demand for radionuclides. Moreover, carbon-based nanofibers are implicated in depleting the ozone layer in the biological system.

**Conclusion and Outlook**

Certain developed NPs coated with antibiotics were found to have a synergistic impact on specified strains causing resistance against specific diseases. Scientists expected that the drug delivery of prepared NPs to the cell is the reason for this synergistic effect. The presence of hydrophobic groups in cell membranes is widely recognized. While developed NPs are predefined as hydrophobic, and hydrophilic (i.e. gentamicin and neomycin). Because of this, NPs may more readily pass through cellular membranes and efficiently enter the target cell Recent years have witnessed remarkable advancements in targeted chemical delivery, improving the administration of therapeutic or diagnostic agents to infected organs. Despite these advancements, several meta-analysis studies suggest the need for additional increases in median delivery efficiency. Consequently, various new methods have been tried to address constraints such as low targeted accuracy or non-specific dispersion. These uses of NPs have proven suppress cell growth, induction of mitochondria-mediated cell death, reduction of medication side effects, and prolonged survival, all contributing to improved antibiotic activities. A special note should be made of the usage of lipid-based and polymeric nanocarriers, which are mainly intended to provide the best possible drug encapsulation efficiency, minimal toxicity, high biocompatibility, and resistivity. Over the past few years, a great deal of work has been achieved in removing the barriers that required conventional medication delivery. Through careful customization of NPs with conjugate carrier attributes. This process simultaneously increases efficacy while lowering elimination through the immune system.

Advancements in NPs targeted medication delivery, but there is still much room for improvement in terms of resistivity efficiency. Only a small number of researchers have used animal models; the majority have focused on in vitro investigations using cell lines. Extended blood circulation, FA-conjugated transporter endosomal escape, and carrier–protein interaction remain extremely challenging to accomplish in vivo. To advance the development of these drug transporters to a therapeutic level it will require consideration of the complex relationships among transportation and eradication procedures, development in the target area, connections to the immune system, and cellular uptake. Challenging to fully address every parameter in antibiotic resistance design, it is crucial to underscore their functional characteristics when they are being developed. It is important to acknowledge that not all newly announced antibiotics have the innate ability.

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Chapter 08

# Development of In Vitro 3D-Intestinal-Chip for Ethical Assessment of Anticoccidial Nanoparticles in Poultry

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## ABSTRACT

All the recent therapeutic approaches have failed to fully control the Eimeria species in poultry, thus there is needed more innovative and novel therapeutic approaches to reduce the economic loss. Firstly, there is an immense need to build a 3-D-intestinal-chip for an initial quantitative investigation of available anticoccidials, host-pathogen interaction, and effects of botanical nanoparticle-based compounds like garlic extract, Olive oil, and Curcumin on various developmental stages of *Eimeria*, especially sexual stages which are bottle-neck for disease development. The final focus should be to determine the efficacy and impacts of different nanotechnology-based anticoccidials at the cellular level after the quantitative analysis of *Eimeria tenella* and its implementation in the commercial industry. The outcomes of applying nanotechnology and 3D-chip-based culture systems are promising, which is evident in recent research outcomes. As 3D-Chip system of intestinal tissue has been given accurate results related to the developmental stages of *Toxoplasma gondii*, a protozoon. New research horizons should be based on modern technology concerning the development of a 3D intestinal chip and the application of nanotechnology, which is more advantageous in terms of ethical, economical, and experimentation time. 3D-chip-based techniques have been recently developed in human studies with significant successful outcomes in protozoal development and the evaluation of therapeutic efficacy. Nanotechnology-based therapeutics have already been under trial *In Vitro* and *In-Vivo* research in broilers with efficient outcomes but the standard outcomes have not been achieved till now. As all the materials, procedures, and protocols are under research in human medicine, experimentation is completely feasible in veterinary medicine.

**KEYWORDS**
Coccidiosis, Eimeria, *In Vitro*, chip, Ionophore, Botanical, nanoparticle

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## INTRODUCTION

Avian coccidiosis is a protozoal disease with heavy economic losses of up to 14.5 billion dollars per year. (Blake et al., 2020). The family of causative agents is *Eimeriidae* and phylum *Apicomplexa*. Eimeria is a microscopic protozoan parasite and has the capacity of spore-formation with a predilection site of replication in enterocytes, which causes weight loss because of the severity of diarrhea and leads to sudden death in some cases. During the Eimeria infection, other attacking pathogens can also cause infection and enhance the symptoms clinically. Seven species are mainly involved in disease occurrence and each species varies from others for the site of attack and occurrence of lesions.(Quiroz-Castañeda and Dantán-González, 2015).

Illness manifests as a clinical intestinal disease characterized by declined feed intake, bloody diarrhea, weight loss, and death. (Wickramasuriya et al., 2022). The commercially existing anticoccidials are facing drug resistance, chemical residue issues in poultry meat, high medicinal costs, and export regulatory legislation in different countries. (Attree et al., 2021). Botanical compounds such as extracted oils and their bioactive molecules have recently become popular because of their anti-inflammatory and antimicrobial effects. (Muthamilselvan et al., 2016; Attree et al., 2021).

There is an immense need to investigate a lot of new anticoccidials at a rapid pace, low cost, and minimum exploitation of animals and birds with the development of alternative technology. For a long time in scientific research, there has been a great concern to adopt ethical guidelines and the majority of research laboratories are following the 3Rs. In animal experiments, the main focus and efforts are based on reduction, replacement, and refinement. Reduction

involves certain approaches and strategies are encouraged that minimize the required animals for experimentation to get maximum information. The second R is a replacement to replace the animals with an alternative method and avoid animal use in experimentation, while the last R is for refinement in all procedures that are adopted in animal housing and husbandry practices. (Traversa and Joachim, 2018).

**Life Cycle of Eimeria**

There are two phases of the life cycle of Eimeria categorized as exogenous and endogenous phases, the first one occurs in the environment and the last one occurs inside the cells of the gastrointestinal tract. Oocysts are shed through the excreta of birds containing a single cytoplasmic mass covered with a hard protective wall and undergo meiotic division for sporulation in conducive environment conditions, especially temperature and humidity. In this process, cytoplasmic mass gets divided into four segments known as sporocysts, with another protective covering. In each sporocyst, two sporozoites are penetrating stages of coccidia into the intestinal wall. The outer covering of the oocyst is so strong that is resistant to getting disrupted by various physical and chemical treatments including sodium hypochlorite. Somehow, the sensitivity of oocysts is more to heat while their hard walls are resistant to any mechanical damage but these are disrupted inside the gizzard of a chicken and resultantly released sporocysts (Dubey, 2019). In the duodenum, sporozoites start an activity with the action of pancreatic enzymes and bile salts, which denature the covering of an anterior pole of sporocysts and make them capable of penetrating the intestinal wall. (Burrell et al., 2020). Sporozoites escape through the Stieda body invade in cytoplasm of enterocytes and consume all the energy stored in the cells through the process of excystation (López-Osorio et al., 2020).

**Efficacy of Available Anticoccidials**

Because of the significant economic impact of coccidiosis, its treatment and prevention are highly demanded, and conventionally chemical compounds, ionophores, and vaccines are being used for that purpose (Noack et al., 2019).

It is a challenging task to choose suitable anticoccidials to minimize the damage from coccidiosis at commercial poultry farms. After conducting multiple studies to check anticoccidial activities through *In Vitro* models, especially for field-isolated strains of *E. tenella*. Efficacy was checked through the count of intracellular sporozoites and schizonts by using staining techniques and applying phase-contrast microscopy. Ionophores, the most commonly used anticoccidials available in the market, are taken through fermentative bacterial species and have a significant effect as antiprotozoal drugs as their name depicts, they disrupt the ionic transport across the surface of protozoal stages like trophozoites and sporozoites (Noack et al., 2019).

The mechanism of action of Ionophores was studied at the ultrastructural level and their anticoccidial effect because of disturbance of osmotic pressure and change in morphologies within 30 minutes of incubation. (Mehlhorn et al., 1983). Minimal inhibitory concentration (MIC) of ionophore compounds was studied which prevented mortality in infected. (Xie et al., 1991). *In Vitro,* a study was best regarded to study the anticoccidial resistance and checked anticoccidial resistance *In Vitro* study, and comparison was made among the ionophore-sensitive strain of *E. tenella* and ionophore-resistant strain isolated from the field. It was observed that the resistant strain showed less uptake of the drug than the level that was required to hinder the development of Eimeria and noted that it was 40 times less than sensitive strains in Porcine Corneal Keratocytes (PCK) cells (Augustine et al., 1987).

Currently, the growth of *E. tenella* is monitored in Madbin-Darby bovine kidney (MDBK) cells which were incubated with anticoccidial drugs, among which salinomycin showed significant inhibition for intracellular development. (Dimier-Poisson et al., 2004). Anticoccidials have three modes of action including inhibiting mitochondrial activity, inhibition of folic acid synthesis, and inhibition of sporulation. (Thabet et al., 2017). For determining the intracellular number of sporozoites in response to the treatment of ionophores, molecular techniques of quantitative PCR (qPCR) were performed but were not compared with the *In Vivo* outcomes of therapeutics. As ideal conditions are *In Vivo* so standard reference to check the anticoccidial or coccidiostat activity is *In Vivo* trials because standard outcomes of treatment trials can only be achieved through *In Vivo* studies for implementation commercially (Jenkins et al., 2014).

The industry is relying on already available compounds to limit the spread of coccidiosis because no other remedy is available. To make this treatment effective rotation programs are adopted to overcome the challenge of resistance in commercial broiler farming, but for the long term, the discovery of novel drugs is the need of the hour (Muthamilselvan et al., 2016).

Previous therapeutic and preventive practices have been facing drastic challenges like drug resistance, drug residues in meat, and vaccine failure. The promising protocols for disease control have opened the door to a new research window for the discovery of alternative patent therapeutics and preventive approaches ( Soutter et al., 2021; Arias-Maroto et al., 2022).

**Currently Available Novel Botanical Compounds**

The compounds derived from a specific part of plants, essential oils (EOs), or chemically processed plant molecules have antimicrobial and immunomodulation properties (Nazzaro et al., 2013). So, these new alternatives which do not have toxic properties and have considerable effects on coccidia development, are integral compounds to incorporate in the therapeutic industry. Many natural extracts from stems, leaves, fruits, and roots have been selected as anticoccidial because of their anticoccidial properties (Ali et al., 2015).

It has been studied those natural oils from clove, thyme, artemisia, and tea trees have oocystcidal properties so they were selected as anticoccidials (Remmal et al., 2011). Moreover, the mixture of thyme, garlic, and oregano oils reduces the oocysts count in the droppings of birds, decreases lesions of disease and overall improves the health performance of the bird (Gaafar et al., 2014)

**Assessment of Botanical Compounds *In Vitro***

Recent research priorities are focusing on herbal compounds and remedies as it is already studied that they have a significant capacity to hinder parasitic activity. Botanical compounds including clove, thyme, Artemisia, and tea tree oils effect were checked on the exogenous cycle of Eimeria during the *In Vitro* study. It was examined that the incubation period of 20 h along with essential oils (EO) resulted in a decrease in oocyst number 5 times (Remmal et al., 2011). There was found to increase in the number of dead oocysts upon the basis of dose, as well as a change in the sporulation activity, and reduction in ATPase enzyme activity in the endoplasmic reticulum, and a reduction in oocyst formation (Naciri-Bontemps, 1976). The effect of Green tea was studied on three various species of *Eimeria* (*E. maxima, E. acervulina, and E. tenella*) and saw consistent disruption in the sporulation process by using 25% tea extract (Molan and Faraj, 2015).

The mode of action of essential oils (EO) was considered to be based on a rapid diffusion process through the membrane of the parasite, destruction of ions transport mechanism, and shrinkage of the cell ultimately leading to cell death. Pomace is considered one of the promising sources of valuable bioactive ingredients, having anticoccidial properties, especially of pulp from olive oil with the capacity of oocyst lysis (Ryley and Wilson, 1972). Curcumin is obtained from Curcuma longa having natural polyphenolic compounds with medicinal properties. For centuries, garlic has been regarded as the most effective herb in medicine, and the effect of turmeric and garlic powder is also documented on the sporulation activity of oocysts with inhibition up to 80% at a maximum concentration of 10g/L (Elkhtam et al., 2014).

Most of the botanical compounds' effect was studied with maximum concentration on sporulation rate of multiple species of Eimeria especially Allium sativum dose rate was kept at 10g/L, Artemisia absinthium 50g/L, Essential oil (EO) of Biarum bovei 50g/L, EO of clove 4g/L, Thyme EO 4g/L, and green tea extract 100g/L (Habibi et al., 2016).

**Endogenous Mode of Action of Alternative Anticoccidials**

The effect of many botanicals on the endogenous phase of Eimeria has been investigated *In Vitro* and seen significant changes in the morphology of sporozoites that damaged the viability and infectivity of the parasite during invasion in MBDK up to 72% by incubation with 72% curcumin (Khalafalla et al., 2011). A combination of phytochemical compounds was also tested of curcumin with Echinacea purpurea extract and oregano and the combined effect of all showed synergism. (Burt et al., 2013). However, there are main issues to working with natural extracts that depend upon their variable composition, which is especially based on multiple factors such as type of plant and procedures of extraction. In order to overcome this limitation and to get beneficial results by selecting nature-identical compounds (Rossi et al., 2020a).

Currently, during a study chemically synthesized thymol and carvacrol-based treatment proved their anticoccidial efficacy on invading sporozoites in MBDK cells. (Felici et al., 2020). Essential oils of garlic and oregano showed a strong *In Vitro* anticoccidial activity with a positive impact on growth performance and intestinal microbes. (Sidiropoulou et al., 2020).

Among herbs, garlic is a crucial herb with significant anticoccidial efficacy and has compounds like allicin, propyl thiosulfinate, and propyl thiosulfinate oxide. In a study, it was reported that a smooth decline in the DNA of intracellular E. tenella after undergoing invasion in MBDK cells after treatment through different concentrations of allicin from 1.8 ng/mL to 180 mg/mL with a percentage decline in 54 to 99% (Alnassan et al., 2015).

In the recent past many protocols were adopted to study the *In Vitro* life cycle of Eimeria but because of the intricacy of its life cycle, it is difficult to study it through the *In Vitro* process. There are proposed many *In Vitro* models however, chicken intestinal epithelial cells (IEC) matched to the natural targeting environment of Eimeria. However, in ovo studies have been conducted successfully to study Eimeria and even check the efficacy of anticoccidial drugs by using CAMS of chicken embryos to study accurate parameters of infection, oocyst activity, and quantification through qPCR. The establishment of well-developed *In Vitro* models is necessary for understanding invasion, biochemical, and development processes as these models provide rapid screening of anticoccidial efficacy, reduction in time, cost, and animal use. The need for alternative compounds is increasing significantly to develop an understanding of their target protozoal developmental stage and mode of action. Control of the exogenous phase requires the implementation in cleaning and disinfection protocols in the farm settings. EOs can definitely enhance the hygiene of farm facilities in alternative to chemicals. However, the required concentration of these mixtures is very high and this could make the process complicated and increase the costs. (Adaszyńska-Skwirzyńska and Szczerbińska, 2017).

In context to the endogenous phase requirement of concentration is lower than the exogenous phase, it clarify that anticoccidial alternatives are more impactful when they are given directly to the host as they find their target and invade the Eimeria at different developmental stages. During *In Vitro* efficacy testing, chemically synthesized nature-identical compounds are evaluated in comparison to EOs, which reduces the challenges like composition variation and development of standardization. This formulation plan is urgently needed to apply for the development of compounds that can be used as feed additives (Rossi et al., 2020a).

In studies, *In Vitro* invasion and development of stages of oocyst, and rate of lysis can provide a complete understanding of the mode of action of botanical and synthetic molecules. Regardless, *In Vivo* research still was the final

point to assess the ultimate impact of coccidia, as the *In Vitro* model was not able to completely replace the live organism. Nevertheless, *In Vitro* technologies are faster and comparatively cheap for a comprehensive understanding of pathogenesis and screening of anticoccidials. *In Vitro* experimentation is encouraged to replace *In Vivo* approaches by culturing specific cells, tissues, or organs in a nutritive and sterile environment (Tannenbaum and Bennett, 2015). Common commercially available cell lines being used in research are derived from multicellular organisms (Gorzalczany and Rodriguez Basso, 2021).

**Establishment of Intestine on a Chip (A New Concept)**

A novel chip-based three-dimensional organoid technique has been developed to mimic *In Vivo* studies by following the 3Rs principles (Achberger et al., 2019). Intestine on a chip (OOACs) is the most advanced chip-based technology to develop intestinal organoids by taking endothelial cells from the duodenum of adult humans. In this system, the cellular structure showed similar parameters of permeability, cell-to-cell junction, and gene expression to the natural human intestine. This is a horizon toward new biological research for drug delivery systems and pharmacokinetics at the cellular level (Gijzen et al., 2020; Kasendra et al., 2020). The synthesis of natural identical compounds to the herbal bioactive molecules by a chemical process in a specific concentration and purity could be used to compensate for the problems of essential oils (Rossi et al., 2020b). A novel chip-based three-dimensional organoid technique has been developed to mimic *In Vivo* studies by following the 3Rs principles. (Achberger et al., 2019). Nanotechnology-based herbal compounds can be used in veterinary medicine for *In Vitro* testing with beneficial outcomes of rapid drug delivery, stability of compounds, lower dose rate, and long-lasting bioavailability (Ramadan et al., 2022). The advanced nanoparticles are being used in preclinical studies in comparison to conventional drugs and provide encouraging results with stable physical properties, better absorption through cell membranes, more bioavailability, and fewer side effects (Swain et al., 2018).

Practical applications of the chip-based-intestinal 3D model have been developed for the sexual stages of the *Toxoplasma gondii* successfully for the first time ex-vivo which are a bottleneck to control the protozoan efficiently inside the cell (Luu et al., 2019; Holthaus et al., 2021). 3D chip-based organoids of human brain tissues have been used for studying the infectious stages of cerebral toxoplasmosis and permanently sorting out the issue by using an animal model, which is different from humans genetically (Seo et al., 2020). Plasmodium also has been studied by using 3D chip-based humans and determining the side effects of the antimalarial drug chloroquine (Achberger et al., 2019). In this experimental model, 16-day-old chickens are purchased after getting permission from concerned authorities. After obtaining ethical approvals from the concerned authority for this purpose, the chicken intestinal tissue is isolated during the postmortem from eighteen days old chicks, a section of 5mm will be cut and saved in phosphate-buffered saline, and 3D chicken intestinal organoid is developed by adopting the protocols (Noel et al., 2017; Derricott et al., 2019; Nash et al., 2021). The processing of *Eimeria tenella* sporozoites is done after obtaining frozen purified *Eimeria tenella* sporozoites from the laboratory of the University and will be processed accordingly (Nash et al., 2021). For the development of the Chicken Intestine Explant Barrier Chip (IEBC) the organoid of the chicken intestine is placed into explants of tissue in the IEBC with a rubber ring on the central well of the chip and then tissue on a mesh is placed inside the chip and fixed and at the end chip is closed with the lid (Amirabadi et al., 2022).

**Applications of Nanoparticle as Anti-coccidials**

A nanoparticle is an ultrafine particle with a size from 1 to 100 nm. (El-Saadony et al., 2020). Nanoparticles have positive aspects of transportation and protection of oxygen, and EOs from degradation and keep them stable for enhancement of the function of products. Nanoparticles have a positive impact on the bioactive compounds and their release. (Liang et al., 2012). Recently, advancements in nanotechnology have made significant improvements in the antimicrobial activity of nanoparticles (Reda et al., 2021).

In commercial poultry prohibition of the use of antibiotics as a growth promoter has made a significant shift in approach for production. Herbal compounds are gaining popularity to gain maximum gut performance as well as anti-oxidant properties. (Gernat et al., 2021). Phytogenic nanoparticles have the best bioavailability in such conditions when the body is under the stress of infections for the betterment of animals' and humans' health (Hafez and Attia, 2020).

The Effect of therapeutic nanoparticles can be observed on various sexual stages of *Eimeria tenella* in the chicken intestinal 3D organoid chip-based system. The preparation of Nanocurcumin is done by following the protocols of (Yadav et al., 2020). Preparation of Garlic-based Zinc oxide Nanoparticles is prepared by loading Garlic extract with zinc oxide nanoparticles and using *In Vitro* (Jari et al., 2021). For Garlic Nano-Hydrogel Preparation, Gamma radiations are utilized for the structuring of sodium alginate in hydrogel solutions and then blended with acrylic acid (Ibrahim et al., 2021). For Curcumin-olive oil nanocomposite (C-Oo. Nc) Materials, an active component of curcumin, is processed for developing a compound with high efficacy, and bioavailability (Ramadan et al., 2022). The internal environment of the protozoal development was completely studied and achieved the desired targets of the *In Vitro* study by using a 3-D chip system (Holthaus et al., 2021).

**Conclusion**

Conclusively, Eimeria being a protozoon has similarities with the *Toxoplasma gondii* in life cycle stages and its development in a 3-D chip system will lead to the investigation of developmental stages in an accurate way with precision

and preclinical treatment trials to overcome coccidiosis will be more practical as nanoparticles are being widely investigated for several veterinary diseases, globally. New research approaches through the application of technology-based concepts will be more advantageous in terms of ethical, economical, and experimentation time. 3D chip-based techniques have been recently developed in human studies with significant successful outcomes in the field of protozoal development and the evaluation of therapeutic efficacy. Nano-based therapeutics have been already under trial *In Vivo* research in broilers with efficient outcomes, but the standard outcomes have not been achieved till now. The application of 3D technology and nanotechnology in the investigation of parasitic biology and therapeutic evaluation can make a revolution in veterinary medicine. All the materials, procedures, and protocols which are required during experimentation are already used in research for humans. Therefore, the availability of research-related material and experimentation is economically and physically feasible.

Implication of nanotechnology in veterinary medicine will overcome the drug resistance challenge and residues issue which is highly important for public health. Moreover, conducting research in the poultry sector through innovative tools is the need of the hour as the industry has gained its maximum potential to sustain food security and on the contrary end it is facing massive challenges of poor feed efficiency, high medicinal costs, mortality in birds, and economic losses. So, it is highly demanded to adopt 3D approaches with precision and effectiveness and the usage of alternative therapeutics in future research horizons for the development of the poultry industry and economy.

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Chapter 09

# Use of Nanoparticles in Modern Advancement of Medicines

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**ABSTRACT**

Nanotechnology is the most emerging technique in the advancement of medicine. In this chapter we will discuss about the modern uses and advancement of the medicine used in human diseases and in veterinary filed. Different types of nanotechnology, their use in diagnosis of the disease and its treatment are also discussed in this chapter. The medicine which is made on nanotechnology is known as nanomedicine. The use of these nanomedicine can cause lower toxic effects, and the efficacy of these drugs are more than excellent which is our ultimate goal.

**KEYWORDS**
Nanoparticles, Nanotechnology, Nanomedicine, Use of Nanoparticles.

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## INTRODUCTION

Nanotechnology is the most emerging technique in almost every modern technique. The word "Nano" comes from a Greek word which means "dwarf" (Singh, 2022). The nanoparticles are very small in size; they are in the range of 1-100nm. The nanoparticles used in the medical field are also known as nanomedicine. There are some unique properties of nanoparticles which include a large surface-to-mass ratio, small size, and high reactivity. These unique properties of nanoparticles make them more advantageous than using traditional diagnostic agents and therapeutics (Chouhan et al., 2021). In therapeutic delivery systems, particles are released on a control basis and only targeted delivery. While in diagnostic approach, nanoparticles are used in the detection of any abnormality which is present on a molecular level such as virus fragments, disease markers, and precancerous cells. The use of nanoscale particles can cause huge modifications in the fundamental properties like diffusivity, half-life, solubility, immunogenicity, and drug release characteristics (Abdellatif et al., 2021). The benefits for using these nanoscale particles are lower therapeutic toxicity, easy administration, more effectiveness, extend the life cycle of product, and reduce cost of health care. The nanoparticles have been used for the last 2 decades to treat various diseases like diabetes, asthma, pain, allergy, infections, and cancer (Hussain et al., 2021). Researchers are still working for advancement of these nanoparticles to improve the healthy lifestyle.

### Therapeutic Nanoparticle Types

The Nanoparticles are mainly categorized as

- Nano-structured
- Nanocrystalline (Rafique et al., 2020)

The subtypes of nano-structured materials are lipid-based, non-polymeric, and polymer-based nanoparticles (Gad, 2023). Dendrimers, nanoparticles, micelles, nanogels, protein nanoparticles, and drug conjugates are examples of polymer-based nanoparticles. The carbon nanotubes, nano diamonds, metallic nanoparticles, quantum dots, and silica-based nanoparticles are examples of non-polymeric nanoparticles. The solid lipid nanoparticles and liposomes are two types of lipid-based nanoparticles (Ranjbar et al., 2023).

### Nano-Structured Particles

#### Polymer-Based Particles

The dendrimers, because of their strong mono dispersity and hyper-branched, compartmentalized framework, are a popular choice for polymers in the medical applications (Chis et al., 2020). This type of nanoparticles has a size range of about 1-5 nm by varying the number of branches. They can be created by spherical polymerization, which causes the dendrimer molecule to become hollow inside. Therefore, when compared to smaller dendrimers, advanced dendrimers having greater than 64 surface groups show greater efficiency in delivering therapeutic medicines (Prajapati and Jain, 2020). These dendrimers have free end groups that can readily alter or conjugated with biomaterials to increase the

permeability of molecules and minimal toxic effects in cells (Chis et al., 2020).

**Nanoparticles**

Synthetic or natural polymer-based nanoparticles offer an alternative for therapeutic applications because of their unique qualities, which include biocompatibility, non-immunogenicity, non-toxic nature, and biological degradation (Idrees et al., 2020). The evenly distributed matrix pattern has been suggested as polymeric nanoparticles. Based on their composition, they can be categorized as either nanosphere or nano capsules. Therapeutic chemicals are encapsulated in a distinct polymer membrane in nano capsules, while in nanosphere, the particles are distributed in the whole polymer structure. A greater concentration of drugs can be delivered to the target location by controlling the release properties of the therapeutic compounds incorporated into polymeric nanoparticles (Begines et al., 2020).

**Micelles**

Water-insoluble medicinal compounds are mostly delivered systemically via polymeric micelles (Begines et al., 2020). They form as aggregates in solution and have a size of about 100 nm. Polymeric micelle component molecules have a spherical shape, with water repellent centers surrounded by a mantle of hydrophilic groups (Kannadhasan and Nagarajan, 2022). Their excellent ability to remain stable in biological systems is ensured by their hydrophilic surface, which also helps to protect them against non-selective uptake by the reticuloendothelial cells. The unique structure of polymeric micelles provides a significant process to transfer the substances for medicinal therapy that allows variable loading capacity, modification of specific ligands along with reduced speed of disintegration (De et al., 2022).

**Drug Conjugates**

Low molecular weight drugs are typically conjugated with polymers, especially in the anti-cancer therapy (Zhang et al., 2021). The polymer-drug conjugates work as very stable and soluble carriers that enhance the EPR effect in cancer cells. Drugs and polymers may develop pH-responsive chemical linkages to create polymeric drug conjugates that are sensitive to pH changes. Because the tumorous area has relatively low pH, the pH sensitivity of the nanoparticle is employed for controlled distribution of medicine in that location (Yu et al., 2020).

**Protein Nanoparticles**

The protein nanoparticles also known as “virus-like particles” (VLPs) are classified as nano-carrier systems that resemble isolated viruses morphologically but lack viral genetic content (Saha et al., 2020). Because they can trigger immune responses specific to specific antigens against cancer cells, virus-like particles and CPs are appealing carrier systems for nanoparticles for the production of cancer vaccines (Pena et al., 2023).

**Nanogels**

A nanogel is a gel particle with similar characteristics but having a smaller size of less than 100 nm (Ghaywat et al., 2021). Cross-linked natural or synthetic polymers provide the nanogels with their swelling characteristic, flexible size, and high-water content. Biosensors, biochemical separation, cell culture, bio-catalysis, drug administration, anticancer therapy, and many other uses for nanogels are among their many uses, some of these uses are the administration of medicines including cytokines, vaccinations, nucleic acids, and nasal vaccines (Abusalah et al., 2023).

**Non-Polymeric Particles**

Non-polymeric particles can be created by graphene. The single-walled nanotubes, multi-walled nanotubes, and C60 fullerenes are different configurations of carbon nanotubes (Darroudi et al., 2023). Research with fullerenes has demonstrated their potential for administering medications such as antimicrobials, antivirals, and antitumor medicines. Furthermore, they can supply free radicals to heal damaged mitochondria.

**Metallic Nanoparticles**

Metallic nanoparticles, which range in size from 1nm to 100 nm, are mostly composed of oxides of cobalt, nickel, iron, gold, and magnetite, maghemite, cobalt ferrite, and chromium dioxide (Shabatina et al., 2020). They can be made and altered to include a variety of molecules, allowing them to be embellished with biological components such as proteins, DNA, and peptides, as well as medicinal substances. Their special characteristics as a carrier go beyond stability and biocompatibility to include magnetic properties. Therefore, by application of magnetic field externally, magnetic nanoparticles can be manipulated (Cao et al., 2020).

**Lipid-Based Nanoparticles**

**Liposomes**

The liposomes are vesicles that are created by hydrating phospholipids that are dry (Has and Sunthar, 2020). These molecules can be created in unique sizes, shapes, compositions, and levels of adaptability, as they contain a wide range of lipid molecules and additional surface modification. The capacity of liposomes to attach with cell membranes and deliver their materials inside the cytoplasm makes them ideal smart carrier systems for targeted distribution, which is one of their

most significant advantages.

**Exosomes**

Different kinds of cells spontaneously produce and secrete exosomes. These extracellular vesicles, with a size range of 30 nm to 150 nm and are produced from endosomes (Joshi et al., 2020), are typically found in bodily fluids. There are growing investigations on the potential applications of exosomes as tissue regeneration agents, cancer diagnostic biomarkers, and medication delivery vehicles for autoimmune disorders and cancer.

**Nanocrystalline Particles**

The drug particles that are carrier-free and have a size of just a few nm are known as nanocrystalline particles, or nanocrystals (Mei et al., 2022). As a very economical method, nanocrystal formulations are frequently created for medications that are poorly soluble in water and have low absorption rates. Decreased volume is typically a proven strategy to improve an agent's pharmacokinetics, with the dissolving velocity acting as the rate-limiting step (Nguyen et al., 2022). The rate of dissolution is accelerated by the crystalline structure's increased total surface area. This property increases solubility, which is crucial, especially when the therapeutic effect of a drug is restricted (Salunke et al., 2022). The types of nanoparticles are shown in Fig.1.

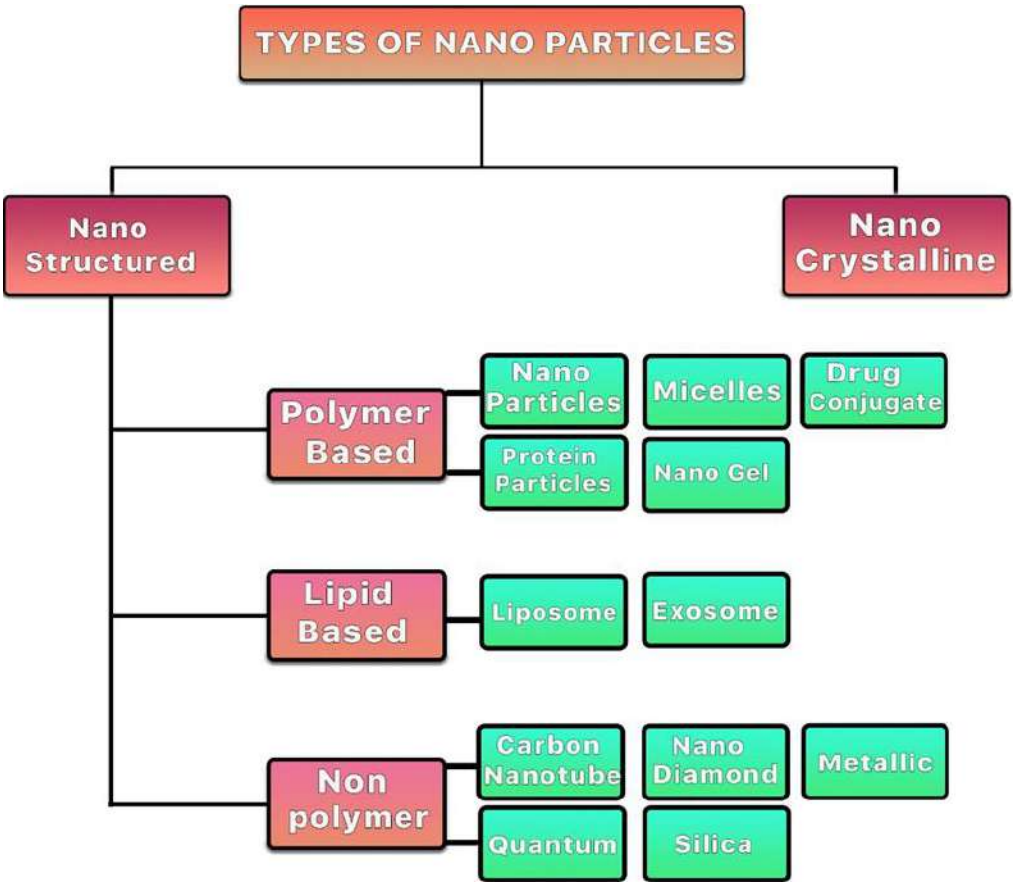


**Fig. 1:** Different types of nanoparticles

**Role of Nanoparticles in Diagnostics**

**Imaging Techniques**

In the past years, there has been significant research done in nanoparticles and their use in the diagnosis and treatment of clinical disorders. The use of nanoparticles in diagnostics is due to their unique combinations of physical and chemical properties that allow the biological molecules to be detected at low concentrations (Bai et al., 2020).

**Magnetic Resonance Imaging**

Powerful magnetic fields and radiofrequency pulses are used to generate the internal structures images for diagnostic purposes (Rotundo et al., 2022). MRI relies on the hydrogen atoms that are present in the water molecules in the body when they are exposed to the magnetic fields. Magnetic Resonance Imaging (MRI) has been the most important advancement in the development of medical diagnosis since the last 100 years after the discovery of X-rays (Hussain et al., 2022). It is applied to every part of the body and has become one of the major tools of radiology for diagnostic purposes.

1. Neurological disorders
2. Cardiac imaging
3. Musculo skeletal imaging
4. Oncological imaging

5. Abdominal and peritoneal imaging (Summers et al., 2021)

**Fluorescence Imaging**

The fluorescence imaging techniques use chemical sensors that enable them to visualize the parameters (Li et al., 2020). Fluorescence imaging has wide applications and enables us to do the diagnosis of diseases and abnormalities (Mohammadi et al., 2022). These applications include:

1. Molecular Imaging
2. Cancer detection and its stage
3. Infectious disease diagnosis
4. Vascular imaging
5. Imaging guided biopsies (MacRitchie et al., 2020)

**Biosensors**

The biosensors are used to convert the biological signals into the measurable form, so that valuable information may be used for the detection of activity of target molecules in the presented sample (Purohit et al., 2020). These are the devices that convert biological signals to other forms with the help of a transducer. They have wide applications in biotechnology, healthcare systems, food safety and environmental monitoring.

The biosensors have three major components:

1. Bio-receptor
2. Transducer (converting device)
3. Output system (Korotcenkov et al., 2023)

**Application in Disease Detection**

The biosensors provide rapid results during the detection of diseases using on-the-spot testing by portable and handheld devices (Biswas et al., 2022). They play a critical role in disease screening by spotting the biomarkers that may be associated with infectious disorders and chronic conditions. The biomarkers after detection facilitate individualized treatment procedures, disease management and therapeutic applications, which may be unique and characteristics for each patient. They may help to monitor the disease states drug responses by the patients and physiological changes in the body (Ngoepe et al., 2013). The biosensors play an important role in the diagnosis because they offer fast and accurate detection of the biological markers in clinical testing samples.

**Nanoparticles for Disease Treatment**

The nanoparticles are of great significance in the treatment of persistent diseases like cancer and neurodegenerative diseases (Rahman et al., 2022). Some branches of the nanoparticles that complicate it are:

- Liposomes
- Solid lipid nanoparticles
- Inorganic nanoparticles
- Micelles
- Nano vaccines
- Nano antibiotics (Carvalho et al., 2020)

**Nano Particles in Cancer Treatment**

In United States increasing number of cancer cases are reported every day. In 2018, the recorded cases were approximately 1.7 million. The nonspecific toxicity as hair loss, loss of appetite, diarrhea and peripheral neuropathy are results of dose limiting toxicity. Likewise, the pharmaceutical limitations such as problems with the aqueous solubility and stability etc. were also recorded. Specific challenge to chemotherapy is multiple drug resistance (MDR). Nanoparticles (1-100nm) can bypass the drug efflux mechanism that's associated with its phenotype; so, it's an alternative to multi drug resistance (Dey et al., 2022).

**Photo-thermal Therapy**

From the past few decades, laser treatment has emerged to be highly effective in cancer treatment. In this process, photo-thermal damage to tumors is achieved by light absorbing dyes (Han and Choi, 2021). The effectiveness of noble metal structures in photo-thermal cancer treatment is based on the phenomenon of strongly enhanced visible and infrared light absorption, plasmon resonance, phototherapy agents have more intense magnitude as compared to conventional one.

**Photodynamic Therapy**

The development of photodynamic therapy (PDT) is an alternative to ablative tumor and sparing function oncologic intervention. This therapy involves the administration of locating tumors photosensitizer (PS) followed by activation of PS by illuminating the tumor with the light of a specific wavelength. The cytotoxic reactive oxygen species (ROS) are generated by the transfer of energy from PS to molecular oxygen. The ROS can oxidize the specific macromolecules for the

ablation to tumor cells (Xie et al., 2023).

**Anti-microbial Applications**

The antimicrobial drug resistance is a major issue in the present age. The antibiotics are not of significance and importance because of their misuse, expensive rates, and some short term and long-term side effects. On the other hand, nanoparticles are of great importance due to their small size and effective treatment because of their ability to destroy pathogenic cells.

**Fighting Drug Resistant Infection**

The alternative to anti-microbial agents and exhibiting remarkable anti-microbial effect by pathogenic destruction is achieved through metal and metal oxides nanoparticles such as zinc oxide and silver. In medicine, anti-microbial drug resistance is a major issue in chemotherapy, expensive rates and side effects all these issues are minimized or excluded with the help of nanoparticles (Rabiee et al., 2022).

**Enhancing Antibiotic Efficacy**

The combination of 4 major conventional antibiotics ceftazidime (CAZ), imipenem (IMI), gentamicin sulfate (GENT), and meropenum (MER), with sliver nanoparticles (starched stabilized) were tested for their antimicrobial effects in comparison to three isolates of *B. pseudoallele*. The effect of combinations with silver nanoparticles showed functional inhibitory concentration (FIC) and fractional bactericidal concentration (FBC) index values ranging from 0.312 to 0.75 µg/mL and 0.252 to 0.625 µg/mL, respectively, against three isolates of *B. pseudoallele* (Allend et al., 2022).

**Safety and Ethical Considerations**

The safety and ethical concerns are our utmost priority in every field of technology. The nanotechnology is a growing field of science nowadays use of nanoparticles measured in nanometers. Its impact on human health as well as on the environment and role in toxicology is also under study. Studying the potential health risks and studying the magnitude of potential risk are two important considerations.

**Potential Risks and Challenges**

The nano toxicology is an emerging sub-branch of nanotechnology to study effects on human health and environment. Chemical risk assessment (CRA) is the most relevant approach to study the toxic effects of nanoparticles. Here, "risk" is defined as if probability of exposure to hazardous material is high, and it ultimately affects human health and environment (high chances to effect) risk is considered as high.

CRA involves four steps.

- Hazard identification
- Dose response assessment
- Exposure assessment
- Risk characterization (Cantoni et al., 2021)

CRA methodology is currently used by the world health organization (WHO) and Organization for economic corporation and development (OECD) and also many other US agencies. The potential risk of nanoparticles is usually studied as '*in-vitro*' and '*in-vivo*'.

***In-vitro***

Today, nanoparticles cytotoxicity testing is totally based on *in-vitro* testing methods for analysis of hazardous chemical agents. Some common features of nanoparticles like charged surface, hydrophobicity, high adsorption capacity, optical and magnetic properties and catalytic activity, are also considered toxic effects (Khan and Hossain, 2022).

***In-vivo***

While performing studies with carbon encapsulation, iron carbide nanoparticles show very strong magnetic properties to be used in biomedical, but assessment of its potential risks was very low (Papadopoulou et al., 2023). However, with a study of iron encapsulated nanoparticles, its effects on a mouse with a time range of 1 week to 1 month showed major toxic effects. After one week, particles were localized in reticuloendothelial cells, particularly of the liver and lungs, but after one year, they were also present in many other organs.

**Conclusion**

Nanoparticles are the smallest units which are used in the advancement of medicines and drug delivery system. These particles are used to target the desired area. There are different types of nanoparticles which are commonly used for the therapeutic and diagnostic purpose. The nano structured particles include polymer, lipid and non-polymer based nanoparticles while other category of the nanoparticles are nano-crystalline particles.

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Chapter 10

# Use of Copper Nanoparticles for Growth Promotion and Disease Control in Poultry Nutrition

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**ABSTRACT**

Copper (Cu) is an important metallic element that is found abundant in the nature. The Cu nanoparticles have promising applications. In poultry nutrition $CuSO_4$ is the primary source. In the body, Cu is present in very minute amounts because most of the it is eliminated from the body through feces, which is hazardous for the environment. Nanotechnology has the potential to solve this problem through the creation of nanoparticles. The Cu-NPs have a tiny size and high ratio of surface to volume, which makes them more accessible. The promising and current uses of nano-Cu in poultry, to meet the demands of a growing world population, more animal-based protein should be produced. The human food chain is impacted by the use of Cu as a feed additive, which may also have an impact on the food safety and/or quality. It is determined that the use of nano-Cu applications has the potential to offer an effective means of lowering the quantity of Cu present in poultry, which can aid in lowering expenses and environmental contamination while boosting animal productivity. Consequently, based on their biological effects, it is necessary to optimize the span and dosage of Cu-NP supplementation for animals. Furthermore, there is a lot more to study about Cu-NP's bioavailability in animals.

**KEYWORDS**
Copper Nanoparticles, Poultry, Growth promoter, Disease control, uses

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## INTRODUCTION

An essential microelement "Copper (Cu)" is necessary for the body's normal physiological processes (Sharif et al., 2021). The ionization capacity of Cu makes it available in ionic form. The ionic capacity of Cu is very crucial for enzymatic reactions in the body (Festa and Thiele, 2011). The major location of dietary Cu absorption is from the stomach to the distal small intestine. This absorption can be actively absorbed with the assistance of particular proteins, or it can be endocytic or pinocytic, the carrier of bivalent metals-I Copper transporters-I (CTR1) (Scott et al., 2018). The Cu acts as an anti-microbial agent and also increases growth in poultry. Because of its poor natural availability, Cu is extracted from Copper sulfate ($CuSO_4$) (Scott et al., 2018). Numerous studies have demonstrated the possible usage consequences of Cu nanoparticles on poultry. Nano-Cu in nutrient embryo feeding has demonstrated encouraging results in concerning with improved immunological reaction, increased production of the breast meat, feed conversion ratio (FCR), and growth performance in broiler chickens (Joshua et al., 2016). When turkey chickens' thigh muscles were supplemented with nano-Cu, the activity of aminopeptidases was considerably boosted (Jóźwik et al., 2018). Moreover, serum Cu rose linearly when Cu-NPs size ranging from 5 ppm, 10 ppm, and 15 ppm were added to clean water (Ognik et al., 2016). The femoral bones' density and strength were enhanced by nano-Cu, reducing the likelihood of fracture in chickens. Additionally, it improved the nuclear antigen for growing cells (Scott et al., 2018). Chickens and other animals primarily obtain Cu from their diets from $CuSO_4$. Nevertheless, the inorganic salt has low bioavailability due to the presence of substances that may hinder absorption. Water and soil are polluted when these minerals are defecated in large amounts, administrating the inorganic minerals in the dietary feed of animals therefore posing a threat to the surrounding natural habitat. Due to their higher ability to digest and absorb along with solidity in the upper GI tract of chicken, organic sources of Cu exhibit lower

excretion than inorganic forms; yet, because they are more expensive and need less dosage of supplements than inorganic Cu, still they have inconsistent outcomes (Bao and Choct, 2009). To improve the bioavailability and absorption and prevent negative impacts on the health and performance of hens, attempts have been made to identify alternate sources of Cu. Depending on the bioavailability of copper, various sources have been added to poultry feed, including tribasic CuO, CuCl, Cu citrate, and $CuSO_4$ at varying amounts (Pang et al., 2009). For financial reasons, the feed business still favors $CuSO_4$. The financial gain by using these micro-sized trace elements, which are employed lately as a key in the disciplines of life sciences, nutrition from minerals, physiological studies, biotechnology, and reproductive studies along with animal medicine, has been modernized by nanotechnology (Peters et al., 2016). The term "nanoparticles" describes particles smaller than 100 nm, where a material's chemical, biological, and physical characteristics are fundamentally different from those of the material in its bulk form (Feng et al., 2009). Furthermore, the efficacy of the active components is increased by their small size, which may also result in a reduction in the applied quantity (Sri Sindhura et al., 2014). The thermal conductivity of cu-NPs is higher because of their lower surface area, it has improved fluid viscosity and thermal conductivity which leads it to different applications like antimicrobials, detergents, and molecular sensors. Copper is involved in enzymatic reactions and processes inside the body, metabolism of Iron, immune system functioning, and formation of red blood cells. It also plays an important role in collagen and elasticity of connective tissues. Copper takes a major part in the composition of dopamine which increases nervous system development (Scott et al., 2018).

**Nanoparticles**

Before using NPs, their new properties must be ascertained using a zeta potential analyzer, transmission electron microscopy, Fourier transform infrared spectroscopy, and UV-Vis spectroscopy. This is required because changes to the properties of nanoparticles (NPs), such as their structure, size, surface area, aggregation, and solvability may biologically affect animals (Sabry et al., 2021).

**Dietary Importance of Copper**

In livestock management, it is very important to measure the quantity of Cu in the diet because its excess and deficiency can cause problems. If Cu is deficient in the body then it can disturb the reproductive system, making hindrance in sperm development. This results in high rates of mortality of embryos during the hatching stage. Feathers also show poor color patterns with less pigmentation, growth will also be slow and body weight will be reduced (Mroczek-Sosnowska et al., 2013). It can weaken the muscles and bones causing scurvy and anemia in several cases (Mroczek-Sosnowska et al., 2013). However, the multifunctional copper protein ceruloplasmin is not affected by Cu deficiency in the liver (European Food Safety Authority, 2016). The excess of Cu may have fatal effects on poultry. So, copper must be provided in optimal amounts according to the requirement, which changes with the growth and development of animals (Kim et al., 2016). The ability to acquire and lose electrons in Cu atoms to produce states of cupric $Cu^{+2}$ and cuprous $Cu^{+1}$ determines the essentiality of Cu. Thus, this modification is essential for enzymes that aid in the metabolic degrading of all significant substrates, including carbohydrates, proteins, and lipids. Due to the decomposition of nucleic acids, lipids and proteins caused by the conversion of cuprous to cupric Cu, tissues, and organs may sustain significant functional damage. As a result, organisms have evolved mechanisms utilizing particular protein carriers that control the uptake, distribution, utilization, and excretion of Cu, lowering the possibility of oxidative damage and regulating the generation of free Cu ions (European Food Safety Authority, 2016).

**Cu Examination in Chicken**

Copper can be examined in chicken through various methods, the most reliable method to check its amount in the body is through liver. The liver indicates the proper concentration of copper in chicken (Kim et al., 2016). The Cu concentration in the liver is concerned with the copper's natural availability in chicken feed (Kincaid, 2000). Furthermore, a decrease in the liver Cu can indicate poor Cu consumption since the liver releases stored Cu when physiological demand is not met. Copper bioavailability in the poultry feed has many variations due to the variations in agronomic conditions and processes (Leeson, 2009). Therefore, many other supplemental forms of copper have been added to the animal diet which include copper citrate, copper oxide, copper citrate, copper sulphate, and chlorides (Kim and Kil, 2015). The bioavailability of trace minerals for animals is in accordance with the ingested trace minerals from a certain source are absorbed in a form that can be metabolized by the animal is known as relative bioavailability (Ammerman et al., 1995). Because using $CuSO_4$ as the primary origin in feed has a few drawbacks, such as interactions with other added components and rheological issues due to its high ability to absorb moisture and its highly reactive chemical nature, researchers are now more concerned with the bioavailability of Cu (Luo et al., 2005). It is also a powerful electron acceptor and an acidic chemical. Therefore, these characteristics have the potential to cause the mixture to solidify in addition to oxidizing, thereby losing its integrity and value. The 20% moisture level of chicken feed can lead to more concentrated $CuSO_4$ crystal surfaces for processes involving the breakdown of imbalanced organic substances such as oils, lipids, vitamins, and enzymes (Marchetti et al., 2000). Numerous research works have contrasted the bioavailability of various forms of Cu with $CuSO_4$. Some hints that compared to $CuSO_4$, Cu citrate is more efficient. Furthermore, supplementation usage levels of 150, 300, and 450 mg Cu/ kg feed, (Liu et al., 2005) explained the bioavailability of cupric oxide, cupric carbonate, and cupric sulphate. Based on levels of Cu in the liver, these values were estimated to be 88.5%, 54.3%, and 0% for the sulphate, carbonate, and oxide,

respectively tribasic Cu chloride (TBCC) has a relative value of 134% when compared to $CuSO_4$, according to feeding supplements up to 390 mg/kg feed, although suggested a value of 112% for TBCC (Arias and Koutsos, 2006).

On the other hand, it seems that reducing the amount of Cu in the excreta is possible with the help of the organic Cu, which is heterocyclic with proteins, peptides (Świątkiewicz et al., 2014). There have been suggestions that chelated copper is more readily absorbed and metabolized and that it may also avoid copper's antagonistic effects on other minerals (Leeson and Summers, 2009). Additionally, research has looked into the possibility of using chelated copper in place of $CuSO_4$ as a pig growth enhancer (Zhao et al., 2014). The Cu absorption is less in the stomach as compared to the intestine and is delayed due to the compound's formation of insoluble compounds upon interaction with phytic acid. The $CuSO_4$ given at 250 mg/kg of feed was found to reduce phosphor retention in broilers by the formation of an insoluble Cu-phytate heterocyclic complex (Banks et al., 2004).

**Cu as an Antibiotic Growth Promoter**

Antibiotics have been used extensively to manage livestock and poultry infections. Antibiotics at subtherapeutic concentrations have also been employed as growth promoters (Sasidharan et al., 2020). To prevent the spread of germs immune to antibiotics and the presence of antibiotic remnants in the animal products that pose a health risk to people, the EU has outlawed the use of many antibiotics. Correspondingly, a great deal of effort is put into identifying environmentally acceptable substitutes that have the same therapeutic potential for altering intestinal microbiota and stimulating animal growth. Correspondingly, a lot of research has been done to identify antibiotic substitutes that have comparable effects on altering gut microbiota, boosting immunity effectiveness, and animal growth is encouraged (Sabry et al., 2021). The Cu is a prospective substitute for antibiotic growth promoters (AGP) due to its potential antibacterial and growth-promoting qualities. For instance, chicks fed 188 mg/kg of extra dietary Cu from TBCC or $CuSO_4$ had development and performance that was comparable to or better than that of chicks given antibiotic growth boosters (Abbasi-Kesbi et al., 2018).

**Use of Cu-Np**

The modern meat and egg-type chicken lines perform far better than older ones, therefore their nutritional needs have changed, particularly with regard to microminerals. The micro minerals are inexpensive, thus there's a chance that chicken diets may contain an excessive amount of Cu supplementation (Alyousef et al., 2019). As a result, a higher concentration of Cu in excreta may be considered a pollutant of the environment. For instance, modern meat are advised to consume up to 25 mg/kg of Cu, which is five times higher than the suggested intake of 5 mg/kg of Cu. This suggests that there's a chance that the excreta contain more Cu. New restrictions that limit the amounts of microminerals in poultry diets have been established as a result of widespread concern about environmental pollution (Additives and Products or Substances used in Animal, 2016). Producing animal protein economically and at a price that people can pay is a challenging operation that depends on a number of factors. The largest issue facing commercial chicken is high feeding costs. Therefore, it appears that the effectiveness and bioavailability of Cu in nanoform could make it a viable substitute for ordinary Cu form in terms of both the environment and the economy (El Basuini et al., 2016).

**Toxicology of Cu-NP**

The high-tech industry and human lifestyle have seen significant changes in the recent decades due to the explosive expansion of nanotechnology (El Sabry et al., 2018). During use or transit, metal-nanomaterials may be discharged into manufacturing systems. Therefore, the danger of health issues in animals may grow due to the contamination or abuse of nano-metals (Abd El-Ghany, 2019). At certain amounts, copper usage, whether in greater quantity or nanoscale form, is harmful to animals. Reactive oxygen species production, which causes the oxidation of big molecules like proteins and lipids, is the main mechanism by which copper is hazardous (Vieira et al., 2009; Wang and Gallagher, 2013). Moreover, Cu-NPs have drawn special attention due to their novel physicochemical characteristics that may have a deleterious effect on animal health (Wang et al., 2014). In agricultural areas, microminerals are widely used in both bulk and nano form. Concerns regarding the possible harmful impacts of microminerals on the environment are growing over time (El Sabry et al., 2018). The effects of excessive Cu supplement dosage in chicken diets were investigated (Skřivan et al., 2006), and discovered that when $CuSO_4$ was added to the basal diet at 0 and 240 mg/kg diet, the Cu content per kilogram of excreta dry matter rose in laying hens from 25.3 to 397 mg/kg. Additionally, found that when the content of copper and other microelements in the broiler feed increased, so did the excretion of these elements (Toor et al., 2023). Cu should be consumed at a lower level in the diet since environmental pollution with Cu rises in tandem with the amount of Cu emitted in animal waste (Sasidharan et al., 2020). Notably, a number of variables can have a great effect on the toxicity and destiny of NPs in the surroundings. The availability of organic materials, water temperature, and the physiological and genetic makeup of fish species can all have an impact on the aquatic system's NP harmful capacity (Song et al., 2015). Consequently, as the use of trace elements applications grows, more research on toxicology is needed to better understand the variables influencing the toxicological potential of nano minerals (Toor et al., 2024).

**Conclusion**

Poultry raised in intensive production systems should receive copper supplements. To preserve animal welfare and ensure that customers may safely consume eggs and meat, it is crucial to consider the source, form, and quantity of

copper. High dietary copper intake, however, may raise the amount of copper litter, which is bad for the environment. Although the amount of copper in diets may be reduced by nanotechnology, the advice to use nano-copper in animal diets is still hampered by the lack of clarity surrounding the paths and fate of nanoparticles. Furthermore, the long-term harmful effects of these particles appear to be extremely dangerous if food or the environment gets contaminated with nano-copper. Enhancing the development and performance as well as lowering the disease factor to promote growth and lessen Cu excreta in the natural habitat are the primary goals of employing Cu-NPs as feed additives in poultry. The Cu-NP is biologically more active than bulk Cu due to its tiny size and high surface to volume ratio. This allows for more effective biological interactions and may reduce the quantity of Cu utilized in the food without having a negative impact on the environment or the performance of chickens. However, in order to get the most out of Cu-NP and prevent any potentially harmful consequences, it's crucial to manage the size and concentration.

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Chapter 11

# Synergistic Effects of Silver Nanoparticles as Alternative Medicine Strategies

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**ABSTRACT**

Nanoparticles are currently used in different areas of medicine, therapeutics, diagnostic field, gene therapy and anesthetic industry. Nanoparticles are effective due to their small particle size and large surface area. AgNPs prove very effective as antiviral, antibacterial, antifungal, anti-inflammatory, antioxidative, anti-diabetics and anticancer agents. Main advantage of AgNPs use is to prevent antimicrobial resistance by damaging or killing bacterial cells and enhancing the efficacy of the drug. Silver nanoparticles synthesized by many different methods like chemical, physical and biological. AgNPs are widely used in the veterinary and human field and improve the quality of life of both animals and humans. In animals AgNPs helps to solve many reproductive problems, parasitic, fungal, bacterial infections, to treat mastitis and as vaccine adjuvant especially rabies vaccine. In humans it is effective in treating, prevention, and diagnosis of different diseases like cancer and wound healing etc. AgNPs show many synergistic effects when combined with different medicines used for specific diseases or problems. Currently the use of nanoparticles is increasing in many fields and further advancements must be made in the use of nanoparticles to improve the health of animals and humans.

**KEYWORDS**
Synergistic, Silver Nanoparticles, Alternative Medicine Strategies

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## INTRODUCTION

Nanotechnology combines the knowledge of different science branches including biology, physics, engineering, chemistry, medicine and pharmacology etc. (Dulta et al., 2022). In the past few years their importance and use has improved and increased in different fields like disease treatment, diagnostic field, prevention indication, gene therapy, regenerative medicine, oncology, anesthetic industry, drug delivery and therapeutics. Nanoparticles are small particles ranging between 1-100 nanometers (Dolai et al., 2021). Silver nanoparticles are most often used as nanoparticles in medication (Almatroudi, 2020). Now a days AgNPs are applied as antifungal, antiviral, antibacterial, anti-inflammatory, anti-angiogenic, anti-tumor, anti-oxidative and also used in water treatment, cosmetics, imaging, textiles, drug carrier, biosensors and human health care. (Jain et al., 2021). In veterinary and human medicine antibiotic resistant bacteria are a major health problem (Palma et al., 2020). Efficiency of antibiotics reduces with rapid development of bacterial resistance (Bassetti et al., 2022). To overcome this problem silver nanoparticles are used in combination with different antibiotics. Silver nanoparticles along with penicillin G show synergistic effect against *Actinobacillus pleuropneumoniae* (Afridi et al., 2022). Mostly antibiotic resistance is reported in bacteria like *Salmonella* spp., *Escherichia coli, Pasteurella multocida, Porphyromonas gingivalis, Actinobacillus* spp. and *Staphylococcus aureus* (Uddin et al., 2020). AgNPs facilitate the penetration of antibiotics in the bacterial cell due to change in permeability of membrane (de Lacerda Coriolano et al., 2021). Use of AgNPs with antibiotics helps to reduce the spread of bacterial resistance, increases the efficiency of antibiotics use and reduces the requirement of drug (Nefedova et al., 2022). Silver nanoparticles along with the antibiotics have good antimicrobial and anti-cancer activity. It also improves wound healing, act as anti-diabetics agent and as vaccine adjuvant (Wang et al., 2023).

**Nanoparticles Synthesis**

Nanoparticles are synthesized basically by biological, chemical and physical methods (Pandit et al., 2022).

**Physical Methods**

By this method nanoparticles formed using a tube furnace at atmospheric pressure by process of evaporation and condensation (Sabouni Tabari et al., 2020). The benefit of this method is that the use of chemicals is absent and speedy method and radiation used as reducing agents (Ijaz et al., 2020). But the disadvantages of this method is high energy utilization and low yield and absence of consistent distribution (Niculescu et al., 2022). High energy radiation, thermal and electrical energy and mechanical pressure cause melting, evaporation, condensation and erosion to form nanoparticles (Said et al., 2022). Most commonly and widely used physical methods include inert gas condensation, laser ablation, flash spray or laser pyrolysis, high energy ball milling, melt mixing and electro spraying (Niedermaier et al., 2021).

**Biological Methods**

To get nanoparticles of different sizes, shapes, and environment friendly, these are synthesized by biological method (Salem and Fouda, 2021). Different nanoparticles like silver, gold, selenium, platinum, titanium, uranite, tellurium, and magnetite are synthesized by using bacteria, fungi, viruses, yeast and mycetes etc. (Osama et al., 2020). Biosynthesized nanoparticles have slow production rate and are not monodispersed, so to overcome these problems it is important to optimize extraction techniques and cultivation methods (Morriss et al., 2024). Molecular, biochemical, and cellular techniques that initiate the production of biological nanoparticles must be properly examined to increase synthesis and improve properties of nanoparticles (Rónavári et al., 2021).

**Chemical Methods**

Synthesis of nanoparticles by chemical methods is done by reduction process and use of reducing agents whose composition depends on the operating situation and conditions at which reaction take place (Abid et al., 2022). Hydroquinones, monosaccharides, garlic acid, ascorbic acid, secondary alcohols, aldehydes, citrate and ethanolamines are organic reductants used for nanoparticles synthesis (Das and Pradhan, 2022). Inorganic reductants include alkali metals borohydrides like sodium borohydride (Suárez-Alcántara and Tena García, 2021).

**AgNPs with Antimicrobial Activity**

Silver nanoparticles attack bacteria and effect cell division and respiratory chain and cause death of bacterial cell (Mikhailova, 2020). AgNPs are effective against both gram -ve (*Escherichia Coli, Pseudomonas, Klebsiella*) and gram +ve bacteria (*Staphylococcus aureus*) (Bruna et al., 2021).

**Mechanism of Action of Silver Nanoparticles as Antimicrobial**

Silver nanoparticles interact and damage bacterial cell wall (Joshi et al., 2020). They change the permeability of the bacterial cell membrane (Mikhailova, 2020). After that change in the permeability of cell membrane, plasma membrane collapse (Salleh et al., 2020). AgNPs interact with the DNA of bacterial cells, inactives the enzymes, impact metabolic processes, change expression of genes and cause destruction of respiratory chains (Bruna et al., 2021). Nanoparticles deliver silver ions which gain entry in the cell of bacteria and produce Relative Oxygen Species (ROS) and results in the destruction of biomacromolecules (Alavi, 2022). Silver nanoparticles have small size and large surface area that result in its high reactivity (Hamad et al., 2020). If the size of silver nanoparticles is less than 10 nm, they are able to penetrate cytoplasm and cause disruption of cellular metabolism and stop biochemical processes (Mikhailova, 2020).

**AgNPs use in Veterinary Health**

In veterinary field nanoparticles play an important role in therapeutics, improving immunity, disinfection, enhance the production, reproduction and health, diagnosing, preventing and treating diseases with reduced side effects (Kareem et al., 2022). The use of nanoparticles as feed additives for animals improve their growth, performance, weight gain and feed conversion ratio (Radi et al., 2021). Nanoparticles used in a small amount and are cheap and enhance the process of fermentation in the rumen of animals (Abdelnour et al., 2021). It helps in solving reproductive problems related to retained placenta, calving, hormones release, oestrus detection and sperms freezing (Poddar and Kishore, 2022). Nanoparticles helps to solve severe problems like Brucellosis, tuberculosis, foot and mouth disease, blood pathogen infections and Mastitis (Abbas et al., 2022). Some devices like nanotubes and nano sensors are used which give advancement to veterinary field (Woldeamanuel et al., 2021). Nano sensor is the device used to diagnose reproductive tract infections, hormonal imbalance problems and oestrus detection (Devi et al., 2022). Some nanoparticles also used as contraceptives in animals (de Brito et al., 2020). Nanoparticles have good therapeutic effects as in combination with drugs given for particular disease enhances the action of drug and work more quickly with least side effects (Yetisgin et al., 2020). It also protects against viral and bacterial infections, reduces the pain and also promote and fasten healing, make sure the bioavailability, stability distribution, specificity and digestion of drugs. It also helps to reduce the concentration, frequency and toxicity of pharmaceutical compounds. Nanoparticles act as adjuvant and improve the immune status of animals and with vaccines improves its performance and effectiveness. Silver nanoparticles are used in the shampoos, soap and ointments of especially pet animals because it freshens and disinfects the surface (Ilangovan et al., 2021).

**AgNPs Ointment for Post-operative Treatment of Caseous Lymphadenitis**

Caseous lymphadenitis is a zoonotic and infectious disease of small ruminants in which granulomas developed in the lymph nodes and internal organs (Rodríguez Domínguez et al., 2021). This disease is caused by *Corynebacterium pseudotuberculosis*, a gram +ve bacterium (İlhan, 2020). In the past iodine solution was used after drainage of lesions which is not effective and have low antibacterial effect and cause cytotoxicity (Ozhathil and Wolf, 2022). Now AgNPs are used as an effective agent due to best antimicrobial activity and wound healing power (Chinnasamy et al., 2021). AgNPs are used as post operative treatment for caseous lymphadenitis (CL) (Adegbeye et al., 2021). After excising the lesions, the wound treated with ointment made by AgNPs mixed with some oils and waxes. The result of this treatment is very fast and excellent. AgNPs also help in lowering purulent or moisture discharge from surgical wounds (Nqakala et al., 2021).

**AgNPs for Mastitis Control**

Mastitis is one of the commonest and biggest problem in dairy cattle and goats nowadays (Jabbar et al., 2020). Almost 90% of environmental bacteria cause mastitis. Because of the antibiotic resistance, the treatment of mastitis with antibiotics is ineffective. AgNPs are the best agents to overcome this bacterial resistance problem (Mateo and Jiménez, 2022). A Study has shown that silver and copper nanoparticles are very effective and safer against the bacteria that cause mammary gland inflammation (Taifa et al., 2022). AgNPs and CuNPs have synergistic effects against many mastitis causing pathogens. These nanoparticles also add in the pre and post dipping disinfectant solutions because these nanoparticles have no toxic effect for animals and humans (Kalińska et al., 2023).

**Green Synthesized AgNPs as Adjuvant in Rabies Vaccine**

Vaccination plays an important role in the prevention of diseases (Sultana et al., 2020)**.** Green synthesized AgNPs are cost effective and easily synthesized (Huq et al., 2022). AgNPs produced by *Eucalyptus procera* proves to enhance the immunity against inactivated rabies virus (Dilnawaz and Misra, 2023). Nanoparticles increase the immune response when used as adjuvant. AgNPs enhances the humoral response to rabies vaccine (Awandkar et al., 2021).

**AgNPs use in Human Health**

AgNPs play an important role in improving human health. SNPs wound dressings are used for the treatment and care of wound cites in humans. (Farahani and Shafiee, 2021). Now a days SNPs coated or used on medical devices which implanted or inserted in human body for long duration and also used in dental equipment and materials (Rajan et al., 2023). SNPs are very effective against pathogens of oral cavity and act as best disinfectant. It is also used in water purifiers and in biosensors. AgNPs also prevent the growth of cancer calls by blocking the metabolic machinery of abnormal cells. These nanoparticles are effective in malaria, leishmania, AIDS, chronic hepatitis and other human diseases (Manna et al., 2023). Main property of these AgNPs is their small size and large surface area so these enter the bacterial cell easily and also enters the target tissue or organ more fastly and enhance the action of drug administered (Manna et al., 2023). Table 1 shows the mechanism of action of silver nanoparticles.

**Table 1:** AgNPs and their mechanisms of action

| Bacteria | Size | Coat | MOA | References |
|---|---|---|---|---|
| Staphylococcus aureus | 55 and 278 nm | Chitosan | Irreparable injury to bacterial cells | (Mirzaie et al., 2020) |
| | 11.23 nm | PEG | Permanent damage to bacterial cells | |
| | 13.5 nm | Naked | Irreparable harm to bacterial cells | |
| Escherichia coli | 9.68 nm | Gelatin | Change the permeability of membrane and effect respiration | (Bouafia et al., 2020) |
| | 13.5 nm | Uncovered | Effect respiration of bacterial cell and alters permeability | |
| | 1 to 10 nm | Uncovered | Effect respiration of bacterial cell and alters permeability | |
| Salmonella typhi | 55 and 278 nm | Chitosan | Alters intracellular ATP levels, stops the replication of bacterial DNA and bacterial cytoplasm membrane injured | (Arshad et al., 2021) |
| | 14 nm | Uncovered | Harm bacterial cytoplasmic membranes and stop DNA replication and alters ATP levels | |
| | 1 to 10 nm | Uncovered | Harm bacterial cytoplasmic membranes and stop DNA replication and alters ATP levels | |
| | 11.23 nm | PEG | | |
| Pseudomonas aeruginosa | 9.68 nm | Gelatin | Damage to bacterial cell that is irreversible | (Ali et al., 2020) |
| | 1 to 10 nm | Uncovered | Effect the permeability and respiration of bacterial cell | |
| | 55 and 278 nm | Chitosan | Irreversible injury to cells | |
| Vibrio cholera | 1 to 10 nm | Uncovered | Damage membrane permeability and respiration | (Chatterjee et al., 2021) |

**AgNPs use in Cancer Treatment**

Cancer is one of the leading causes of death worldwide. AgNPs proves to be very effective in diagnosis and treatment of cancer cells due to antitumor activity (Huy et al., 2020). Most common type of cancers appear in humans are lungs, breast, liver, thyroid, cervical, prostate and colorectal. The synergistic combination of chemotherapy drugs and AgNPs are very beneficial, minimize drug resistance, improves efficacy of drug and minimize side effects. When x rays interact with AgNPs it results in the release of secondary electrons which interact directly with DNA and cause DNA strand breakage. These electrons also produce ROS by ionizing water molecules and leads to further damage to cancerous cells like mitochondrial dysfunction, inhibition of DNA replication, lipid peroxidation, endoplasmic reticulum stress and finally death of cells. Radiosensatizing effect of AgNPs decreases as their particle size increases (Fathy, 2020). Table 2 shows the use of AgNP's in combination with anticancer drugs

**Table 2:** Combination of anticancer drugs and AgNPs

| Anticancer drugs | Used substances | Combination method | References |
|---|---|---|---|
| Imatinib | AgNPs and *Eucalyptus procera* | Biosynthesis method | (Jabeen et al., 2021) |
| Methotrexate | Tri sodium citrate, AgNPs, NaOH and NaBH4 | Conventional heating | (Rozalen et al., 2020) |
| Doxorubicin Alendronate | N-hydroxy Succinate and AgNPs | Use of microwave | (Jampilek and Placha, 2021) |
| Epirubicin | AgNPs and epirubicin | One pot synthesis | (Abdel-Hameed et al., 2022) |
| Doxurubicin | Graphene oxide dispersion, AgNPs and Azadirecta indica | Biosynthesis method | (Palai et al., 2019) |

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**Conclusion**

Livestock plays an important role in the lives of humans such as eggs, meat and milk consumption from domestic animals is a primary source of living. Our basic aim is to improve the health, enhance the production of animals and upgrade human health. This is done by diagnosis, prevention and treatment of diseases that appear in animals and human and by improving their nutrition. Nanotechnology is an emerging industry. Nanoparticles currently proves very effective and beneficial to solve these problems because of their super qualities or characters like antibacterial, antiviral, antifungal and anti-inflammatory effects and enhances the effectiveness of drugs by improving their action and delivery to specific sites. In future more advancements must be made in nanoparticle synthesis and use and more awareness will be spread over the world regarding their advantages. NPs show very effective results especially in the field of human and veterinary medicine.

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Chapter 12

# In-silico Drug Designing and Development a Breakthrough in Pharmaceutical Industry

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**ABSTRACT**

*In silico* drug design uses computing techniques and algorithms to simplify the process of identifying, improving, and assessing prospective candidates for drugs. CADD (Computer-Aided Drug Design,) allows experts to effectively investigate extensive chemical spaces, anticipate interactions among drugs and targets, and evaluate the pharmacokinetics and cytotoxic features of drug molecules. This is achieved by merging many fields of study, such as molecular modelling, computational biology, and cheminformatics. The use of CADD methods, such as virtual screening, structure-based drug design, and ligand-based drug design, has culminated in significant progress in the discovery of prospective lead compounds, the enhancement of current therapies, and the reutilization of authorized pharmaceuticals. In addition, artificial intelligence and machine learning have played a significant role in decreasing the duration and expenses related to the pharmaceutical manufacturing procedure, thereby becoming an essential element in decision-making for improved and safer therapeutic agents. This chapter emphasizes the crucial importance of CADD in speeding up the process of discovering new drugs.

**KEYWORDS**
CADD, Drug Discovery, Drug Designing, In-silico techniques, Machine Learning in Drug screening

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## INTRODUCTION

The need for new drugs with a wide margin of safety and high efficacy is the topmost demand of the medical and healthcare sector. The rise in antimicrobial resistance and drug tolerance highlights the dire need of the pharmaceutical industry for new potential drug availability. Since drug development and discovery is not only quite strenuous and capital-intensive but also time-consuming. Even after making a lot of efforts promising results are not certain. Various surveys conducted in past reported that a drug used to take 10-15 years and about US$2 billion investment to become available for use in healthcare facilities (Berdigaliyev and Aljofan, 2020).

These circumstances have forced scientists and industrialists to find out some other cost-effective ways for drug designing and development. Nowadays the pharmaceutical industry has found various ways including high throughput drug screening and artificial intelligence-based drug modelling which are highly cost-effective and expeditious. Similarly *in silico* method for screening potential drug candidates allows the scientists and researchers to reduce the hassle and make the process of drug development fast (Chang et al., 2023).

*In silico* drug designing is the use of computer-based modelling and simulation techniques for discovering and designing drugs that after further studies and clinical trials can be launched in the market for use in the health sector. Currently used *in silico* techniques include structure-based drug design (SBDD), ligand-based drug design (LBDD), virtual screening (VS), molecular docking, molecular dynamic simulation, ADMET prediction, and many others. With the advancement in this sector, other useful techniques based on machine learning algorithms, artificial intelligence and De novo drug design are also making a mark in drug development and designing (Selvaraj et al., 2021).

Drug Repurposing also act as an extremely helpful tool for finding out the new uses of the already available drugs. It assists the industries by reducing the cost of clinical trials and helps the practitioners to use the already available drug for addressing other diseases as well. The enormous amount of data gathered in recent years about drugs, their structures,

and their mode of action has allowed us to link drugs to various diseases. Despite this so-called "big data" era, the vastness of the knowledge and complexities create hurdles in understanding the whole scenario. Overall, these *in silico* techniques have opened the door of opportunities and ease for pharmaceutical industries, scientists, practitioners and stakeholders (March-Vila et al., 2017).

*In silico* drug design and development is a very enormous area which is yet to be explored further. It allows the pharmaceutical industries to save time, cost of testing, reduce trial labour, and give a head start allowing to streamline the drug discovery process. The current status of antimicrobial resistance, emerging and re-emerging diseases is putting a lot of emphasis on finding new drugs and utilize time efficient rapid technologies to ensure the optimized provision of healthcare facilities to the people of the world (Shaker et al., 2021). The current chapter aims to highlight the working and benefits of various *in silico* drug design and development techniques which are already in use by pharmaceutical industries. The future technologies which can be used for the above-mentioned purpose and ensure promising results will be discussed here.

**Evolution of Drug Discovery Techniques**

Initially the drug discovery techniques were not based on a well-designed and elaborate framework. The techniques involved various "hit and trial" methods, accidental discoveries, in vitro, in vivo assays, and medicinal chemistry. These techniques involved screening of various plants, microbes, and animals extract keeping in view their medicinal characteristics. The extracts were carefully selected and were available for testing and trial in the healthcare sector. Similarly, accidental discoveries like that of penicillin also served as a source of novel drugs. Medicinal chemistry involved the restructuring and refinement of various available drugs on the basis of their structure-activity relationships (SAR) and organic chemistry. This could significantly improve their potency, efficacy, and pharmacokinetics. Scientists also relied on performing *in vitro* and *in vivo* assays using various compounds against animals, their enzymes, receptors, proteins, or cells. These compounds were tested on lab animals putting their health and welfare at risk (Dias et al., 2012).

These techniques of old times proved to be extremely time-consuming and didn't stand for some promising results. The advanced techniques involving *in silico* methods have covered these drawbacks and set the pharmaceutical industry on finding new potential agents by giving the tools based on virtual screening. *In silico* computational methods have proved to be game changers in pharmaceutical industry and delivered many useful compounds including atazanavir, saquinavir, indinavir, and ritonavir against human immunodeficiency virus (HIV). Raltitrexed and norfloxacin are also outcomes of CADD (Computer-aided drug discovery) (Shaker et al., 2021).

**Pharmacodynamic (ADMET) Prediction**

Other than drug designing the *in-silico* approaches also proved helpful in giving a head start for clinical trialing. The ease purposed by ADMET prediction analysis helps the researchers to find out the pharmacokinetics (PK), toxicity, and potential side effects of the screened compound. Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) prediction utilizes various rules and theories which are based on human physiology and response abilities (Ferreira and Andricopulo, 2019). The main cause of a drug candidate's failure to proceed through clinical trials is undesirable toxicity and infelicitous PK properties. Using ADMET prediction based on various principles and theories, we can rule out the maximum drug candidate which shows a chance to present unlikely results during clinical trials allowing the pharmaceutical industries to save a huge investment and time (Jia et al., 2020).

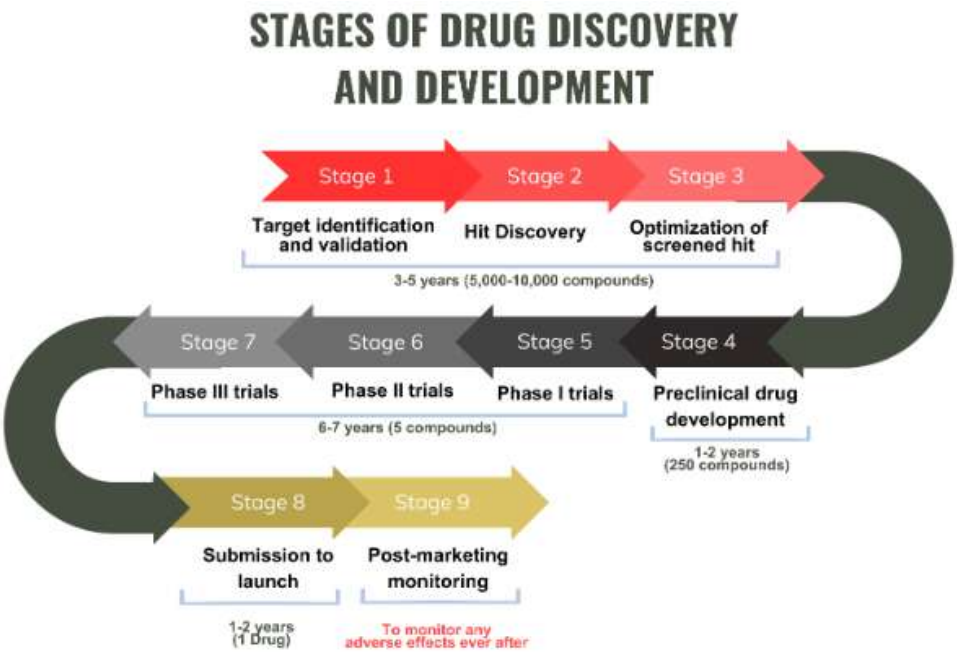



**Fig. 1:** Steps involved in drug discovery and development.

**Computer Aided Drug Discovery (CADD)**

The recent advancement in technology has led to the adoption of computer-based tools which do not require any high-end machines. These computer-aided drug discovery technologies have proved to be quite nifty in reducing time and expenses required for drug development. Without utilizing a large team of experts and laboratories CADD allows the researchers to find out the best hits with use of their individual computers or sometimes a super cloud based computer is utilized (Niazi and Mariam, 2023). The overall steps involved in drug discovery and development are outlined (Fig. 1).

CADD assists with reducing the burden of the first four stages along the reduction of cost and labour. Further it helps in optimizing the compounds for later stages. CADD technology utilizes various techniques among which most commonly used are structure-based drug design, ligand-based drug design, and many others (Fig. 2). Moreover, now a days approaches are made to integrate these technologies with machine learning and artificial intelligence which will not only assist in designing the compounds but will also help in VS of the compounds from a library of $10^6$ million (Gupta et al., 2021).

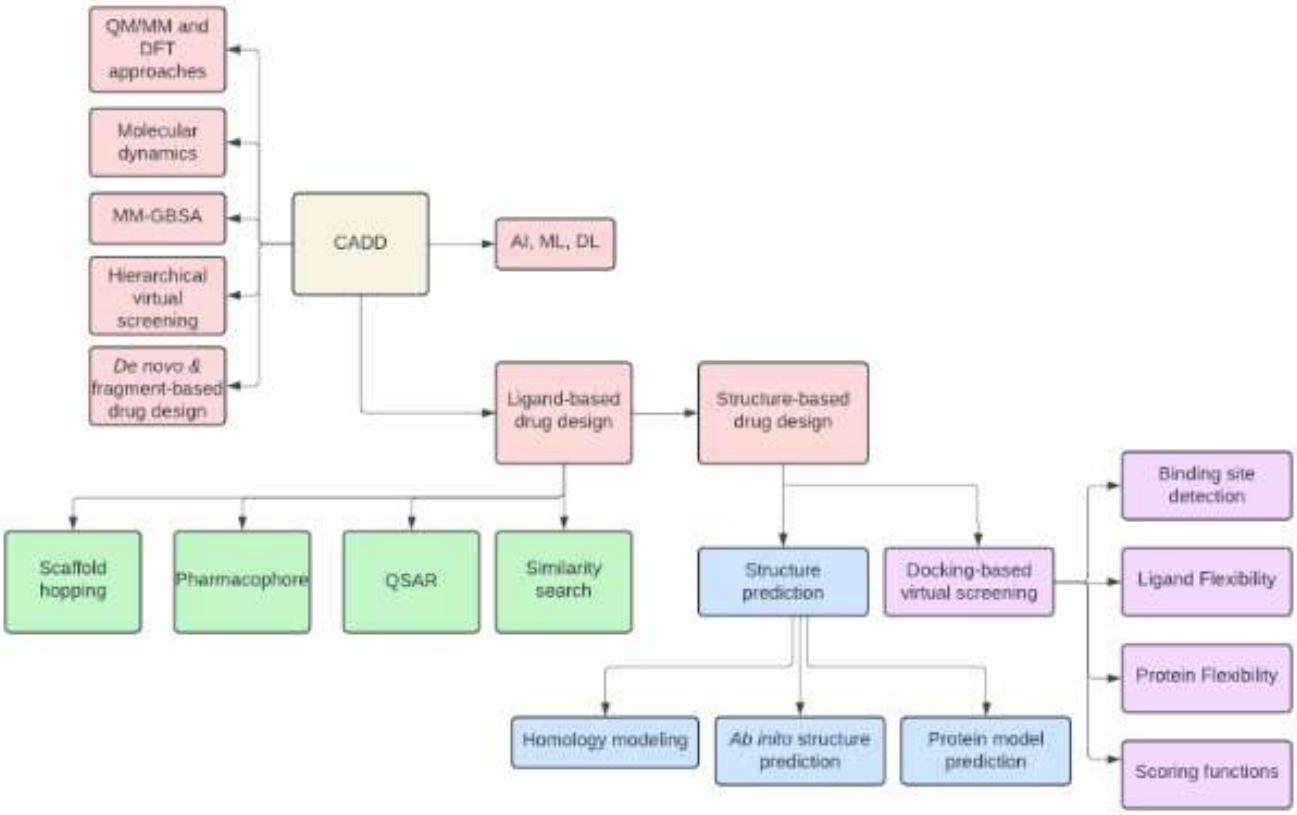



**Fig. 2:** Various *In silico* Approaches being utilized in today's era for drug discovery and development (Niazi and Mariam, 2023).

**Structure-based Drug Design**

The functional properties of a protein are defined by its structure. Similarly, the structure-based drug design requires the three-dimensional (3D) conformation of the protein being targeted in the body. Normally the 3D structure of the protein can be acquired by various experimental techniques including NMR spectroscopy, X-ray crystallography and cryo-electron microscopy. The 3D protein structure can be accessed from the online repositories including UniprotKB/TrEMBL, and PDB. According to the reports of March 2024, the UniprotKB/TrEMBL databases contained more than 248 million sequencies yet only 218,500 structures were deposited on Protein Data Bank (PDB).

Unfortunately, if a desired protein structure is not available online the researcher opts for homology modeling or *ab initio* structure prediction techniques.

**Homology Modeling**

The prediction of a protein structure by simply aligning its sequence along a homologus protein is termed homologus modeling. The homologus protein acts as a template for constructing the model. The method is divided into three steps:

1. Identification of template
2. Alignment of template protein along sequence
3. Construction of model

For the first step the protein sequence is identified from a database, or it can be done experimentally by available techniques. PSI-BLAST is considered more appropriate since it relies on profile-based approaches to detect residual

patterns. Generally, to ensure effective homology modeling, it is crucial to have a minimal sequence identity of at least 30%. Less than 30% identity will produce abject results while an identity score of more than 40% will allow to minimize the error chances in alignment. At the level of more than 40% alignment identity about 90% of the main-chain atoms are supposed to be modeled with ~1Å RMSD, while the loop and side chain orientations may present most of the structural changes (Zhao et al., 2023).

For the second step pairwise, alignment is utilized which is divided into two categories.

1-Global alignment aims to align the sequences when they are almost similar in length. It utilizes the EMBOSS needle and EMBOSS stretcher tools based on Needlemann-Wunsch algorithm (Madeira et al., 2019). It presents the concept of "gap penalty" in case of gap in alignment and works on basis of "dynamic programming" (Bellman, 1966).

2-Local alignment aims to align the sequences which are dissimilar by identifying the regions which share similarities. It utilizes the tools EMBOSS water and LALIGN which works on the basis of Smith-Waterman algorithm. It counts the first row and column as "0" and unlike global alignment it does not offer any negative scoring.

Multiple Sequence Alignment (MSA) can be used to improve the accuracy of the alignment if the sequence chosen contains low homology. MSA can be performed using MUSCLE, T-Coffee, ClustalW, MEGA 7, and RStudio as well. MSA can align the sequences in a tree branching order placing the sequences with higher homology first and the lower homology ones after them. A characteristic example of MSA utilization was presented in the discovery of Alanine-Serine-Cysteine transporter (SLC1A5). The data at that time for SCL1 family protein for human was in lesser quantity due to less experimental evidence (Garibsingh et al., 2018).

Following the alignment, the next stage (third step) is to develop the model. This process begins by constructing the backbone, followed by the creation of loops, and finally addressing the side chains. To form the polypeptide backbone, it is important to copy the coordinates of protein residues from the template. However, it is crucial to accurately identify and remove any gaps, since failing to do so may result in their amplification during subsequent phases. This amplification might potentially lead to disruptions and structural changes in the final protein. Loop modelling and prediction is done by either of the two ways as:

1-Knowledge-based method utilizes the experimental data available on databases.

2-Energy-based method utilize the *ab initio* modeling for predicting protein folds for example identification of LPRK2 (leucine-rich repeat kinase 2) loop prediction (Guaitoli et al., 2016).

Lastly the side chains are constructed on the backbone and the model is optimized by the help of mechanic force field which enhances the model's quality. The side chains often exhibit restricted conformations (rotamers) and may be anticipated using methods such as SCWRL (Wang et al., 2008) to minimize the overall potential energy. After the model is completed, a ligand-based technique may be used to enhance the accuracy of the homologous model.

Homology modelling offers cost-effective and extremely precise findings; however, it does have certain limits. The main limitation of homology modelling is that it depends upon the availability of template. Moreover, accuracy of alignment possesses a high place in homology modelling (Hameduh et al., 2020).

### *Ab Initio* Protein Structure Prediction

*Ab initio* protein structure prediction is the technique for predicting the structure of the protein without using any template. It gives a way forward for filling in the space created by homology modelling *in silico* drug development. The approach relies on Anfinsen's thermodynamic hypothesis, which states that the three-dimensional arrangement of a protein with normal functioning is the one that has the lowest Gibbs free energy in the total system. The conformation of a protein structure is dictated by the intermolecular interactions that exist between the atoms. This highlights the significance of the arrangement of amino acids in a particular setting (Kuwata, 2020). It is classified in two categories as:

1. Physics-based methods like ASTRO-FOLDS and UNRES perform prediction on the basis of quantum mechanics instead of structural data.
2. Knowledge-based method utilizes the low-energy local structures which are checked experimentally and are available in fragment library.

Molecular dynamics refinement generally uses physics-based approaches. CASP (Critical Assessment of Protein Structure Techniques) evaluates protein structure prediction techniques. These approaches are expensive and only work for short protein structures. (Pearce, 2023).

Knowledge-based methods propose a cost-effective computational technique as the fragments of the sequences are picked from the available libraries and splice the 3D structure of these proteins. The selected sequences are then arranged, and the top fragments are chosen on basis of Monte Carlo sampling algorithm to generate a pool of structures having suitable local and global interactions. These selected sequences are called "decoys" (Zaman et al., 2020). In this method the protein conformation is simplified opened up and then top ranked fragments are chosen randomly to determine the torsion angle which will replace the previous in the protein chain. Based on metropolis criterion, energy of conformation is determined and those with lower energy are accepted. The whole process is repeated multiple times for different parts of protein structure i.e, sidechains, and loops until the design is complete. I-TASSER and QUARK are famous tools for knowledge-based *ab initio* approach (Pearce et al., 2022).

### Protein Model Validation

The predicted structure must be verified by validating their stereochemistry, rotational angles, bond lengths, and

torsion angles. This validation can be carried out using WHATCHECK and Ramachandran plots. Ramachandran plot checks the protein backbone dihedral angles ϕ (N—Cα) and ψ (Cα—C). It determines the quality of the model by assessing the torsion angles of the peptide conformation.

To determine spatial features including 3D conformation and statistical potential Verify3D can be used. Other tools for protein model validation are:

MolProbity
Iris
SWISS-MODEL
*Coot*

Moreover, in order to check experimental distance constraints cross linking mass spectrometry (XL-MS) can be used (O'Reilly and Rappsilber, 2018).

**Docking Based Virtual Screening**

Molecular docking is a method of discovering new drugs by calculating the binding affinity and suitability of a ligand to its receptor. As receptors are highly specific in binding to external or internal substances therefore an attachment of a nearly identical structure having higher affinity can lead to the blocking of that receptor or modify its working physiology (Fan et al., 2019). As both the ligand and receptors are considered dynamic entities and have a very crucial role in ligand-receptor binding. There are three main objectives of molecular docking:

1) Predicting the binding mechanism of a ligand to the receptors.
2) Virtual screening is used to uncover a new and promising therapeutic candidate from a collection of small compounds.
3) Prediction of binding affinity of ligands and receptors based on the scoring functions (empirical, semi-empirical, force field and knowledge based) (Torres et al., 2019).

Some common programs used for molecular docking and their working principles are elaborated in Fig. 3.

For docking based screening of the drugs there are various steps as: Binding site identification, Receptor Flexibility, Protein/Receptor Flexibility, and scoring function.

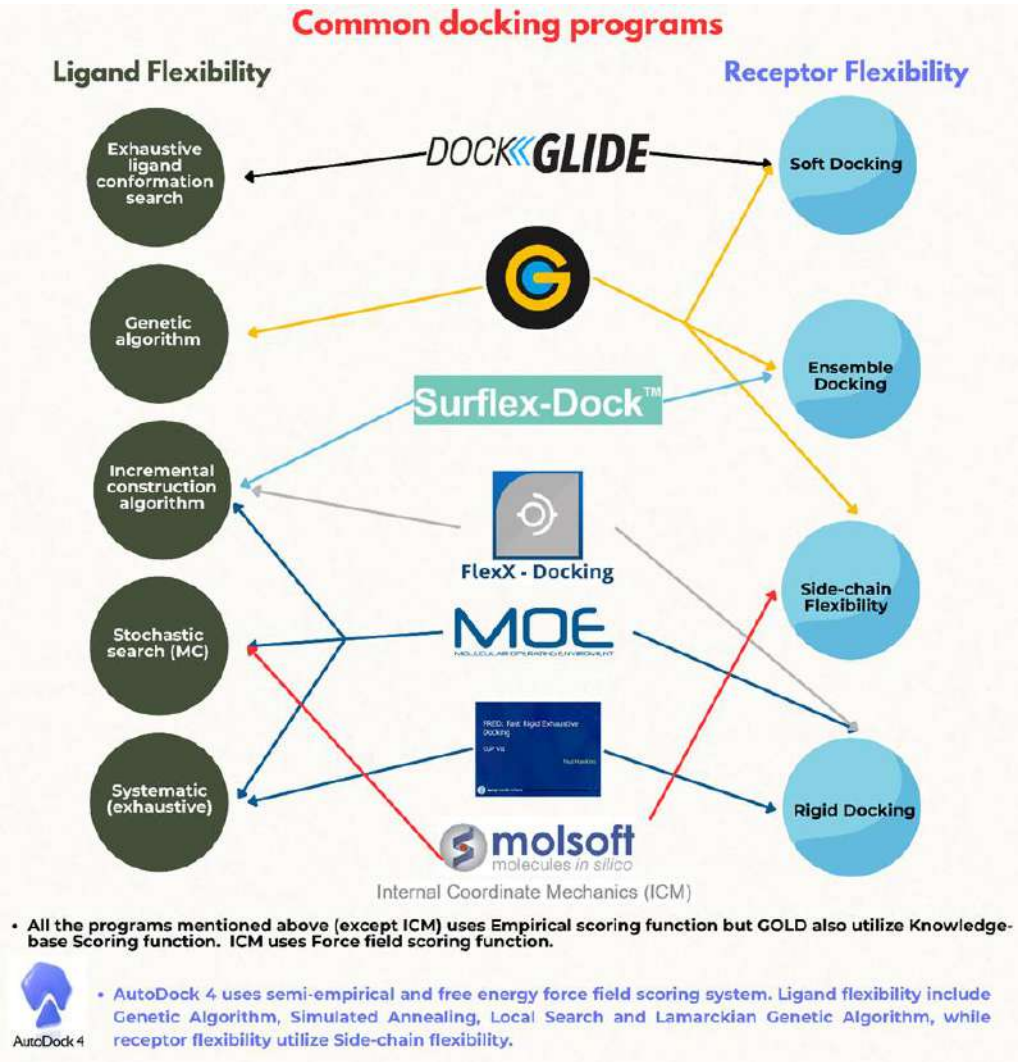



**Fig. 3:** Some common docking programs and their working functions.

**Binding Site Identification**

The location of a binding site for ligand attachment on receptor or protein is one of the most essential steps of molecular docking. Most of the structures available on the databases i.e, PDB are holo structures (Ligand-bound) having both molecular pockets and geometries well defined. However, in the case of apo structures (Ligand free), traditionally three methods are used to identify binding sites.

1) Template-based method (firestar, 3DLigandSite, and Libra); uses protein sequence to identify residues and conserved regions of a protein.
2) Geometry-based method (CurPocket, Surfnet, and Sitemap); identify the size and depth of the cavities to select the clefts and the pockets.
3) Energy-based method (FTMap and Q-SiteFinder); examine the energetically favorable binding sites present on the surface of a protein.

These programs facilitate the identification of vital information pertaining to orthosteric binding sites, prospective allosteric binding sites, hot spots on protein surfaces (which may affect protein-protein interactions) and enable additional analysis of previously discovered binding sites to develop more effective medications. Identification of hidden binding pockets (Cryptic pockets) provide a solution to make the target proteins druggable which were once considered undruggable (Vajda et al., 2018).

**Ligand Flexibility**

Databases including ZINC, DrugBank, ChemBridge, and Enamine can be used to obtain the small molecule structures (Ligand structures). There are several ways of performing conformational sampling of ligands:

- **Systematic search**, i.e, Exhaustive search uses brute force analysis but can cause trouble in computing hence, rule-based approaches are preferred.
- **Rule-based method,** i.e, Incremental construction algorithm produces conformation on the basis of identified structural preferences. Torsion angles and ring conformations sampling mostly breaks the conformations which are joined to result a low energy conformation molecule.
- **Stochastic search** utilizes the probabilistic criterion to select or accept the randomly changed degree of freedom of ligand. There are different search methods in stochastic search i.e, Monte Carlo (MC) sampling, genetic algorithms-based sampling, and distance geometry sampling (Shukla and Tripathi, 2020).

**Protein Flexibility**

Protein flexibility is an essential parameter to check the biological function and subtle changes which can be done by the docking results. There are 4 types of methods for handling protein flexibility:

**1) Soft docking:** Allows the overlapping between protein and ligand to a smaller degree in docking calculations by relaxing the inter-atomic vdW interaction.
**2) Side chain flexibility:** Keeps the protein backbone fixed and only allows sampling of side chains by changing their confirmation in essential torsion degree of freedom**.**
**3) Molecular relaxation method:** The first step involves using stiff protein docking to position the ligand into the binding site. Subsequently, the protein's structural framework becomes more relaxed, while adjacent side chains are often manipulated using techniques like Monte Carlo (MC) and Molecular Dynamics (MD).
4) **Protein ensemble docking** Align the ligand with a set of fixed structures of proteins that exhibit different conformations, enabling the flexible receptor to be properly represented. The docking findings are acquired for each validation individually and then reevaluated (Harmalkar and Gray, 2021).

The water molecules pose a great challenge in protein-ligand docking and requires extra computational efforts to optimize the results.

**Scoring Function**

Once all the possible binding modes are identified their quality is evaluated by the help of a scoring function. These functions estimate the binding energy and mode to rank the potential drug candidates. Force field-based, Empirical-based, and Knowledge-based approaches are the primary classifications of scoring functions that use distinct algebraic formulas to evaluate the modes (Li et al., 2019). The equations of the scoring functions are presented in Fig. 4.

**Ligand-Based Drug Design (LBDD)**

If the information related to protein structure is not enough, active ligand is used to highlight the important features which becomes the bases of biological activity. The ligand-related data is applicable in ligand-based drug designing. The SBDD and LBDD are mostly integrated to get better results for hit identification during virtual screening (Vázquez et al., 2020). Some important ligand-based drug designing methods are:

**Similarity Search**

Similarity search follows the hypothesis that structurally similar molecule shares similar physical and biological properties and activities. Structural representations and their similarity's quantitive measurement are two main points for similarity search. The mathematical equation for quantification of similarity in molecules is given in Fig. 5.

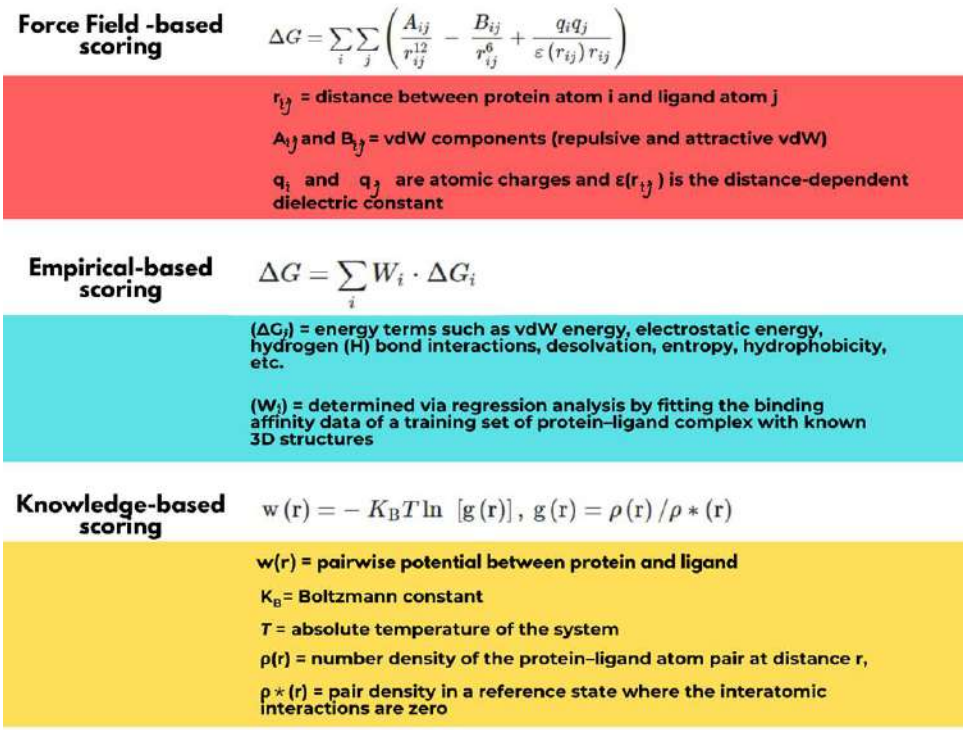
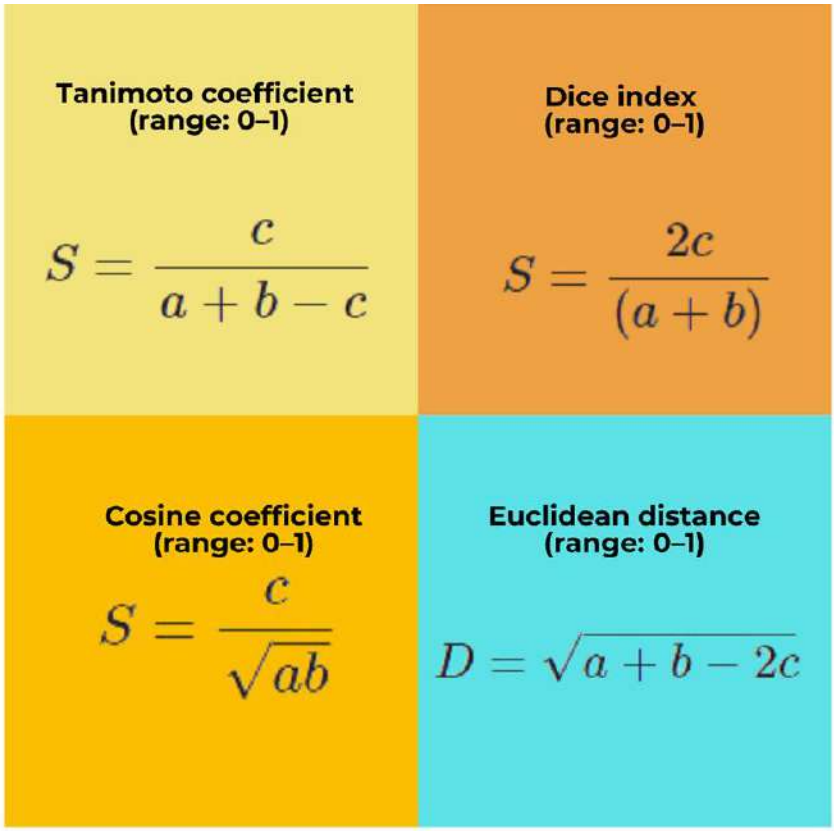



**Fig. 4:** Mathematical equations for Scoring Functions




a is the number of bits present in molecule A, b is the number of bits present in molecule B and c is the number of bits present in both molecule A and B. S denotes similarities and D denotes distances where S=1/1+D.

**Fig. 5:** Mathematical equations for quantification of Similarity in molecules.

Various Molecular Fingerprints are used to elaborate the structure of the molecule while their measurement depends on 1D, 2D, and 3D descriptors. Molecules are divided into sequence of bits and common bits are assessed to find out the similarity between molecules. Molecular fingerprints includes:

- Topographical fingerprints
- Strutural keys
- Circular fingerprints

- Pharmacophore fingerprints (Gao et al., 2020)

These fingerprints are used for synthetic compounds mostly however, for natural compounds "Natural Compound Molecular Fingerprints" (NC-MFP) are utilized (Seo et al., 2020).

Assessment and quantification of the similarity betwixt two molecules (A and B) can be done by various metrics. These metrics give the value between 0 and 1.

The most common example of its application is the identification of CDK8 inhibitors which employed W-18 and W-37 molecules and found WS-2 molecule in return as potent agent (Wang et al., 2017).

**QSAR Modelling**

Quantitative Structure-Activity Relationship (QSAR) model is the one that derive the correlation between experimental determined activity and calculated properties of a group of molecules. 1D-QSAR and 2D-QSAR are considered as "Classical" QSAR methodologies while 3D-QSAR is the advanced type of modelling.

1D-QSAR: aims to correlate biological activities and molecular properties

2D-QSAR: it correlates biological activity with ligand structure (2D) and also utilize descriptors.

3D-QSAR: it considers the three dimensional spatial presentation of molecules. It was used for producing NR3C1 as a target for maslinic acid analogue (Alam and Khan, 2017).

**Pharmacophores and their Validation**

Pharmacophores are the molecular frameworks that have essential characteristics for biological activity of a drug. Presently these are the ligand's electronic and steric characteristics in its 3D spatial arrangement. There are six classical pharmacophores;

1) Hydrogen bond donors
2) H ydrogen bond acceptors
3) -ve ionic pharmacophores
4) +ve ionic pharmacophores
5) Hydrophobic regions
6) Aromatic regions

It was used in the identification of *Mycobacterium tuberculosis* MurG inhibitors (Saxena et al., 2018).

Before using pharmacophore model its validation is performed on the basis of Yield of actives, Sensitivity, Specificity, Enrichment factor, Goodness of hit list (*GH score*). After model validation small molecule databases are screened against it and the compound with relevant features is taken as a "hit compound".

**Scaffold Hopping**

It identifies the iso-functional molecular structures which have significantly altered molecular backbone. Scaffold hopping utilizes an active compound and replaces its "Cores" resulting in a compound with similar biological activity. The changes and alterations can range from minor to major extensive modifications and are classified into 1°, 2°, 3°, and 4° (Acharya et al., 2023). It was employed in the identification of new indolopyrazinoquinazolinone scaffold 2 (Wang et al., 2019).

**De novo and Fragment based Drug Design**

De novo drug designing is termed as the production of novel compounds having required characteristic physicochemical and pharmacological properties. A clear target constraint must be identified before performing De novo drug design. For example, the production of Carbonic anhydrase was one of the milestones achieved by the said technique.

Traditionally this method used atom drug design which gave highly diversified results for new molecules, but current approaches employ the fragments of the compounds which provide ease for synthesis but lacks diversity (dos Santos Nascimento, 2023).

**Hierarchical Virtual Screening**

HVS (Hierarchical Virtual Screening) is termed as the use of both SBDD and LBDD techniques for finding the hit compounds. It employs the strengths of both the methods in a stepwise way leading to the screening of molecules and compounds generating lead compounds with desired properties (Fig. 6). For example the discovery of rocaglamide derivatives to treat skin infections (Dos Santos et al., 2022).

**Molecular Mechanical/Generalized Born Surface Area (MM-GBSA)**

MM-GBSA is a robust methodology used to determine the binding free energy by evaluating the disparity in the free energies across different constituents of the system, such as proteins, ligands, and complexes, the equations for derivation are given in (Fig. 7). It works on the principle of induced fit model and treat the protein and ligand as flexible entities; therefore, it is considered more precise but an expensive method (Salo-Ahen et al., 2020).

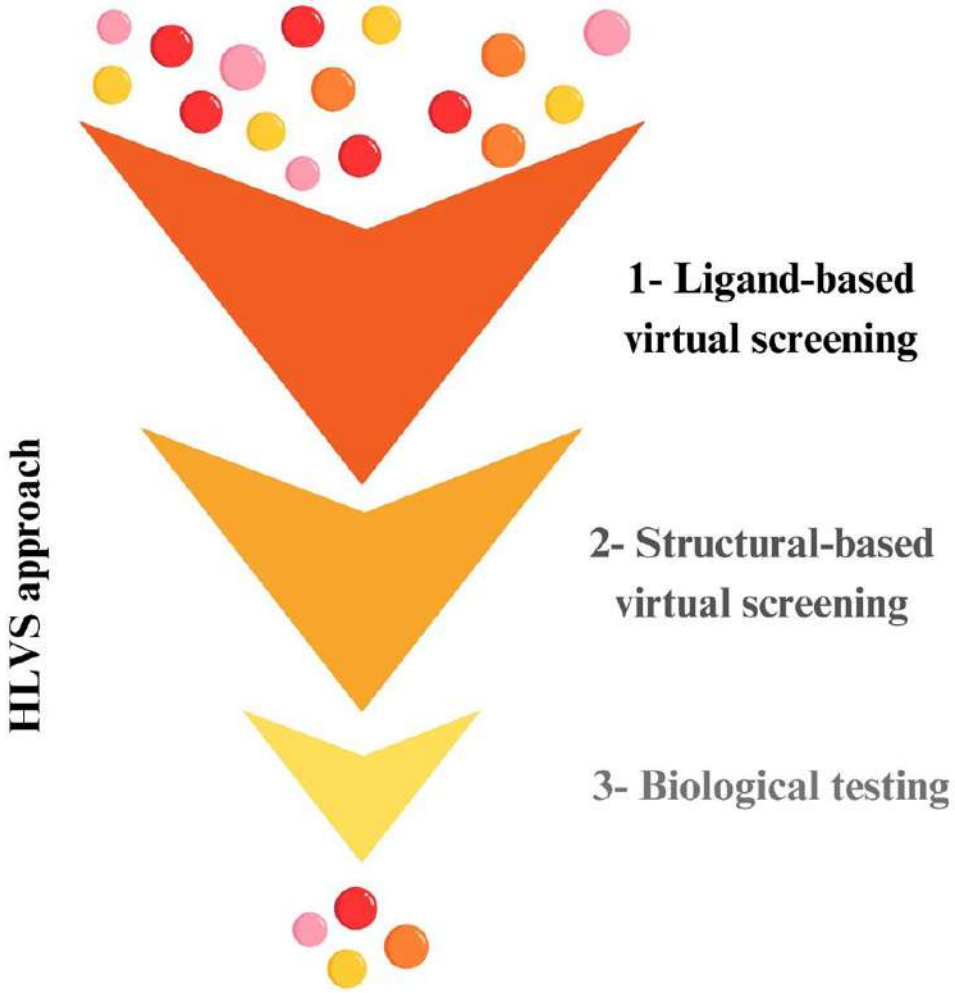



**Fig. 6:** HLVS approach for screening compounds.

**Equation for Estimation of Total Binding Free Energy**

$$\Delta G_{bind} = E_{complex} - E_{ligand} - E_{receptor}$$

$$\Delta G_{bind} = \Delta H - T\Delta S = \Delta E_{MM} + \Delta G_{sol} - T\Delta S$$

$$\Delta E_{MM} = E_{int} + \Delta_{ele} + \Delta E_{vdW}$$

$$\Delta G_{sol} = \Delta G_{PB/GB} + \Delta G_{SA}$$

$$\Delta G_{SA} = \gamma SASA + b$$

$\Delta E_{MM}$ = Change in the gas-phase molecular mechanics (MM) energy
$(E_{int})$ = Internal energy
$\Delta_{ele}$ = Electrostatic energy
$\Delta E_{vdW}$ = vdW energy
$\Delta G_{sol}$ = Sum of electrostatic solvation energy
$\Delta G_{PB/GB}$ = Polar contribution
$\Delta G_{SA}$ = Non-polar contribution
SASA = Solvent accessible surface area
$\gamma$ = Surface tension constant
$b$ = Correction constant
$-T\Delta S$ = Conformational entropy calculated by normal mode analysis

**Fig. 7:** Equation for the derivation of Total binding free energy.

**Molecular Dynamics (MD)**

Molecular dynamics is a highly useful and smart tool for assessment of mechanism of action of pathogenicity/therapeutic effect. The technique has improved in the area of algorithms and force field parameters. MD assesses the effect of each individual particle and its motion in a model system similarly, in pharmaceutics it assesses the mutations, phosphorylation, ligand bindings and protonation effects on proteins. Majorly MD provides information about the dynamic structures of biomolecules and accurate energy values of ligand-receptor complex hence help in identification of hit compounds (Tuccinardi, 2021).

**QM/MM and DFT Approaches**

One of the most important factors in drug designing and discovery is its pharmacodynamic studies. Pharmacodynamics is termed as the body's response towards an administered drug. Quantum mechanics and molecular mechanics provide an estimation of the pharmacodynamic (ADME) properties of a drug. The molecular mechanic technique utilizes molecular descriptors and electronic interactions of a system. While QM provides more practical results

which are highly congruent to theoretically derived results. Quantum mechanics (QM) analyses the positioning of bindings and provides an assessment of the energy for both natural systems and drug-receptor complexes. MM is less expensive than QM and DFT (Density Functional Theory) (Fig. 8) however, all these are used to analyze smaller areas of a protein i.e, binding site (Tzeliou et al., 2022).

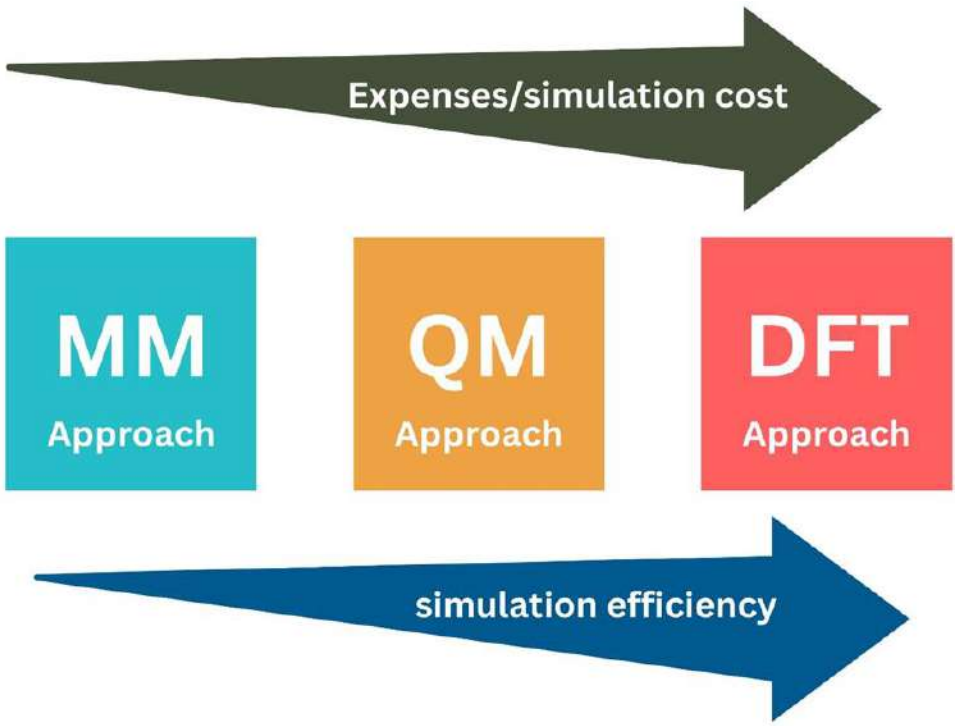



**Fig. 8:** Efficiency and cost comparison of MM, QM, and DFT approach

**Revolution of Artificial Intelligence and Machine Learning in Drug Design**

Since the invention of machine learning (ML) and artificial intelligence (AI) the scientific world has got a new outlook on the world. AI assists scientists and researchers with the elaboration of chemical structures, synthesis of ligands, tissues and organ targeting capacity of compounds, development of models, and 3D structure generation of proteins. AI and ML can assist the process by identifying and short listing the novel or alternative compounds for a disease (Fig. 9). The algorithms can predict an error free ligand-protein interaction thus assisting the researchers with precise modelling and shortlisting of compounds. In short, these techniques should be focused on the integration of chemical and physiological principles of the body and drug manufacturing for a precise and accelerated decision and triaging of compounds (Peña-Guerrero et al., 2021).

The only challenge here with these technologies is the diversity of data and rules which need to be combined with the algorithms to facilitate decision making process.

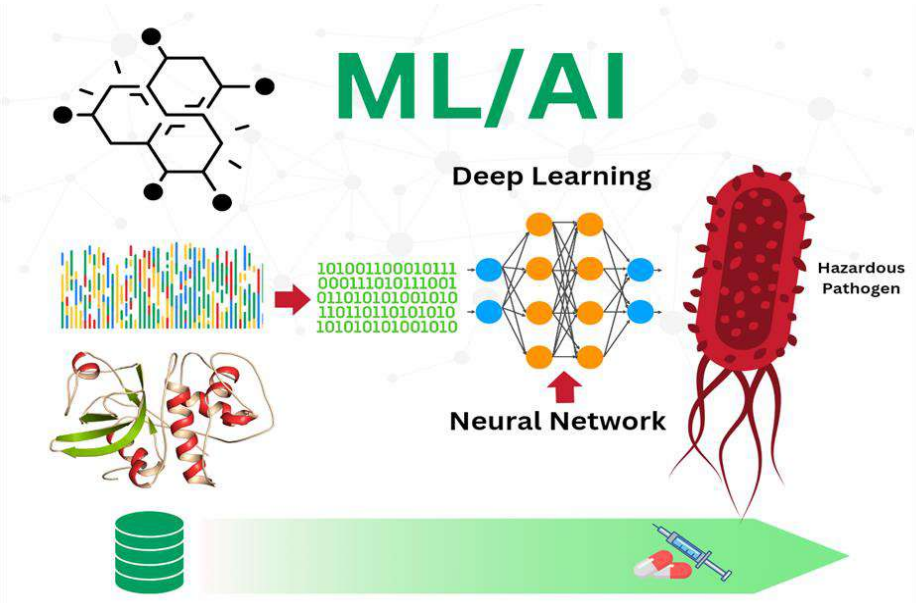



**Fig. 9:** ML and AI techniques for prediction of drug design.

**Conclusion**

*In silico* drug design and discovery techniques are highly useful and trouble-free tools which enable the users to find out the most desirable drug hit for development. These tools allow the researcher to reduce the time, cost, labor, and laboratory use. CADD has led to the discovery of very useful compounds for highly pathogenic and important ailments. SBDD and LBDD are most utilized techniques for development of drugs up till now however, their integration with the AI and ML can help the researchers to develop new compound in a less expensive and hassle-free scenario. The ADMET prediction techniques allow the scientist to short list the drugs which are less likely to cause any issues in the clinical trials leading to the better adjustment and market survival of the novel entities. However, the large amount of data in this big data era is the uphill challenge for drug discovery and their development. The grip on this huge amount of knowledge is the only key to finding out more suitable and precise techniques for development. This issue can be tackled with artificial intelligence, machine learning, and deep learning technology. Although the need of clinical trials is essential and hold a high place in the process, CADD and *in silico* tools are extremely useful and provide a modern solution for streamlining drug discovery process.

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Chapter 13

# Use of Nanoparticles in Diabetes Treatment

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## ABSTRACT

Increased concentration of sugar in plasma is known as diabetes and it is divided into two types: type 1 and type 2 diabetes. Several limitations have been identified in the methods of conventional treatment for diabetes. The nano-particles have been actively used for overcoming such limitations in diabetes treatment. The applications of nano-particles in the treatment of diabetes include the use of nano-particles for insulin delivery, diabetic retinopathy, diabetic cardiomyopathy, peripheral neuropathy, and diabetic foot ulcers. Recent advances also include the use of zinc oxide nanoparticles in the treatment of diabetes, the use of nanotechnology in the regeneration of islet beta cells, and the use of nano-particles in diabetic wound healing. The nanotechnology for treating diabetes has been proven to be more efficacious than conventional methods. This chapter highlights the introduction of diabetes mellitus and nanoparticles, limitations of conventional methods of treating diabetes, applications of nanoparticles in diabetes mellitus, and future perspectives.

**KEYWORDS**
Diabetes mellitus, Nano-particles, Nanotechnology, Insulin, Beta cells

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## INTRODUCTION

### A Brief Overview of Diabetes

The raised levels of plasma sugar are a hallmark of Diabetes Mellitus, which is a chronic metabolic illness. Type 1 diabetes results from the pancreas producing insufficient insulin, whereas type 2 diabetes arises from the body's inability to respond to the insulin produced by the pancreas properly. In lower-middle-income nations, nine countries list diabetes among their top 10 leading causes of death, while in higher-middle-income countries, six nations do so, and in high-income countries, ten countries do the same. Globally, about 422 million persons suffered from diabetes mellitus (DM) in 2014, based on data provided by the World Health Organization (WHO). The incidence of DM is predicted to continue rising. By 2040, projections from the International Diabetes Federation (IDF) suggest that the figure will rise to 642 million (Dragan Lovic1, 2020).

### Categories

There are three primary forms of diabetes mellitus (DM). Type 1 DM (T1DM of unknown cause) is characterized by an insulin shortage and requires lifelong insulin variant injection for therapy. It is alternatively referred to as juvenile or childhood-onset diabetes or insulin-dependent diabetes. Type 2 diabetes (T2DM), often termed adult-onset and noninsulin-dependent diabetes, stems from insulin resistance, which is the body's failure to appropriately respond adequately to the produced insulin (Fig 1). The state of high blood glucose during pregnancy in women who have never had diabetes before comes last: gestational diabetes. In this instance, there is a significant chance that the child may grow up with DM (Kaul et al., 2013).

### Epidemiology (Disease Distribution and Transmission)

The main drivers for rising planetary incidences of type 1 diabetes which is less frequent are yet unknown. It is believed that environmental changes are altering diabetes-associated alleles, with geographic and ethnic variations in type 1 diabetes incidence and prevalence serving as the main contributing factors. The rates are lowest in Japan and the Pacific region and highest among Caucasians. Type 1 diabetes is not limited by age; however, approximately 50–60% of cases manifest before reaching the age of 20 (Baynes, 2015).

Ninety percent of those with diabetes have type 2, which is the most prevalent variety. Similar to type 1 diabetes there are significant regional and ethnic differences, although obesity is the main factor contributing to the rising incidence (Baynes, 2015).

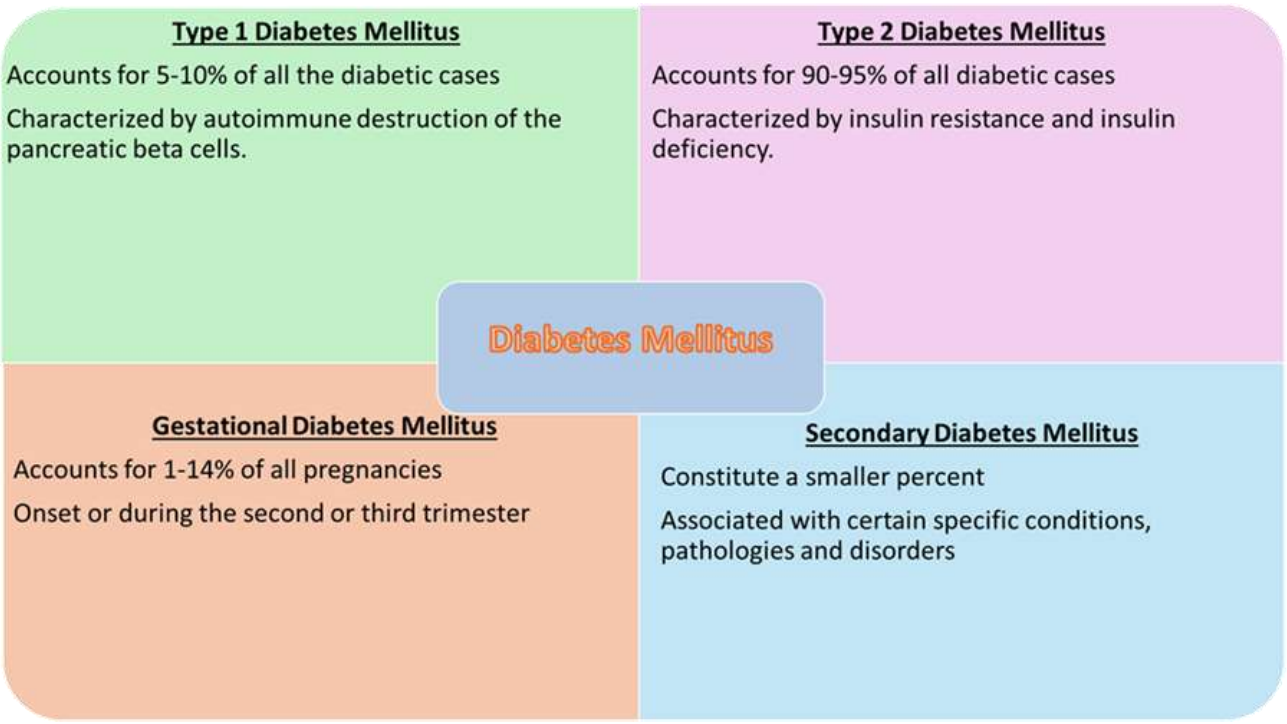


**Fig. 1:** The image above shadows the types of diabetes and their prevalence around the globe along with the characteristics of each type. The major portion of the diabetic population suffers from type 2DM but it can be prevented with lifestyle modifications along with pharmacological treatment. The most dangerous type is type 1 DM as it is not preventable with lifestyle modifications but needs lifetime insulin administration for survival.

Estimates of the prevalence of obesity in more obese societies fluctuate between 6-8% in the UK to 50% in the Pima Indians of Arizona. Research conducted on immigrants worldwide has shown that well-nourished populations face a risk of type 2 diabetes that is 2–20 times greater than lean populations of the same racial background (Baynes, 2015)

**Etiology**

Insulin insufficiency, which results from the autoimmune destruction of pancreatic β cells and leads to type 1A diabetes, is the cause of T1DM. Other underlying reasons for their insulin secretion abnormalities are hereditary. The deficiencies in glucose sensing by β cells along with other genetic and acquired disorders contribute to the condition (Yau et al., 2021).

Malfunctions in how insulin interacts with its target tissues (muscle, liver, and fat) result in type 2 diabetes mellitus (T2DM), a condition that can be exacerbated by various factors and often increase the beta cells' ability to secrete insulin diminishes, while the majority of patients with type 2 diabetes mellitus in the United States and Europe are overweight or obese (Yau et al., 2021)

**Diagnosis:**

**Table 1:** Criteria for diagnosing diabetes. Based on plasma glucose levels, diabetes can be diagnosed using either the 2-hour plasma glucose (2-h PG) value or the fasting plasma glucose (FPG) value from a 75-g oral glucose tolerance test (OGTT). This criteria also gives information on prediabetes glucose levels. (Phillips, 2012)

| Parameter | Normal tolerance | Glucose impaired Tolerance | Glucose Diabetes Mellitus |
|---|---|---|---|
| Plasma glucose levels during fasting mg/dl (mmol/L). | Less than 100(5.6) | 100-125(5.6-6.9) | Equal to or greater than 126(7.0) |
| Two hours post-glucose administration mg/dl(mmol/L) | Less than 140(7.8) | Equal to or greater than 140-199(7.8-11.0) | Equal to or greater than 200(11.1) |
| HbA1c | Less than 5.7 | 5.7-6.4 | Equal to or greater than 6.5 |

**Oral Glucose Tolerance Test**

In this test, 75 or 100 grams of glucose solution should be consumed for the usual glucose tolerance-3 test (Phillips, 2012)

**Complications**

In the following section, complications and prevention of diabetes mellitus are described (Tripathi and Srivastava, 2006)

**Acute Complications**

Among these are non-ketotic hyperosmolar condition (NKHS) and diabetic ketoacidosis (DKA). Type 1 diabetes primarily leads to the former, while type 2 diabetes primarily causes the latter.

**Chronic Complications**

- Diabetic retinopathy
- Neuropathy
- Nephropathy
- Hypertension
- Infections
- Cardiovascular disease

**Indications and Expressions**

- Blurred vision
- Fatigue
- Increased thirst or polydipsia
- Increased appetite or polyphagia
- Elevated blood sugar or hyperglycemia
- Frequent urination or polyuria
- Weight loss

**Management and Prevention**

Type 1 diabetes is not preventable.

People may, however, take a few precautions to help avoid type 2 diabetes. Among the strategies to help avoid type 2 diabetes are;

- Keeping a healthy weight
- Maintaining a healthy diet with minimal intake of processed foods, saturated fats, and added sugars, high fiber low fat content, smoking and alcohol cessation, minimal intake of artificial sweeteners,
- Engaging in regular exercise
- Medical treatment (management)

**Nanoparticles**

The nanoparticles are novel dosage forms with differing compositions and properties from their respective counterparts. The nanoparticles are particulate dispersion or solid particles in a size range of 10-1000 nm (Biswas and Wu, 2005).

Nanoparticles are the basic components of nanotechnology. The nanoparticles possess unique properties due to their small size such as stability, surface area, high reactivity, strength, and sensitivity. On the nano-metric scale, nanoparticles are categorized into organic nanoparticles which include dendrimers, liposomes, and micelles the others are inorganic nanoparticles which include metal and metal oxide-based nanoparticles, and carbon-based particles. The nanoparticles are of different dimensions, sizes, shapes, and structures. The nanoparticles can either be of zero dimension, one dimension, two dimension, or three dimensions. They exist in spherical, hollow core, cylindrical, tubular, spiral, and conical, flat, or irregular shape. The nanoparticles can be amorphous or crystalline having uniform or rough surfaces. Depending on the method of preparation, drugs can be entrapped, encapsulated, dissolved, or attached to a nanoparticle matrix. The drug release rate can be controlled by nanoparticles, nano-capsules, and nanospheres preparation. In nano-capsules, the core of the drug is surrounded by a specific polymer membrane. At the same time, nanospheres are homogeneous systems in which the drug is uniformly dispersed within the polymeric matrix. The nanoparticles synthesized from chemical methods have toxic effects due to the adherence of toxic chemicals on the surface. Preparation of nanoparticles by using microorganisms is an eco-friendly alternative to chemical and physical methods. Currently, metallic nanoparticles like silver, copper, zinc, gold, titanium, magnesium, and gold are prepared using different microorganisms (Mohanraj and Chen, 2006)

Therapeutic and diagnostic nanoparticles have two categories: 1) Organic nanoparticles and 2) Inorganic nanoparticles. Inorganic nanoparticles have been used successfully for preclinical studies whereas organic nanoparticles have been used successfully in various applications ranging from vaccination to long-lasting depot delivery systems.

**Methods of Preparation**

The following methods are most frequently used for nanoparticle preparation:

- De-solvation
- Dialysis

- Ionic gelation
- Nano-precipitation
- Solvent evaporation
- Salting out
- Spray drying
- Supercritical fluid

Other methods we can use to prepare nanoparticles include Vacuum deposition and vaporization, Gas condensation, Chemical vapor condensation (CDC), Chemical Vapor Deposition (CVD), Chemical precipitation, Mechanical attrition, Sol-Gel techniques and electro-deposition. All these methods are widely used in the preparation of nanoparticles.

**Advantages**

The major functions of this novel drug delivery are to control the particle size, control the release of drug at a predetermined rate, site-specific targeting, sustain the release of the drug at auction site to acquire increased therapeutic efficacy and reduced side effects, surface characteristics can easily be manipulated to achieve active and passive targeting after parenteral administration.

**Limitations**

Nanoparticles do have limitations:

- The nanoparticle dosage form leads to particle-particle aggregation due to small size and large surface area.
- Due to the small size, handling of nanoparticles becomes very difficult.

The small size of nanoparticles causes their clearance by the reticuloendothelial system through opsonization.

**Applications**

The nanoparticles have significant applications in various fields including food, cosmetics and sunscreens, electronics, catalysis, construction, renewable energy, space exploration, transportation, medicine, and bioengineering.

**Limitation of Conventional Drug Delivery System**

Currently, the medications utilized in the clinical management of diabetes may encompass insulin as well as non-insulin oral hypoglycemic agents. The traditional diabetes treatment using antidiabetic medications can lead to hypoglycemia, posing significant risks to patients including behavioral and cognitive disturbances, seizure and brain damage, and potentially fatal outcomes. Hence, there is a critical need for the development of an advanced drug delivery system capable of achieving sustainable and controlled drug release (Zhao et al., 2020).

**The Limitations of Conventional DDS**

The medication must be administered at a precisely controlled rate and directed to the target site with utmost accuracy to achieve optimal efficacy and safety. The traditional antidiabetic medication's failure to accumulate at the intended site could potentially induce severe side effects to other organs because they have limited specificity for the target site. Furthermore, the traditional dosage forms cannot intelligently adapt to the wide fluctuations in glucose concentration, leading to a heightened risk of hypoglycemia (Zhao et al., 2020).

The diverse pH, environment and digestive enzymes serve as primary biochemical obstacles for oral drug delivery systems. The pH level fluctuates significantly throughout the gastrointestinal tract, gradually increasing from stomach to the colon ranging from 1-8. This transition from acidic to alkaline environments influences drug efficiency and bioavailability (Lou et al., 2023). The desired therapeutic concentration of antidiabetic drugs cannot be effectively achieved in specific target areas due to their susceptibility to chemical instability and proteolytic degradation in the harsh physiological environment (Zhao et al., 2020).

In the management of diabetes mellitus, the primary method of treatment often involves administering insulin via subcutaneous injection. However, patient adherence to this approach is often compromised due to concerns related to discomfort, trauma, pain, and the risk of local infection, skin necrosis, and nerve damage. To address these challenges, nanoparticles have emerged as potential carriers for insulin, offering alternative, more patient-friendly routes of administration that eliminate the need for injections (Souto et al., 2019).

The limited effectiveness of oral insulin administration stems from its low bioavailability and inadequate therapeutic impact, largely due to rapid chemical clearance. When insulin is ingested orally it encounters various obstacles in the gastrointestinal tract including acidic conditions and enzymatic degradation which can degrade the insulin molecules before they can be absorbed into the bloodstream. Additionally, the large size and hydrophilic nature of insulin molecules make it challenging for them to pass through intestinal epithelium and enter systemic circulation effectively. As a result, only a small fraction of ingested insulin enters into the bloodstream leading to rapid clearance (Souto et al., 2019).

Various strategies have been explored to ensure plasma drug concentrations remain above the minimum effective concentration while avoiding toxicity. One such approach involves administering multiple doses at regular intervals. However,

repeated dosing with conventional drug delivery systems can result in poor patient adherence (Adepu and Ramakrishna, 2021).

**Role of Nanoparticles in the Diagnosis of DM**

The nanocarrier-mediated drug delivery is a method of administering drugs that has several advantages over traditional methods. It offers selective targeting, enhanced cellular intake, and accumulation, improved stability, prevents offsite degradation, and prolongs the active agents' half-life in the bloodstream. This approach enhances the therapeutic efficacy of pharmaceutical agents at the intended site while minimizing off-target normal cytotoxicity (Debele and Park, 2022).

The nanoparticles (NPs) have unique biological, physical, optical, chemical, and magnetic properties that make them valuable for early disease detection and prevention. For example, their small size and specific binding ligands on their surfaces make NPs useful as imaging probes for diseased tissues or organs. Additionally, NPs can be tailored to have longer circulation times and better targeting in the body by adjusting their size, surface charge, and other properties. This makes them useful as contrasting agents for various biomedical imaging techniques, such as magnetic resonance imaging (MRI), for early diagnosis of diseases like diabetes. The NPs have a high surface-to-volume ratio, making them an ideal matrix for enzyme immobilization. This enhances enzyme-substrate interactions and enzymatic activity. Various materials, such as magnetite NPs, gold NPs (AuNPs), and carbon nanotubes, have been investigated for their potential use as matrices for enzyme immobilization. (Debele and Park, 2022).

The integration of NPs into sensors offers several benefits, including increased surface area, better electron transfer from enzyme to electrode due to excellent conductivity and small bandgap, improved stability, and the ability to incorporate additional catalytic steps. In the field of diabetes sensors, nanotechnology is commonly used to enhance standard enzymatic electrochemical glucose detection or for direct detection of glucose oxidation at an electrode, also known as non-enzymatic glucose sensors (Debele and Park, 2022).

**Application of Nanoparticles in Insulin Delivery**

The development of insulin through the use of biocompatible and biodegradable nano-carriers is crucial. The nano-carriers help to protect insulin from stomach acid and enzymatic degradation, thus improving its effectiveness in the intestines. The uptake of nanoparticles depends on various factors such as surface charge, shape, size, muco-adhesive properties, and the method of administration. Furthermore, the characteristics of pharmaceutical agents, such as hydrophobicity, molecular weight, pH stability, and ionization constants, also play a significant role in the cellular uptake. Understanding these mechanisms is necessary for creating effective delivery systems for oral protein drugs. Currently, researchers are focused on optimizing nano-carrier design to protect macromolecules from the gastrointestinal environment and prolonged intestinal permeation. This could potentially enhance the effectiveness of insulin (Debele and Park, 2022).

**Nanotechnology and Diabetic Complications**

**Diabetic Nephropathy (DN)**

The major reason for nephropathy is usually diabetic nephropathy (DN). There is no satisfactory preventive measure and treatment available for DN. The pathogenesis of DN is complicated, various factors and conditions exaggerate the occurrence and condition of DN, which include: glucose imbalance metabolism, endothelial nitric oxide synthase production, inflammatory reactions, along renal hemodynamic changes. Therapeutic drugs for DN significantly reduce the production of renal fibrosis factors such as TGF-β1 and fibronectin as well as inflammatory cytokines including MCP-1 and TNF-α. ("Nanotechnology in the Treatment of Diabetic Complications: A Comprehensive Narrative Review," n.d., #)

**Diabetic Retinopathy (DR)**

With an expansion in patients with diabetes, diabetic retinopathy is one of the major reasons for blindness. The combination of retinal damage, thickened basement membrane, blocked micro-vessels, and impaired blood-retinal barrier function lead to retinal edema and neovascularization. The silicate nanoparticles developed by some researchers showed anti-angiogenesis effects on retinal vein occlusion by inducing the formation of the protein (VEGF) vascular endothelial growth factor. ("Nanotechnology in the Treatment of Diabetic Complications: A Comprehensive Narrative Review," n.d., #)

**Diabetic Cardiomyopathy (DCM)**

Some studies showed that cardiac myopathy is due to the formation of free radicals that cause injury to cardiac muscle cells due to diabetes mellitus which leads to abnormal myocardial diastolic function. The common pathological features of cardiomyopathy are myocardial fibrosis and apoptosis. The PSS-loaded nanoparticles made with the help of synthetic polymer Poly (lactic-co-glycolic acid (PLGA) have shown significant improvement in DCM with improving cardiac diastolic and systolic function and ventricular wall motion. ("Nanotechnology in the Treatment of Diabetic Complications: A Comprehensive Narrative Review," n.d., #)

**Peripheral Neuropathy (DPN)**

The peripheral neuropathy is a chronic disease and a major reason for foot ulcers and amputation. Microvascular

changes and glucose metabolism disorder, deficiency of nerve growth factors, and vitamins are considered the factors of pathogenesis in DPN. The short-term treatment with curcumin nanoparticles showed a reduction in complications of DPN in T2DM. ("Nanotechnology in the Treatment of Diabetic Complications: A Comprehensive Narrative Review," n.d., #)

**Diabetic Foot Ulcer (DFU)**

The diabetic foot ulcers are a severe complication in diabetic patients and can result in amputation and death of the lower limb. The treatment of diabetic foot ulcers includes the establishment of a clear and optimal environment for the foot wound which facilitates its prompt healing. The process of wound healing is complex and involves various stages that must occur in a specific sequence. As such, the perfect wound dressing should be non-allergenic and non-toxic, while also maintaining moisture in the wound area, facilitating gas exchange, protecting the wound from microbes, and absorbing wound drainage.

The nano-technology has been extensively used in research related to wound dressing for diabetes with material like gold, chitosan, curcumin, and silver being extensively studied. There is an increasing interest in biopolymers that share similar structures, biocompatibility, and biodegradability with natural skin. Additionally, metal nanoparticles, such as silver and gold are suitable choices for the wound dressings, mainly due to their antibacterial properties and low toxicity levels.

The PLGA microspheres made with recombinant human EGF nanoparticles (NPS) embedded in wounds of diabetic rats have shown the fastest cure rate by increasing the proliferation rate of fibroblasts. ("Nanotechnology in the Treatment of Diabetic Complications: A Comprehensive Narrative Review," n.d., #)

**Application of Zinc oxide Nanoparticles in the Treatment of Diabetes Mellitus**

The use of zinc oxide nanoparticles (ZnO-NPs) is increasing day by day in a number of industrial products. The most popular metal, ZnO-NPs, have a wide range of biological applications due to their perfect biocompatibility, economics, and low toxicity. Zinc is widely recognized for its ability to maintain the structural integrity of insulin and play an effective role in the secretion of insulin from pancreatic cells. This is the reason that ZnO NPs are effective in the treatment of diabetes (Jiang and Cai, 2018).

A recent scientific study delved into the potential anti-diabetic properties of RSW extract, ZnO nanoparticles, and ZnO-RSW conjugate. To assess their efficacy, the researchers employed amylase and glucosidase inhibition assays. These tests allowed them to evaluate the ability of the aforementioned compounds to inhibit the activity of enzymes responsible for the breakdown of carbohydrates, which can lead to an increase in the blood glucose levels. The results of this study might have contributed to the development of new treatments for diabetes as -amylase inhibitors (Bayrami, 2018).

The results of one study revealed that only ZnO-NPs show high inhibition of pancreatic amylase. Low inhibition was shown by only RSW whereas the ZnO-RSW possesses a higher percentage of inhibition as glucosidase inhibitor. The superiority of conjugated ZnO-RSW nanoparticles is evident in their excellent performance against crude murine pancreatic glucosidase, outpacing the individual ZnO nanoparticles and RSW extract. Consider adopting these nanoparticles for enhanced activity against this enzyme (Kitture, 2015).

**Effect of ZnO NPs on Lipid Profile**

The combination of zinc and extract can significantly improve the body's ability to break down blood glucose, leading to a marked reduction in the lipid utilization and ultimately decreasing the risk of developing hyperlipidemia. What's particularly exciting about this is the potential correlation between the insulin-like action of ZnO-NPs and the plant extract. This promising mechanism could offer significant benefits if further studied. According to some studies, zinc may have a similar effect to a-blockers in the short-term treatment of dyslipidemic patients. A recent study examined the changes in HDL levels in different groups during a test period. The results showed that all groups that received treatment had a significant increase in their HDL levels. However, none of the treatment conditions resulted in a significant decrease in TG levels, except for the ZnO-NPs extract which showed a significant decrease in cholesterol levels (Bayrami et al., 2018).

ZnO-NPs exhibited antibacterial, antidiabetic, antioxidant and anticancer effects. Zinc is a primary trace mineral element and has an important function in the metabolism of lipids in the liver. It stimulates lipophagy in hepatic cells, decreasing lipid accumulation and promoting lipolysis. ZnO-NPs conquer liver fibrosis induced experimentally by thioacetamide and modulate liver enzymes via reducing oxidative stress (Kitture, 2015).

**Regenerative Medicines**

The diabetic regenerative medicine has contributed to the clinical management of diabetes mellitus and its associated problems, resulting in discoveries and advancements. Furthermore, the development of nanotechnology has given diabetic regenerative therapy a boost. The regeneration of islet β cells, retinal tissue, nerve tissue, and wound tissue cells can all be directed correctly by a nano stent. Conductive nanomaterials stimulate the formation of numerous distinct tissues. Countless other benefits and wound-healing properties of nanoparticles have resolved numerous possible challenges in the practical application of regenerative therapies (Matveyenko and Vella, 2015).

**Advances in the use of Nanotechnology in Islet and Islet β Cell Regeneration**

Two methods of supplementing β cells are conceivable because of the lack of insulin production induced by islet β cell function i.e., Replacement through the transplanting of islets or β cells from human embryonic stem (hESC) or induced pluripotent stem (iPSC) cells from cadavers. It happens via progenitor differentiation (neogenesis) or transdifferentiation of developed cell types.Utilizing biodegradable polymer biomaterials as drug carriers to create a nano-controlled medication system, nanotechnology offers targeted cell transplantation with good biocompatibility(Contera et al., 2020).

**The Process of Stimulating Natural Regeneration**

It takes place via progenitor differentiation (neogenesis) or the change of differentiated cell types (trans-differentiation). The research on pluripotent stem cells (embryonic stem cells (ESC) or inducible pluripotent stem cells (iPSC)) and allogeneic islets, particularly porcine islets, has been compelled by immunological rejection and a lack of organ donors. Using biodegradable polymer biomaterials as drug carriers to create a nano-controlled drug system, nanotechnology is targeted for cell transplantation that is both biocompatible and precise. It can enhance the differentiation, implantation, and survival of stem cells when combined with stem cells. Regenerative medicine is being aided by the use of nanomedicine, nanomedicine carriers, nano-contrast agents, and nanosensors of various kinds. The goal of islet cell transplantation is to permanently heal diabetes by using mesenchymal stem cells' limitless capacity for proliferation and creating insulin-like insulin production polymer implantation into the body (Garbayo et al., 2020).

Through this approach, the long-term survival of these cells has been established through successful encapsulation and transport that can shield them from dispersion, destruction, and immune attack. The PLA nanoparticles were used because they were non-toxic, had a long half-life, were easily biodegradable, and had good bio-compatibility (Gao et al., 2013).

Utilizing their small molecular weight, nanoparticles can seamlessly transport drugs and imaging agents through a variety of barriers, allowing them to be implanted into the body or regenerated islets or β cells to produce an accurate and clear image. In addition, nanoparticles can alter their surface groups, such as polyethylene methylation and methylation, which help them avoid being removed by macrophages and maintain a stable presence in the body. As such, monitoring islet function is essential (Wang et al., 2014).

Using biopolymers as its foundation, multifunctional nanoparticles (NPs) are engineered as specialized nano tools to monitor the functionality of transplanted tissues and cells. The primary focus of their actions is the β cell membrane-expressed glucagon-like peptide 1 receptor, or GLP-1R. One naturally occurring ligand for GLP-1R is glucagon-like peptide-1 (GLP-1). High affinity exists between them. Its short half-life in the body prevents it from achieving blood sugar management at a fine level. Therefore, GLP-1 simulations are used in the current treatment. Exendin-4, a synthetic version of the naturally occurring peptide, is the most well-known. It combines chitosan-PGA nanoparticles with detecting agents for enhanced multimodal imaging techniques such as MRI, SPECT, and multispectral photoacoustic tomography (MSOT)) (Dinnyes et al., 2020).

According to recent research, MSC-derived nanoscale exosomal miR-146a reverses islet β cells' dedifferentiation through the miR-146a-5p/β-catenin pathway, enabling its differentiation into the necessary cell subtypes, enhancing β cell function, and lowering insulin resistance to better serve diabetic patients' needs.(He et al., 2021)

**Nanoparticles in Diabetic Wound Healing**

A wound is an interruption in the epidermal layer of one's body. Generally, wounds result from any external invasion of skin-like injury. So, it's normal that anyone can get wounded in daily life. The diabetic patients are the ones who are susceptible to delayed wound healing which ends up in horrific amputations (Qin et al., 2022).

Normal wound healing has four phases including hemostasis, inflammation, proliferation, and remodeling. Soon after the injury, hemostasis begins immediately to produce clots through fibrin and release pro-inflammatory mediators like cytokines and growth factors through platelets. These cytokines activate the inflammatory phase by accumulating monocytes and neutrophils in the wound area. Hemostasis takes 2-3 hours. The inflammatory phase can take weeks or even months in chronic disease (Diabetic Foot Ulcer). Whenever injury occurs it causes blood vessels to rupture and formation of extracellular matrix which closes the wound. During the proliferation phase, endothelial cells and fibroblasts proliferate to direct angiogenesis and new extracellular matrix formation. The old ECM is broken down by proteases. The surface of the wound is covered by crust in the remodeling phase which can last for months (Qin et al., 2022).

Diabetic wounds are impaired due to hypoxia which is either the result of reduced $O_2$ supply or increased $O_2$ usage by the wound during neuropathy and inflammation, respectively. Hypoxia increases the ROS levels resulting in delayed wound healing. Diabetic wounds have reduced angiogenesis and increased inflammatory mediators (Qin et al., 2022).

Diabetic individuals often experience poor wound healing due to various physiological factors. These factors include:

- Impaired migration and proliferation of keratinocytes and fibroblasts.
- Abnormal cytokine and growth factor function.
- A compromised angiogenic response.
- Impaired response to infection.

These factors can significantly impede the wound healing process in individuals with diabetes.

**Polymeric Nanoparticles**

They are usually used embedded in scaffolds. Chitosan is studied based on its properties like biocompatibility, biodegradability, and antimicrobial activity. Chitosan NP loaded with drug curcumin, embedded in collagen scaffold heals the wound faster. It is also involved in decreasing inflammation and promoting angiogenesis (Gupta, Sharma et al. 2022).

**Silver Nanoparticles**

Silver nitrate and silver sulfadiazine have been used but they have certain limitations of staining and irritating the tissues (silver nitrate) and inactivating silver at the wound site (silver sulfadiazine). ACTICOAT, silver loaded dressing has been introduced to deal with these limitations. These dressings can release additional silver when the silver is inactivated by wound fluid, maintaining sustained and steady active silver availability. The silver has wide antimicrobial activity by inhibiting DNA replication (Choudhury et al., 2020).

**Hydrogel**

The nanocomposite hydrogel, based on alginate and positively charged Eudragit nanoparticles, has been used to load Edaravone, a drug having wound healing properties by scavenging ROS. This HG loading increases its scavenging capacity, stability, and solubility at the wound site (Fan et al., 2019)

An effective approach towards the treatment of chronic wounds is scavenging reactive oxygen species (ROS). This hydrogel serves this purpose well. Solubility and stability of Edaravone is increased by Eudragit nanoparticles while the Alginate hydrogel servesfor protection and sustained release of Edaravone. The dose dependent approach of this hydrogel shows the dual role of ROS where low dose speeds up the wound healing while high doses inhibit it.

**Ferulic Acid Nanoparticles**

Diabetic Rats are tested with Ferulic acid nanoparticles for their hypoglycemic and wound healing effects. The FA-NPs are prepared by encapsulating ferulic acid in PLGA (poly-lactic-co glycolic acid) having an average size of 240 nm and spherical. The FA-NP dispersion is for oral administration while FA-NP-based hydrogel is prepared using Carbopol 980, for topical administration. The FA-NPs increase hydroxyproline which plays a role in collagen stability thus speeding the epithelialization of wounds (Bairagi et al., 2018).

**Future Perspectives**

Exogenous insulin is the only cure currently available for Type 1 DM since it is an autoimmune disease. By understanding the mechanisms, and various changes in the pancreatic beta cells as the disease progresses, several different immunotherapies are designed. The main concern in this respect is the quick clearance and inability to maintain glucose homeostasis for a longer duration. Recent studies have shown that combining nanotechnology with these immunotherapies not only provides the targeted and controlled delivery of immune modulators to desired localized sites but also slows down disease progression (Nigam et al., 2022)

Moreover, nanotechnology is being employed in preparing artificial cells and implants. Biocompatible scaffolds, mimicking extracellular matrices, have been prepared by employing nanoparticles for tissue regeneration (Simos et al., 2021). Even at this emerging stage, it is expected that this combinational approach will provide means to treat this autoimmune disorder shortly.

In type 2 DM, the nanotechnology is being used for targeted delivery and controlled release (over an extended period) of oral hypoglycemic agents. Metallic nanoparticles, the nanoparticles containing trace elements involved in glucose homeostasis like zinc, vanadium, chromium, selenium, and lithium, have also been developed. Nanoparticles containing glucose-responsive smart polymers have been developed that release the drug when the blood glucose level is high and vice versa. In another scenario, these nanoparticles are designed first to release the initial dose of the drug providing the immediate effect followed by slow release to attain glucose homeostasis for a longer period (Simos et al., 2021). Formulations developed by nanotechnology are found to be effective in treating various DM complications like diabetic retinopathy, diabetic nephropathy, cardiomyopathy, peripheral neuropathy, and diabetic wound healing.

A novel therapeutic approach to treat type 2 DM is gene therapy which is the non-viral delivery of GLP-1 plasmid DNA (p-DNA) complex coding for a specific protein. The protein is synthesized in the cells of the small intestine on receiving the p-DNA complex and goes to systemic circulation. Here, nanotechnology serves for the targeted delivery of genes (Simos et al., 2021).

Overall, treatment with techniques and formulations employing nanotechnology and smart polymers are more efficacious than conventional treatments at the same potency (dose). However, the reproducibility and stability of nano-carriers are some of the setbacks being encountered. It is anticipated that soon researchers, with in-depth knowledge of nanotechnology, will successfully overcome the current limitations and introduce further novel approaches to combat DM. (Simos et al., 2021)

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Chapter 14

# Use of Nanoparticles to Enhance Immune Response

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**ABSTRACT**

Nanoparticle technology in the modern era serves as various functions but their role in health conditions and generation and modulation of immunity makes it more important in the medical field. It is previously used as a carrier of drug delivery that not only delivers the drug to the targeted site but also prevents its side effects and reduction in dosage. The same discovery lead to its use as a potent vaccine in which adjuvant when mixed with these particles results in the potent activation of immune cells and also increased antigen exposure leading to almost dual development of memory T cells. In recent advances, nanoparticles are being used in diagnostics, and with their immune modulation effect, it is nowadays widely used in therapeutics. Its vital role in the prevention of cancer is considered remarkable. Along with that it also initiates better and stronger immunity against any infection and research is done to study its role as an antimicrobial. These advances in nanotechnology research lead to exploring vast functions in many industries. Nanoparticles are engineered, processed, and modulated because their physiochemical properties are different in different roles in the human body. These physiochemical properties include size, shape, electric charge, surface area, roughness, and modulation. In every field of medicine NPs serve to enhance immune response in one way or another.

**KEYWORDS**
Nanoparticles, Immune system, Medical, Treatment, Diagnosis

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## INTRODUCTION

The immune system plays a vital role in the body's defense against any infectious particle. There are many cells, cell mediators, and organs involved in this system. The immune system is of two types; innate and acquired. The innate immune system is the body's first line of defense and the acquired immune system is the defense that the body gains with exposure to different infections. Vaccines come under acquired immunity.

A nanoparticle is a small particle that ranges from 1 to 100-nanometers in size and exhibits various physical and chemical properties. Nanoparticles can occur naturally, be produced by combustion, or be engineered for performing specialized functions. Nanoparticles can play a major role in clinics and improve pharmacokinetic and pharmacodynamic properties. It serves as a carrier to transfer drugs to the targeted organ effectively protecting the release of active ingredients throughout the drug delivery system (Mohanraj and Chen, 2006).

Therapeutic and diagnostic nanoparticles fall into two categories; organic and inorganic nanoparticles. Inorganic nanoparticles have evolved successfully as preclinical studies and also emerging in clinical trials. Organic nanoparticles are commonly used in clinics as in gene therapy applications, drug delivery systems, and cancer treatment. Organic nanoparticles with lysozymes are used as vaccines to treat bacterial infection and arthritis. Protein-based nanoparticles are basically used for the treatment of cancer (Anselmo and Mitragotri, 2016). =

Nanoparticles are not only beneficial in drug delivery systems but also play an important role as antimicrobials. They can react with bacteria in different ways including releasing various reactive oxygen species (ROS) that the walls of

different bacterial cell walls leading to the internalization of nanoparticles or the release of electrons that interrupt the electron transport chain. These reactive oxygen species have various effective therapeutic effects by denaturing the protein, damaging the bacterial DNA, and inhibiting it from replication (Dizaj et al., 2014).

Now, the traditional drug delivery system is replaced by a controlled drug delivery system. This will lead to the transportation of the drug to desirable organs preventing it from affecting other organs which leads to a reduction in the side effects of that drug. When the required dose is achieved to the targeted organ with less residual volume then it will lead to a lower the drug's dosage. Not all nanoparticles can serve this function. Nanoparticles including polymers, dendrimers, liposomes, magnetic nanoparticles, and silicon or carbon material can serve as rug carrier carriers. Nanoparticles have enhanced reactive areas that can cross the brain brain blood-brain barrier and tissue barrier (Wilczewska et al., 2012).

The interaction of nanoparticles with the immune system can be related to two different mechanisms. First, the nanoparticles target only foreign particles in regard to the treatment of different diseases. In this case, nanoparticles are considered to escape from the immune system or immune cells. Secondly, nanoparticles are specifically targeted to immune cells when nanoparticles serve the purpose of vaccines and for improving the quality of treatment as anti-cancer, anti-viral, and anti-inflammatory therapy. For all medical, industrial, and environmental implications it is necessary for nanoparticles to escape immune cells (Dobrovolskaia et al., 2016).

The innate immune system is rapid in onset while the adaptive immune system produces a slower but specific reaction. Pattern recognition receptors (PRR) recognize the pathogen-associated molecular pattern (PAMP) leads to the activation of the innate immune system. This subsequently leads to gene expression change and the release of cytokines. This release leads to the recruitment of innate immune cells such as macrophages, neutrophils, and natural killer cells; all for the clearance of pathogens. Activation of adaptive immunity occurs as a result of innate immune cell signaling and antigen presentation via major histocompatibility factor in antigen-presenting cells leads to the release of dendritic cells and macrophages (Du et al., 2017).

Nanoparticles can stimulate or suppress the immune response. Some responses are desirable or some undesirable in both cases. For vaccine purposes and antitumor effects, there is a desirable requirement of immune stimulation. Undesirable immune stimulation happens when there is inflammation, anaphylaxis, and hypersensitivity reaction. Desirable immunosuppression by nanoparticles is achieved when treatment of inflammatory disorder and autoimmune disease, prevention of allergic response, and transplant acceptance are required. While undesirable immunosuppression can result in lowering body response against infected and cancerous cells and myeloid suppression or thymic suppression (Zolnik et al., 2010).

Interaction of nanoparticles with immune cells occurs in different ways to reduce inflammation. Interaction of nanoparticles with neutrophils for the depletion of inflammation-related neutrophils, modulating migration, based neutrophil-based drug delivery, and neutrophil membrane-coated technique. Interaction of NPs with monocyte or macrophages inhibiting monocyte regression and production, modulating mobilization and recruitment, depleting hyperactive macrophage, and modulating macrophage polarization. Nanoparticles with dendritic cells regulate antigen presentation. With natural killer cells, enhance cytotoxicity towards unwanted cells. With inflammatory cells it reduces hypersensitivity and cytokine production, scavenging ROS and RNS and eliminating cell-free DNA. With T lymphocytes, deplete auto-reactive T-lymphocytes, inhibit auto-reactive B-lymphocytes, and induce T-cell related immune tolerance (Liu et al., 2022).

**Protein Corona**

Interaction of NPs with the protein of body fluid forms protein corona that leads to easy recognition by innate immune cells. There are hard and soft protein corona exists exist. This protein corona has three types of effect including opsonization of NPs, colloidal destabilization, and stealthiness of NPs. Opsonization of NPs directly leads to NP toxicity or phagocytized by monophagocytic cells (MPC) and decreases circulation efficiency leading to a decrease in target efficiency. Colloidal destabilization leads to undesired aggregation which subsequently faces phagocytosis by MPC or accumulation in filtering organs. Stealthiness of NPs can directly lead to toxicity or increased circulation time leads to positive target efficiency (Rampado et al., 2020).

**Physiochemical Properties of Nanoparticles and Cellular Uptake**

Size, shape, stiffness, roughness, and electric charge all effect the cellular uptake of nanoparticles and the presence of protein or lipid corona resulting in hydrophobicity and hydrophilicity that ultimately lead to ligand binding or cellular uptake. There two types of cellular uptake; active which is shifted directly to lysosomes after cellular uptake and other type is passive uptake of nanoparticles. Both active and passive transport of nanoparticles occurs (Sabourian et al., 2020).

Nanoparticles having a size of 25-100nm are considered as therapeutics. NPNP size ranging from 30-200nm can lead to cellular uptake but doesn't trigger an immune response. Non-spherical NPs are not only efficient in cellular uptake but also trigger multiple interactions with cell surfaces as compared to spherical NPs. Nps having more negative charge leads to a reduction in electrostatic interaction with plasma proteins. Soft NPs reduce immunogenicity and enhance tumor accumulation as compared to their hard part (Kiio and Park, 2021).

**Nanoparticles as Immunomodulation**

There is a need for new technology to overcome the outcomes faced in previous treatment strategies like side effects off target, loss of bioactivity during circulation, and inadequate immune stimulation. NPs serve as carriers for efficient drug delivery systems preventing side effects of the drug and protecting the active substance of the drug leading to modulation of the immune e system (Feng et al., 2019).

Nanoparticles can not only deliver drugs effectively to the targeted sites but also carry adjuvant and antigens to secondary lymphoid organs. Biogenic NPs exhibit multiple antigen-presenting cells so that adaptive immunity can develop against multiple antigens. That's why it is significantly used in the immune modulation of cancer therapy and improves therapeutic effect (Cheng et al., 2020).

Specific NPs are used for targeting specific immune cells. For example, NPs target the mononuclear phagocyte system (MPS). The purpose behind these clinics is to modulate excessive monocytic activity during severe inflammation and restoration of peripheral immune tolerance. This leads to a reduction in the potential of pathological immune cells in controlling different diseases like West Nile virus, myocardial infarction, encephalitis, and inflammatory bowel disease. The combination of NPs with autoantigens is used against experimental autoimmune encephalitis in immune autoimmune models by restoring peripheral immune tolerance (Getts et al., 2015).

**Innate Immunity and NPs**

Innate immunity is basically responsible for taking action against foreign particles in the body. That's why NPs first initiate innate immune response rapidly. Nanoparticles interact with plasma protein and complement system leading to the uptake of innate immune cells of the body including neutrophils, macrophages, and dendritic cells. Interaction of NPs with any cells has both positive as well as negative effects. Interaction of NPs with neutrophil desirably degranulation, inflammatory activation, and cell death. Inflammatory activation and cell death are common with all types of interaction that may be desirable or not. In interaction with dendritic cells, it modulates antigen presentation. NPs interaction with macrophages is responsible for the reactivity (Boraschi et al., 2017).

Cells of the innate immune system particularly macrophages and dendritic cells are responsible for generating stimulation of the adaptive immune system depending upon safety and hazard. Specific cytokines and chemokines are released when NPs interact with immune cells. The interaction of NPs with the adaptive immune system involves a series of cytokine cascades (Petrarca et al., 2015).

Therapeutic NPs are considered for the treatment of cancer, blood, lungs, and heart, infectious and inflammatory diseases. Clinical applications include tissue regeneration, retinal implants, cartilage and joint repair, antimicrobial therapy, targeted drug delivery, and metabolic disorders (McMillan et al., 2011).

**NPs and Adaptive Immunity**

Whenever there is a foreign particle or antigen either due to infection or vaccine, adaptive immune cells activate to encounter it. It also stimulates when the innate immune system fails to encounter antigens. Adaptive immunity is of two types; humoral and cell-mediated immunity. Bioengineered NPs through specific intracellular pathways are responsible for stimulation of adaptive immune response and also help in increased antigen presentation. NPs interact with dendritic cells, B-cells, and macrophages of the adaptive immune system in blood circulation (Ghosh et al., 2022).

For medical applications, it is necessary to initiate an adaptive immune response. NPs are considered to initiate strong immune reactions and break tolerance as it target the specific immune cells also known as adaptive immune cells (Duschl, 2014).

**NPs in Development of Vaccine**

NPs are considered as best adjuvant and antigen carriers. The vaccine works on the mechanism of stimulation of innate immune response and then adaptive immune response and biodegradable poly glutamic acid NPs work on same mechanism. NP action as vaccine development is dependent on toll like receptors (TLR 4) and MyD88 signaling pathways (Uto et al., 2011).

NPs are intensively used as vaccines because they enhance antigen exposure to immune cells due to their multiple antigen-presenting cells. NP result in antigen-specific immune response. Vaccines alone exhibit lesser immune response as compared to combination with NPs. In combination of the vaccine with NPs, spleenocytes increase the secretion of cytokines, improved the generation of memory T-cells and antigen-specific IgG antibodies, and efficient induction of dendritic cell activation and T-cells differentiation (Zhang et al., 2014).

Specific cell interaction and intra-follicle lymph node transport are determined by its size. Smaller NPs of size 5 to 15nm clear within 48 hours while larger NPs having size 50-100nm persist for more than 5 weeks. NPs having a large size 50-100nm generate five folds more production of antibodies and one hundred seventy five folds more delivery of antigen at follicular dendritic cells or dendrites (Zhang et al., 2019).

In the case of a subunit vaccine, high adjuvant dose is required to initiate strong CD8+ effect of immune cells. But, with combination of NPs with adjuvant enhance the dual uptake of adjuvant and antigen by cross protecting dendritic cells. This enhances the potent CD8+ effect and generates cells effective memory cells against antigens. This process will generate immunity at a lower dose (De Titta et al., 2013).

NPs are used in enhancing the production of IgA and IgG subtypes that enhance antibody production and the production of dendritic cells, B and T lymphocytes that are modulated by NPs releases cytokines that clear the pathogen from the body. This mechanism is the basis of the development of vaccines (Sengupra et al., 2022).

**NPs in the Treatment of Cancer**

In innate and adaptive immunity, macrophage plays a key role in establishing anti-tumor activity. In some cases, macrophages are programmed to the M2 phase which instead of producing anti-tumoral effect promotes metastasis and establishment of the tumor. In this case, NPs are used to reeducate the M2 macrophage about its activity. Dying tumor cells release tumor antigen that is encountered by reeducated macrophages so that activate and infiltrate cytotoxic T lymphocytes. This is why NPs initiate a strong immune response (Wang et al., 2022).

In cancer treatment, there is a need to develop different tactics than radiotherapy. Radiotherapy have many side effects on other organs as it is not specific to tumor cell therapy. NPs are an emerging technology that develops from research-based to treatment-based strategies (Davis et al., 2008).

Nanoparticles as a drug delivery for cancer are widely used. Now, new combination therapies are developed to enhance the effectiveness and reduction in exposure to cancer treatment. NPs combined with chemo radiation promote effective drug delivery via synergism and through antagonism suppress drug resistance against cancer (Hu et al., 2010).

For NPs to serve as anti-tumor therapy, it is essential to have certain features:

1. Maintain their stability in the vascular system (blood)
2. To escape from reticuloendothelial cells
3. To escape from mononuclear phagocytic system
4. Accumulate in tumor epithelium
5. High-pressure penetration in tumor fluid
6. Reach the target and prevent interaction with other cells than the tumor (Equisquiaguiree et al., 2012).

**NPs as Carriers for the Delivery of Drug**

NPs serve this function when detected by immune cells or macrophages and lead to phagocytosis, delivering drugs to macrophages and silently eliminating them from the body (Emst et al., 2021). There are two types of drug targeting by NPs; active targeting and passive targeting. Active targeting is known as the uptake of NPs with opsonization; combining with a protein or complement cells to internalize in the body of the host.

Macrophages play an essential role in uptaking and targeting the NPs to the targeted site. That leads to the development of different types of NPs for delivery and recognition that involve metallic, polymeric, solid lipid, and liposome NPs. Another is passive targeting which requires modulation of NPs with surface modification, charge, or size. Passive targeting promoted the accumulation of NPs at the targeted site based on their anatomical and physiochemical features (Colino et al., 2020).

**NPs use in Therapeutics**

NPs are detected by immune cells and have inflammatory effects by producing ROS, RNS, antigen presentation, PRR-dependent gene upregulation, inflammasome activation, pyroptosis, and necrosis. This effect of NPs is used in therapeutics like treatment of cancer, vaccine delivery, and immune activation (Emst et al., 2021).

For designing NPs for therapeutics, it is necessary to consider the retention time and clearance of the body. NPs got entry into the bloodstream via opsonization. This will lead to either filtration in the liver, kidney, or spleen or face clearance from the bloodstream by phagocytosis. For this, NPs are designed by toping the surface so that they can pass the process of opsonization and are easily taken up by the cells this process is called active targeting of NPs (Yetisgin et al., 2020).

In biodistribution and circulation, the size of NPs plays a vital role. NPs having size less than 10nm can be easily eliminated by physiological system. Therapeutic NPs having a size less than 100nm no longer blood bloodstream circulation time. NPs having a size of more than 200nm can be easily cleared by the reticuloendothelial system (RES) by phagocytosis. NPs having size range from 20-200nm can easily accumulate in tumor cells because they can be filtrated from the kidney and can't be recognized in RES (Yetisgin et al., 2020).

The shape of NPs plays an important role in therapeutics. Rod shaped NPs can easily internalize the human body because they mimic like rod shaped bacteria than other shaped therapeutic NPs. These targeted NPs can easily be uptake by endosomes thereby activating immune cells (Yetisgin et al., 2020).

For targeted delivery and clearance, the surface charge of NPs also plays a key role. Therapeutic NPs having electric charge -10 to +10mV are less prone to nonspecific interaction and phagocytosis. pH sensibility of NPs is closely related to the electric charge of NPs. For example, acidic NPs having pH less than 6 is considered to be targeted to lysosomes and then release their substance (Yetisgin et al., 2020).

In the modification of therapeutic NPs, surface modification plays an important role. As most commonly used modifying agent is polyethylenere glycol (PEG) that result in cellular uptake but inhibit uptake from non-specific proteins. That's why length, density and shape of PEG matters in surface phagocytosis and hydrophilicity. Advantage of using PEG as surface modifier is to reduce accumulation in non-targeted site and prevent phagocytosis (Yetisgin et al., 2020).

**NPs as Anti-inflammatory and Healing**

Mode of action involved are angiogenic factors, mineral antioxidant, matrix components, and anti-inflammatory factors. This effect leads to aging effects, autoimmune disease, chronic inflammation, and degenerative diseases (Emst et al., 2021). NPs not only used in treatment but also provide rapid healing of wounds. As wound dressing is the major concern for healing, topical application of nanoparticles not only provides their effective pharmacokinetic approach but in different healing phases provides accurate movement and NPs deliver peptide structure and exogenous growth factors (Rajendran et al., 2018).

NPs affect the healing process of the wound by realignment, collagen deposition, and in wound healing and skin regeneration (Naderi et al., 2018). The combination of NPs with the drug provides a new aspect in clinics. These results may not be achieved by using conventional methods of drug because they provide controlled release, molecular targeting, biodegradable, and is multifunctional combination therapy (Kamaly et al., 2012).

**NPs in Medical or Diagnostics**

These NPs go undetected to immune cells and are used for medical and diagnostic purposes (Emst et al., 2021). NPs are intensively used in DNA diagnostics, cellular imaging, bioseparation of specific cells, immunohistochemistry and immunoassays. These NPs detect abnormal changes in the body. Some NPs are fluorescent that show fluorescent throughout their path in the body and can be detected. Different NPs are in use for diagnostic purpose including gold nanoparticles, quantum dots, and superparamagnetic NPs. These all are used for in vitro diagnostic purposes (Azzazy and Mansour, 2009).

In conclusion, nanoparticles are effective in enhancing the immune response. Nanoparticles are widely used in targeting the drug to the desired organ for years, but nowadays it is widely used in other field of medical and diagnostics. Nanoparticles use in therapeutics, anti-inflammatory, healing promotion are prevailing nowadays but its toxicity is still a concern.

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Chapter 15

# Use of Nanoparticles in the Diagnosis of Cancer Treatment

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**ABSTRACT**

Cancer remains a major worldwide health concern that requires modern techniques of diagnosis and treatment. Nanoparticle NPs have emerged as promising tools in cancer management due to their unique biomedical properties. NPs include biological characteristics that make them very suitable for imaging, targeted drug delivery, and theranostic applications. These properties include their small size, high surface area-to-volume ratio, and flexible surface characteristics. Metal-based NPs such as silver, Copper (Cu), selenium (Se), and palladium (Pd) show promising aspects for cancer treatment in the areas of imaging, drug delivery, and targeted therapy. Metal-based NPs have unique advantages like improved targeting, regulated drug release, and multimodal imaging characteristics. NPs have the potential to improve cancer diagnosis through earlier detection and more precise tumor characterization through improved imaging modalities. Gene therapy, immunomodulators, and chemotherapeutic drugs can all be delivered directly to tumor locations by using a NPs flexible delivery system. When NPs are functionalized with targeting ligands, like peptides or antibodies, they can bind to cancer cells selectively, improving medication accumulation and decreasing off-target effects. Stimuli-responsive NPs have the ability to release therapeutic particles in response to specific stimuli inside the tumor microenvironment, improving treatment outcomes.

**KEYWORDS**
Cancer, Nanoparticles, Biomedical properties, Copper

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## INTRODUCTION

Cancer is the most prevalent cause of death and disability globally and is emerging as a major health concern (Kocarnik et al., 2022). Approximately 9.6 million cancer mortality occurred in 2018, impacting 18.1 million individuals globally (Rajput et al., 2022). By 2040, a diagnosis of above two-thirds of the world's cancer cases is predicted. Cancer causes about 30% of premature mortalities in those between the ages of 30-69 (Ghufran et al., 2023). Cancer is becoming more common for a variety of causes, including aging populations, poor dietary habits, smoking, and limited access to healthcare. Cancer is treated by the application of biological substances such as plant anti-metabolites, terpenoids, alkylating compounds, and alkaloids that harm DNA (Patel et al., 2022). Current chemotherapy has many problems, the majority of which can be related to problems and the absence of target specificity, which lead to insufficiencies and uneven medical results. Chemotherapeutics are destructive to normal cells in the hair follicles, bone marrow, macrophages, and digestive tract when normal cells also multiply quickly. Mucositis, organ failure, myelosuppression, anemia, baldness, and thrombocytopenia are the consequences of chronic contamination (Singaraju et al., 2020). This may lead physicians to modify, stop, or push back the suggested course of treatment. Anticancer medications are more toxic, and chemotherapy resistance lowers their effectiveness. Cancer treatments like radiation, chemotherapy, and surgery are now accessible and usable. Unfortunately, the biggest problem with current cancer treatments is that they attack both cancerous and healthy cells. NPs are naturally occurring, biocompatible, stable, and nontoxic materials, they can be a useful tool for medication delivery. Metal NPs have the potential to overcome challenges related to conventional therapy (Gavas et al., 2021). metal NPs enhance gene silencing, targeting, and drug administration, therefore serving a beneficial and effective role in cancer therapy. Targeted ligand-functionalized Metal NPs improve tumor energy deposition control. Metal NPs are used not only for therapeutic purposes but also as a diagnostic tool for imaging cancer cells (Păduraru et al., 2022). Therapeutic systems based on metal NPs have the potential to revolutionize cancer therapy and control by enabling targeted and regulated medication release in addition to providing simultaneous diagnostic and treatment. The sensitivity, invasiveness, and specificity, of traditional diagnostic techniques are frequently limited, emphasizing the need for novel alternatives. NP has become an extremely useful tool for the diagnosis of cancer. They present unmatched chances to increase the

sensitivity of detection, enhance imaging modalities, and allow for the targeted delivery of diagnostic chemicals (Kemp and Kwon, 2021). NPs are the perfect solution for solving the difficult problems associated with cancer detection because of their small size, high surface area-to-volume ratio, and adaptable surface characteristics. Drug distribution is delayed at the target site by both metabolic and physical barriers. Cellular and non-cellular mechanisms limit drug activity at the cancer level, raising the risk of mortality and recurrence (Haider et al., 2020). Because of the unique functional and physical properties of nanotechnology, there has been a significant surge in scientific interest in recent decades. Cellular and non-cellular mechanisms impede drug activity at the cancer level, raising the risk of recurrence and mortality because of the unique functional and physical properties of nanotechnology. These uses are practical and cost-effective due to the special physical and chemical properties of NPs, such as their structure, large surface area, small size, and chemical makeup (Salem et al., 2022). Utilizing NPs in the treatment of certain diseases, such as cancer, may be valuable. The medicinal usage of NPs themselves, as well as their use in drug delivery systems and diagnostics (nanoimaging), are all interesting applications for NPs. This introduction provides the basis for a more thorough examination of the complex role that NPs have played in transforming cancer diagnosis, emphasizing how they can change the face of cancer detection and enhance patient outcomes.

**Role of NPs for Cancer, Therapeutics, and Biomedical Properties**

The potential of nanotechnology in medical diagnosis and treatment is increasingly recognized. Advances in this field have yielded improved materials for biomedical use. NPs have a wide range of uses due to their unique properties. Multifunctional NPs can carry hydrophobic substances, specifically target disease cells, extend the half-life of a drug, facilitate drug entry and formation at sites of drug resistance and tumors, enhance the safety and acceptance of medications, and encourage the advancement of alternative technologies (Zhao et al., 2020) (Fig. 1)

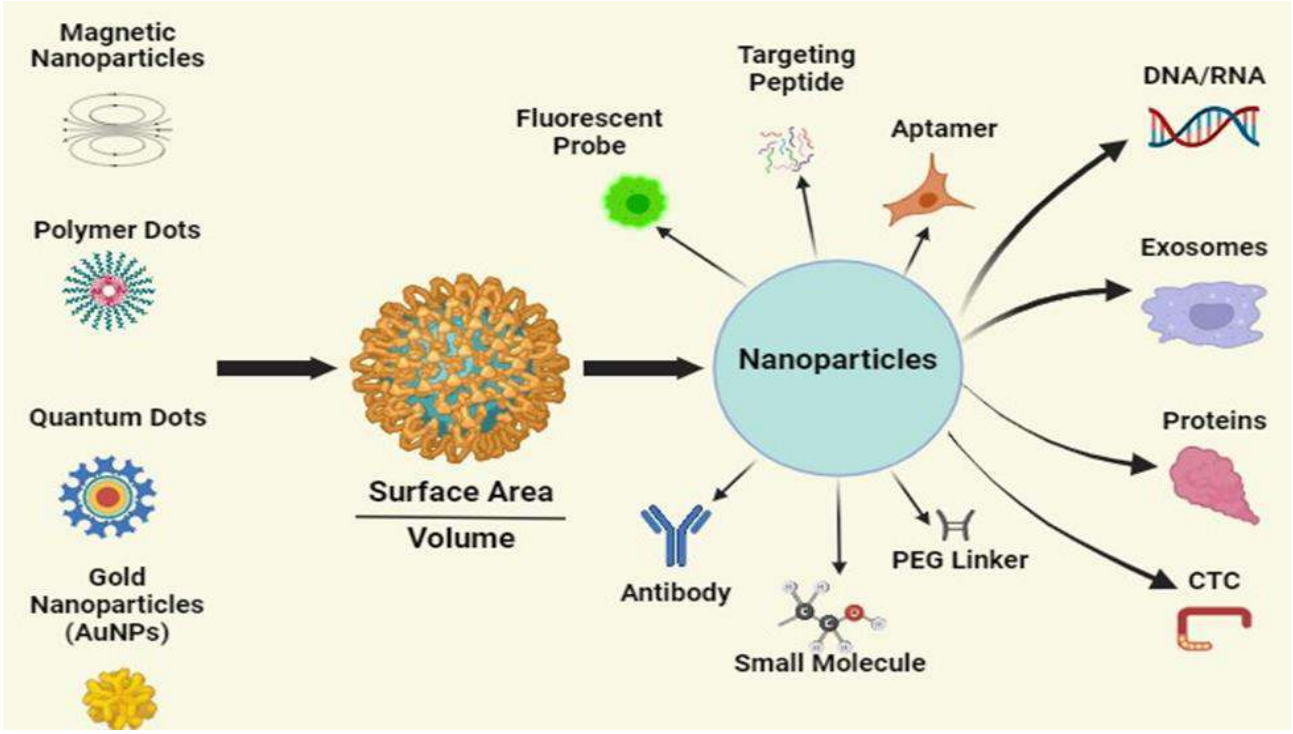


**Fig. 1:** Nanotechnology enhances the detection and diagnosis of cancer (Retrieved from Biorender)

NPs are used in medical applications due to their unique properties, which include their higher surface-to-mass ratio, quantum nature than other elements, and ability to absorb and transport other molecules like proteins and drugs. NPs are available in a variety of compositions since their starting components can include metals, biological lipids, biological lipids, silica, carbon, phospholipids, and other polymers (Ren et al., 2022). Because of their high surface-to-volume ratio and narrow pore size (between 1 and 100 nm), NPs are effective theranostic methods for tumor surveillance and treatment (Ghaferi et al., 2021). When NPs are directly coupled to various biomolecules, effective drug administration and anatomical and functional imaging are made possible. The delivery of NPs involves three different processes, systemic distribution without RES sequestration, extravasation from intratumoral capillaries, and diffusion and penetration into malignant cells (Zhou et al., 2020). Because NPs gather at large levels in tumor cells, they are powerful anti-cancer weapons. Producing larger than 50 nm inhibits the sequestration of RES. Solid tumors have a unique microenvironment, NP is a useful material for imaging and therapeutic delivery. Tumor microenvironment components include extracellular matrix, lymphocytes, inflammatory cells, and signaling molecules. Solid tumors differ from healthy tissue in that they have transparent

microvascular arrangements with capillary pores that range in size from 120 to 1200 nm, which makes NP penetration easier (Trujillo-de Santiago et al., 2019).

**Metal NPs in Cancer Therapeutics**

Nanomaterials have dimensions ranging from 1 to 1000 nm and possess several distinct properties not found in microscopic particles or larger quantities of material (Khan et al., 2019). Because of their huge specific surfaces, high surface activity, strong antioxidant qualities, biocompatibility, and exceptional solubility for chemical changes, nanomaterials have the potential to be exploited in a wide range of biological applications. Polymeric micelles, carbon nanotubes, quantum dots, liposomes, graphs, magnetic, and metallic NPs are commonly utilized in biological applications (Zahin et al., 2020).

**Silver-Based Nanoparticles**

Ag NPs are important because of their various characteristics, including chemical, optical, physical, and magnetic properties, which depend on size and shape and offer benefits (Fahmy et al., 2019). Ag NPs can be found in a wide range of products, including electrical chemicals, composite fibers, biosensors, antimicrobials, and cosmetics. Ag NPs can also be used in cell electrodes, drug delivery, filters, medical imaging, and nanocomposites because of their increased affinity for functionalization, higher resolution, and improved light absorption (Pryshchepa et al., 2020). Ag NPs show remarkable surface plasma resonance (SPR), making them perfect for application in multiple domains, including photo-controlled delivery systems, protein or gene transport, biosensing, and catalysis (Baranwal et al., 2023). Ag NPs can suppress cell division and have antiproliferative effects on cancer cells. Ag NPs can be used to identify a variety of cancers, including cervical, hepatic, lung, and prostate cancers (Andleeb et al., 2021) (Fig. 2).

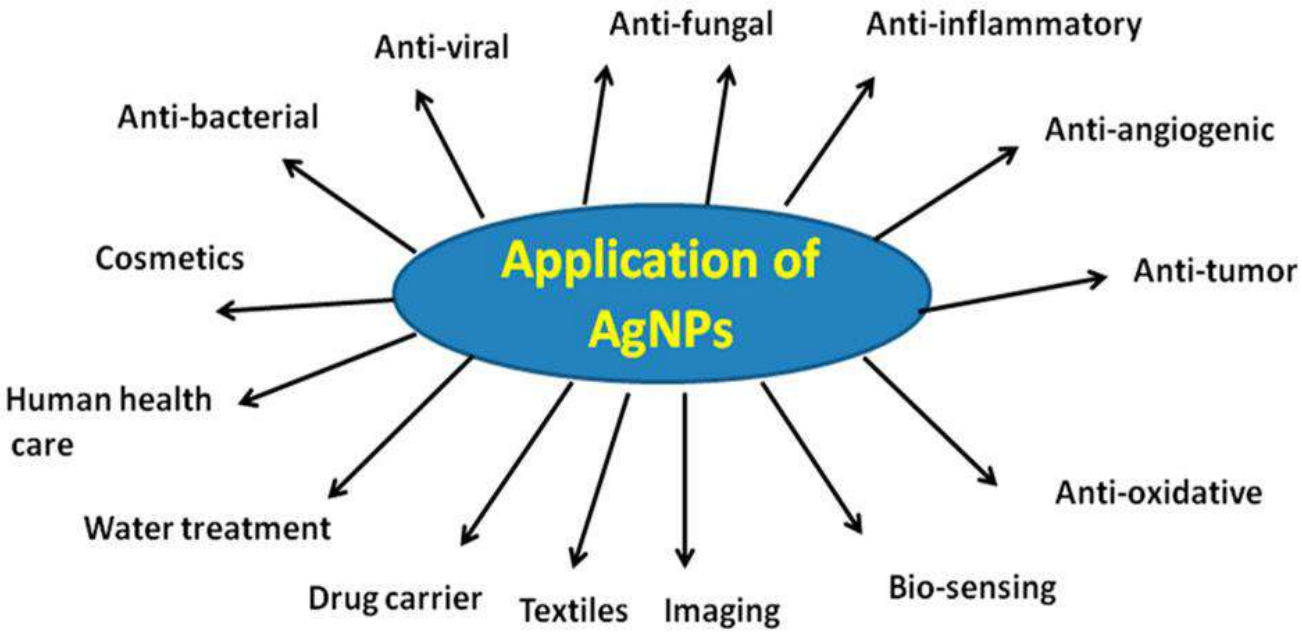


**Fig. 2:** Various applications of AgNPs (Zhang et al., 2016)

**Palladium-Based NPs**

Pd-based nanomaterials (Pd NPs) are highly promising for use in biomedical applications because of their outstanding optical characteristics, high levels of stability, and high levels of biocompatibility, under physiological conditions (Phan et al., 2019). Pd NPs have shown great promise as medicinal agents and contrast agents for imaging cancer. Distinctive examples of 2D nanomaterials with strong NIR absorption, excellent photothermal stability, high photothermal transformation efficiency, and high biocompatibility are Pd nanosheets (Pd NSs) (An et al., 2021).

**Copper-Based NPs**

Cu chalcogenide NPs exhibit improved efficient light-to-heat transformation, near-infrared absorption, and tumor-specific thermal destruction when subjected to a near-infrared laser (Wang et al., 2021). The optical imaging capabilities and fluorescence signal of smaller copper NPs are displayed. Cu NPs also offer image-guided therapy and an adaptable method to administer drugs. Because of their lower cost, greater cytotoxic effect on cancer cells at small dosages, and longer constancy period, copper NPs are more useful than Ag and Au NPS. Novel copper-containing NIR-absorbing nano-formulations have been developed and further verified for PTT, including monodispersed CuTe nanorods, copper selenide (Cu2-xSe) nanocrystals, copper bismuth sulfide (Cu3BiS3) nanocubes, and nanoplates (Halevas and Pantazaki, 2018).

**Selenium-Based NPs**

Se NPs exhibit significantly lower toxicity. SeNPs provide an excellent way of drug administration for a variety of medications (Ferro et al., 2021). The various anticancer activities of Se can be divided into three primary classes: formation of reactive oxygen species (ROS), alteration and chromatin binding, and thiol modification. Se shape and amount have a major impact on its toxicity, biological activity, and cancer-prevention potential (Tan et al., 2018). The most chemopreventive and medicinal form of Se is methylselenocysteine which has better biological activity. Artificial forms of organic Se have been developed that have greater anticancer activity than their natural counterparts. Se-based drugs, such as ebselen, have shown promising anticancer activity against colon, liver, and breast cancer. Se promotes the prevention of cancer by minimizing DNA damage caused by the production of adducts from dimethylbenz(a) anthracene, which is an indicator in the development of liver, colon, and breast cancers (Rai et al., 2023).

**NPs for Medical Imaging**

Medical imaging is frequently used for investigations into biological processes, to identify abnormalities, and to monitor the progression of diseases. Imaging methods are essential for both cancer detection and treatment. Several NPs, including iron oxide NPs, have structural, magnetic, optical, and acoustic qualities that make them useful for improving imaging (Israel et al., 2020). To locate and remove malignancies, introducing NPs into target tissues can enhance contrast and image guidance. For example, in cryosurgery, NPs can increase the imaging quality of the ice ball and the tumor, allowing for more precise coverage and enhanced therapeutic efficacy. The motion of a magnetic field can be by introducing magnetic NPs, which may modify optical scattering and allow one to detect originally incoherent inelastic scattered light (Yu et al., 2021).

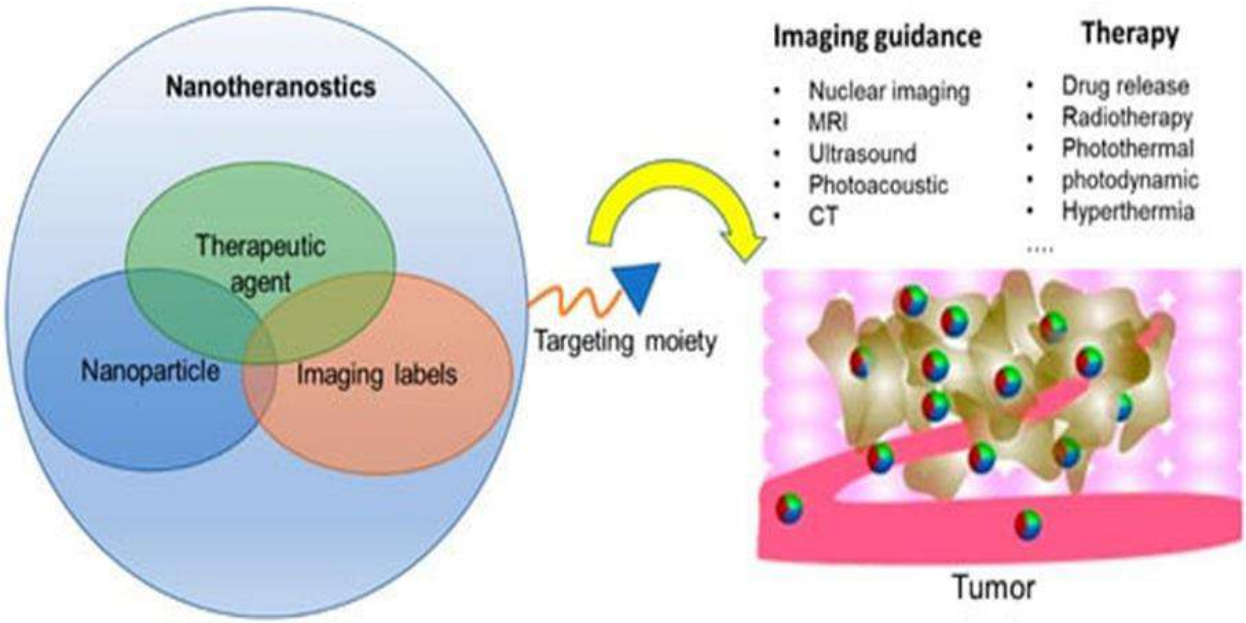


**Fig. 3:** Overview of the multifaceted imaging and therapeutic capabilities of nano theranostics (Dennahy et al., 2022)

Imaging methods can be enhanced by using the special optical and magnetic capabilities of NPs. Quantum dots are semiconductor NPs that, depending on their size, emit light at various wavelengths, which makes them perfect for fluorescence imaging (Pandey and Bodas, 2020). Because of their magnetic characteristics, iron oxide NPs can be utilized as contrast agents in MRI, improving tumor visibility. Light is strongly absorbed and scattered by Au NPs, which makes photoacoustic imaging more effective (Fig. 3). These NPs improve imaging's sensitivity, specificity, and resolution enabling accurate tumor localization, early cancer identification, and treatment response tracking (Mantri and Jokerst, 2020).

**Targeting of Cancer Cells by Metallic NPs**

One effective method of cancer diagnosis and treatment is the targeted application of metallic NPs to individual cancer cells (Khursheed et al., 2022). The capacity of Cu NPs to cause oxidative stress in cancer cells has caused interest as a possible anticancer treatment. Cu NPs have been shown to specifically target cancer cells while protecting healthy cells. Cu Nps improve cancer cells' receptivity to radiation treatment, which encourages tumor remission. Ag Nps specifically target cancer cells by interacting with proteins on the cell surface and producing ROS, which destroys the cell (Takáč et al., 2023). Because of their strong optical characteristics, Au NPs have also been examined as imaging agents for cancer diagnostics. These particles are being considered as potential anticancer therapy since they can induce apoptosis in cancer cells. Zn NPs selectively target cancer cells and induce apoptosis by blocking anti-apoptotic proteins and activating caspases (Chen et al., 2021).

**Targeted Delivery Systems**

Chemotherapy still has problems with over-accumulation in healthy tissue and insufficient target enrichment in areas of malignant tumors (Baranwal et al., 2023). The inhibition of the division of fast-dividing cells, such as hair follicles, gastrointestinal cells, lymphocytes, and bone marrow, can lead to mucositis, hair loss, and even death. Better treatment outcomes and increased efficiency in targeted cancer diagnosis and therapy are made possible by the application of inorganic NPs in drug therapy and genes. Targeted drug distribution is more successful and has fewer adverse effects than normal therapy because it actively distinguishes between healthy and cancerous cells. Chemotherapy drugs can be actively or passively targeted by NPs to tumor cells (Raj et al., 2021). NPs are essential for the targeted delivery of immune drugs. Nano-engineered MSCs combine nanotechnology with cell therapy to precisely target tumor regions and prevent drug-loaded NPs from being removed from the body (de la Torre et al., 2020). It may be possible to reduce peripheral and systemic toxicity, improve the selectivity of killing cancer cells, and enable dosage escalation by targeted drug delivery to tumors. Combining focused approaches could help resolve some of these problems. It is possible to modify NPs so that they specifically target cancer cells and spare healthy tissues. This is achieved by binding particular targeting ligands to the surface of the nanoparticle, such as peptides or antibodies (Marques et al., 2020). These ligands identify and bind to specific biomarkers and receptors that enhance cancer cells, allowing for the selective formation of NPs at the tumor location. Through conjugation or encapsulation, therapeutic chemicals like gene therapy vectors, small interfering RNA (siRNA), or chemotherapeutic drugs can be delivered directly to cancer cells through NPs (Kumar et al., 2022). This focused strategy increases the concentration of medication inside the tumor, reducing off-target effects and minimizing systemic toxicity.

**Current Limitations of Metal NPs and the Challenges**

There are several limitations and challenges in using metal NPs in the observation and management of cancer. Metal NPs can build up in tissues and organs, where they can be poisonous and have negative effects (Attarilar et al., 2020). A comprehensive evaluation of the biodistribution and toxicity is necessary before their application in medicinal situations. NPs are more dangerous than bigger particles because of their tiny physical size. Carbon black is not harmful, even if carbon nanotubes and fullerene pose a serious risk when breathed into the lungs (Yin et al., 2022). In bacterial cells, titanium oxide NPs cause oxidative stress and improve toxicity. Many chemicals that are safe in bulk become harmful at the nanoscale. The instability and aggregation susceptibility of metal NPs may restrict their efficacy and selectivity in the fight against cancer. The occurrence of agglomeration in metal NPs can lead to alterations in their physicochemical properties and a reduction in their therapeutic effectiveness (Manuja et al., 2021). Aggregation in biological systems can be brought on by a variety of environmental factors, including pH variations or the presence of biomolecules. Chemotherapeutic chemicals used to treat cancer are administered without specificity, causing harm to both healthy and malignant tissue, leading to a low level of effectiveness and a high level of damage (Anand et al., 2022). Chemotherapeutic drugs would be well served by controlled drug delivery systems, They would direct the causes to the tumor location, increasing the medication's concentration in cancer cells while protecting healthy cells.

**Future Perspectives**

NPs can enhance a variety of imaging modalities, including optical imaging CT, and MRI. Combining NPs with contrast agents and targeting ligands can improve the sensitivity resolution, specificity and of cancer imaging. Chemotherapy can be administered more effectively and with fewer adverse effects when NPs are used as delivery systems for specific drugs (Yan et al., 2020). They can minimize harm to healthy tissues by encasing anticancer medications and delivering them straight to tumor locations. Furthermore, stimulus-responsive NPs can release medication in reaction to particular stimuli, including changes in pH, temperature, or the activity of certain enzymes in the tumor microenvironment. NPs provide the chance to integrate several therapeutic modalities into one system (Banerjee et al., 2017). Gene therapies, immunotherapeutic medicines, or chemotherapeutic medications can be incorporated into NPs to provide specific therapies and synergistic effects. This strategy has the potential to overcome medication resistance and enhance treatment results. Theranostic NPs combine therapeutic and diagnostic properties into a single system (Shrivastava et al., 2019). These multipurpose NPs have the ability to monitor treatment response, administer medicine, and diagnose cancer concurrently. They have the power to completely transform specific medicine and make it possible to track the effectiveness of treatments in real time. NPs have the potential to significantly improve the effectiveness of immunotherapies, such as immune system checkpoint inhibitors or cancer vaccines. To effectively stimulate an immune response towards cancer cells, they can be utilized to deliver adjuvants, antigens, or immunomodulatory drugs directly to immune cells or tumor locations. It is possible to target specifically and modify the tumor microenvironment with NPs (Thakkar et al., 2020). By inhibiting metastasis, enhancing drug penetration into cancers, normalizing aberrant blood arteries, and modifying immune responses, they can improve treatment outcomes overall.

**Conclusion**

NPs in cancer treatment and diagnosis provide an achievable approach to enhance patient outcomes. NPs have the potential to transform cancer diagnosis and therapy through the improvement of imaging technologies, the facilitation of theranostic techniques, and the possibility of specific medication delivery. NPs have the potential to significantly advance

personalized medicine and enhance the treatment outcomes for cancer patients with continued research and development.

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Chapter 16

# Natural Products Loaded with Nanoparticles for Wound Healing

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## ABSTRACT

The skin is the largest organ of the body and the first line of defense, and it can sometimes suffer from wounds due to physical and chemical sources. To avoid complications from chronic wound healing, the use of nanoparticles has become a popular method. These nanoparticles, with their unique properties and diverse applications, are promising for advancing wound management. This comprehensive review delves into the uses of various nanoparticles, encompassing silver, gold, and zinc oxide for wound healing. The antimicrobial, anti-inflammatory, and regenerative attributes of nanoparticles, notably silver, and gold, have been extensively studied and proven effective in combating bacterial infections, expediting wound closure, and fostering tissue regeneration. Incorporating natural products such as *Azadirachta indica* (neem) and *Curcuma longa* (turmeric) further amplifies the therapeutic potential of nanoparticles. Alginate-based dressings enriched with nanoparticles offer prolonged drug release and enhanced stability, rendering them highly promising for clinical adoption. Despite challenges such as nanoparticle safety and regulatory hurdles, ongoing research endeavors persist in exploring innovative strategies for nanoparticle-based wound dressings to meet unmet clinical needs an enhance patient outcomes in wound management.

**KEYWORDS**
Skin, Wound healing, Natural product, Nanoparticle, Wound management

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## INTRODUCTION

The skin plays a vital role in our survival by serving as a sensory organ, regulating physiological and thermal balance, storing essential nutrients, offering both passive and active protection, and reacting to physical harm and damage (Xu et al., 2015). A wound is characterized as a disturbance in the cellular, anatomical, and functional integrity of living tissue, resulting from various factors such as physical, chemical, thermal, microbial, or immunological factors. Essentially, it entails a breach in the epithelial continuity and may involve structural and functional impairment of the underlying normal tissue (Mulkalwar et al., 2015).

Wounds can be closed or open, such as abrasions, lacerations, avulsions, ballistic and excised, or surgical wounds and are also classified based on the number of skin layers affected. Damage to the epithelial tissue (epidermis) is a superficial wound, which heals quickly through the regeneration of epithelial cells. A partial thickness wound extends into the deeper dermal layer, potentially involving damage to blood vessels. In contrast, a full-thickness wound extends beyond the subcutaneous fat layer, requiring the synthesis of new connective tissue for healing. Full-thickness wounds generally take longer to heal due to the contraction of tissue, whereas those having partial thickness, heal quickly (Lodhi et al., 2017).

This study aims to evaluate the effectiveness of various nanoparticles particularly those loaded with natural products.

This study will explore anti-microbial, anti-inflammatory and regenerative properties of different nanoparticles incorporated with natural products and their application in modern wound healing.

**Importance of Wound Healing**

The intricate regulation of wound healing relies on several critical factors, notably the wound environment, which is abundant in extracellular matrix (ECM). This environment plays a significant role in driving the wound healing process (Rodrigues et al., 2019; Murphy et al., 2012). The process of wound healing is a multifaceted and dynamic sequence of events, encompassing various stages such as the initiation of inflammatory responses, regeneration of parenchymal tissue, migration and proliferation of parenchymal tissue cells, synthesis of extracellular matrix proteins, tissue remodeling, and the eventual enhancement of wound strength (Mathew-Steiner et al., 2021).

Wound healing process engages different cell types, along with growth factors, cytokines, the extracellular matrix, and various enzymes. Cell types involved include platelets, neutrophils, monocytes, macrophages, fibroblasts, keratinocytes, endothelial cells, epithelial cells, and myofibroblasts. Notably, fibroblasts have traditionally been acknowledged as pivotal cells in wound healing, contributing significantly to all three phases of the process (Monika et al., 2021).

The process of healing aims to restore the cellular and anatomical continuity of an organism while minimizing tissue damage and removing nonviable tissue debris. It optimizes tissue perfusion, oxygenation, and proper nutrition to create a healing environment. Key events in wound healing encompass inflammation, proliferation, and migration of connective tissue cells, synthesis of extracellular matrix including collagen, migration, and proliferation of epithelial cells leading to re-epithelialization, and migration and proliferation of endothelial cells leading to neovascularization of the wounded tissue (Lodhi et al., 2017).

**Challenges in Wound Healing**

Various local condition such as infection and inflammation of wound influence healing as skin serves as a fertile ground for microbial colonization. Chronic wounds exhibit a more intricate microbial flora than previously acknowledged, featuring complex structures and diverse populations (Martin et al., 2010). Chronic wounds that fail to heal present a significant risk to health and overall well-being. Patients frequently endure pain, reduced mobility, excessive exudate, unpleasant wound odors, and limited quality of life (Deufert et al., 2017), As a consequence, there are significant disruption, morbidity, and considerable indirect costs incurred by social and healthcare systems (FrykbergRobert and G, 2015). Chronic wounds stem from a disruption in the typical wound healing process. Various factors contribute to this dysregulation, such as microbial biofilms, heightened expression of inflammatory cytokines, elevated levels of proteases, and reactive oxygen species (ROS). These factors impede the transition from the inflammatory phase to the proliferation and re-epithelialization phases. Furthermore, excessive matrix metalloproteinases (MMPs) have been identified as a contributing factor to delayed healing (Caley et al., 2015). Consequently, the wound fails to close, does not undergo healing, and progresses to a chronic state (Han et al., 2017).

Choosing an effective clinical approach to wound management depends on identifying the underlying cause of each wound. This involves addressing factors such as (1) the removal of nonviable (necrotic) tissue, known as debridement; (2) managing inflammation or infection; (3) regulating moisture levels (avoiding excessive wetness or dryness); and (4) assessing the condition of the tissue surrounding the wound (Robson et al., 2006). This approach traces its origins back to ancient Greek and Roman medicine (Bhattacharya and S., 2012), where the removal of these impediments to healing was advocated to enable the completion of the healing process. Debridement is viewed as advantageous for wounds, as it initiates the healing cascade anew by reverting it to an acute state. By removing necrotic tissue, debridement reveals healthy and well-vascularized tissue, promoting cell proliferation and migration (Han et al., 2017).

Apart from eliminating deceased and necrotic tissue, debridement is effective in diminishing, if not eradicating, inflammatory agents, damage-associated molecular patterns (DAMPs), and pathogen-associated molecular patterns (PAMPs). Furthermore, debridement plays a role in eliminating "extracellular traps" and microorganisms present in the wound. The presence of microorganisms in wounds has historically been regarded as harmful (Shedoeva et al,2019).

**Natural Products in Wound Healing**

Natural substances possessing medicinal attributes can aid in the process of wound healing. Numerous investigations have explored the healing potential of such substances, which exhibit anti-inflammatory, antioxidant, antibacterial, and pro-collagen synthesis properties. The therapeutic efficacy of these substances may stem from their bioactive phytochemical constituents belonging to diverse chemical groups including alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, and phenolic compounds as described in Table 1 (Thakur et al., 2011).

Different bioactive agents may exert specific effects on wound healing properties. For example, saponins have been shown to boost pro-collagen synthesis (Ibrahim et al., 2018), whereas tannins and flavonoids demonstrate antiseptic and antibacterial properties (Kováč et al., 2022). Phytochemicals can influence one or multiple stages of the wound healing process. Additionally, they are readily absorbed by the outer layers of the skin (Tsala et al., 2013). These characteristics make natural products and their phytochemical constituents crucial in wound healing, and they serve as inspiration for the development of novel synthetic compounds for this purpose (Ibrahim et al., 2018).

Natural products have been widely employed in wound care management, yielding notable results. Examples include

*Curcuma longa* (turmeric), vitamin E, honey and sea cucumbers. These bioactive agents typically influence one or more stages of the healing process. It's estimated that around 50% of medications approved between 1981 and 2010 originated from natural sources. Among them, 28% were semi-synthetic, 17% mimicked natural compounds, and 5% were natural entities. In the realm of anti-cancer therapeutics, out of 175 anti-cancer drugs approved from 1940 to 2010, 48.6% were either derived from natural sources or were natural products (Gurnani et al., 2014).

**Table 1:** Natural products and their role in healing

| Phase Modulators | Natural Products | Role in healing | References |
|---|---|---|---|
| Modulators of the immune cell function | Vitamin A | Epithelial tissue development, cellular differentiation and boost immune system function | Tsala et al.,2013 |
| | Vitamin C | Enhances Neutrophil function, improved immune cell function | |
| Modulators of skin cells (fibroblasts and keratinocytes) | Vitamins like A, B3, C, E | growth, differentiation and maintenance of epithelial tissues, epithelial cell proliferation | Nesterova et al., 2012 |
| | Alkaloids like Aconitum baikalense | stimulate the growth from fibroblast precursors | |
| Modulators of collagen synthesis | Vitamin C | synthesis of collagen, improves tensile strength of wound | Sudha et al., 2011 |
| | Quinone compound embelin | Boosts epithelialization and granulation process, enhances tensile strength | Swamy et al., 2007 |
| | Plant flavonoid, catechin | Regulates antioxidant activity | Madhan et al., 2005 |
| Modulators of angiogenesis | Vitamin C | Enhances neutrophil and fibroblast function and blood vessel formation | MacKay and Miller et al, 2003 |
| | β-sitosterol | Regulates endothelial cell function | |
| Modulators of the extracellular matrix | flavonoids kaempferol and quercetin | Reduces scar formation | Cho et al., 2010 |
| Modulators of cytokines and growth factors | Taspine Alkaloids | Increases fibroblast population in wound | Villegas et al., 2001 |
| | Anthocyanins Flavonoids | inhibits pro-inflammatory cytokines | Nizamutdinova et al., 2009 |
| | Terminalia chebula Fructus Retz extract of tannin | matures wound | Li et al., 2011 |
| Modulators of the oxidant-antioxidant balance of the wound microenvironment | emblicanin A and B | Reduces oxidative stress during healing | Majeed et al., 2009 |

**Nanotechnology and Nanoparticles for Wound Healing**

Nanotechnology is a swiftly advancing interdisciplinary scientific domain merging material science and engineering. Nanoparticles (NPs), typically sized between 1-100 nanometers (nm), exhibit distinct properties compared to their larger counterparts. They feature unique physicochemical, optical, and biological attributes that can be tailored for specific applications. Throughout history, elements like silver, gold, copper, and titanium have been utilized for various therapeutic purposes (Syed et al., 2012). Nearly two decades ago, nanoparticles were initially mistaken for bacteria due to their diminutive size and were dubbed "nanobacteria" (Kawai et al., 2011). Subsequent investigations clarified that nanoparticles are distinct from typical bacteria, leading to the adoption of the term "nanoparticles" (Young et al., 2010). Continued research unveiled nanoparticles as atypical proteins or minerals under specific conditions (Mitchell et al., 2016). More recently, nanoparticles have been characterized as a collection of non-living entities or objects possessing diverse chemical and physical characteristics (Schlieper et al., 2011).

Numerous materials act as source materials for nanoparticle production, with nanomaterials often cultivated in the presence of serum, adopting a pleomorphic shape. Typically, these nanoparticles vary in size from 20 to 500 nm (Kawai et al., 2011). The physical, chemical, and morphological structure of nanoparticles can be manipulated by adjusting the composition of proteins and ions within the culture medium (Abdelarheim and Saleh al-aboodi, 2013). This variability allows nanoparticles to be categorized as a diverse group comprising multiple distinct entities, each possessing unique characteristic features, rendering them highly applicable in medical technology (Banerjee et al., 2010).

Nanoparticles with chemically synthesized structures, polysaccharides, polymers, metals, and bioactive compounds derived from plants, when combined with active drugs, demonstrate effectiveness in combating human pathogens such as bacteria and viruses. They offer efficient treatment options for various pathological conditions (Shankar et al., 2016). Over the past few decades, numerous nanomaterials tailored for biological applications have undergone extensive research. These encompass liposomes, dendrimers, quantum dots, fullerenes, carbon nanotubes, graphene, as well as iron and titanium oxide, and gold and silver nanoparticles (AgNPs). More recently, nanoparticle-based delivery systems for ions, such as calcium and oxygen, have been employed to stimulate angiogenesis (Vila et al., 2013).

Nanoparticles can be integrated into biomaterials and scaffolds, forming nanocomposite smart materials. These

materials can contribute to wound healing by leveraging their antimicrobial (Lu et al., 2012), selective anti- and pro-inflammatory (Liu et al., 2014), and pro-angiogenic properties (Nethi et al., 2014). Furthermore, nanoparticles can serve as gene delivery vectors, altering intracellular gene expression and protein synthesis relevant to the wound healing process (Charafeddine et al., 2015). Moreover, they can influence wound healing by impacting collagen deposition and realignment (Kwan et al., 2011).

Wound healing can follow two main pathways: primary intention, where the wound edges are brought together and sutured, or secondary intention, where the wound is allowed to heal openly, involving granulation tissue formation, contraction, and re-epithelialization. The overall process of wound healing encompasses sequential and overlapping phases: hemostasis, inflammation, proliferation, and remodeling (Chhabra et al., 2017).

**Nanoparticles Used in Wound Healing**

Wound healing continues to pose significant challenges, underscoring the importance of effective wound management. When biomaterials are integrated with nanoparticles, they can serve as promising materials for wound dressings. Nanotechnology offers diverse strategies for regenerative medicine, including the molecular engineering of self-assembling biocompatible nanoparticles, which has emerged as a prominent area of research. Nanomaterials contribute to improved wound healing and burn treatment by enhancing regenerative processes. Typically ranging from 10 nm to 1000 nm, nanoparticle sizes are much smaller than pathogens targeting cells. Their unique physical and chemical properties confer remarkable and varied biological activities. Notably, their high surface-to-volume ratio facilitates interactions with cell surfaces and penetration into internal cellular environments, leading to heightened therapeutic efficacy (Monopoli et al., 2012).

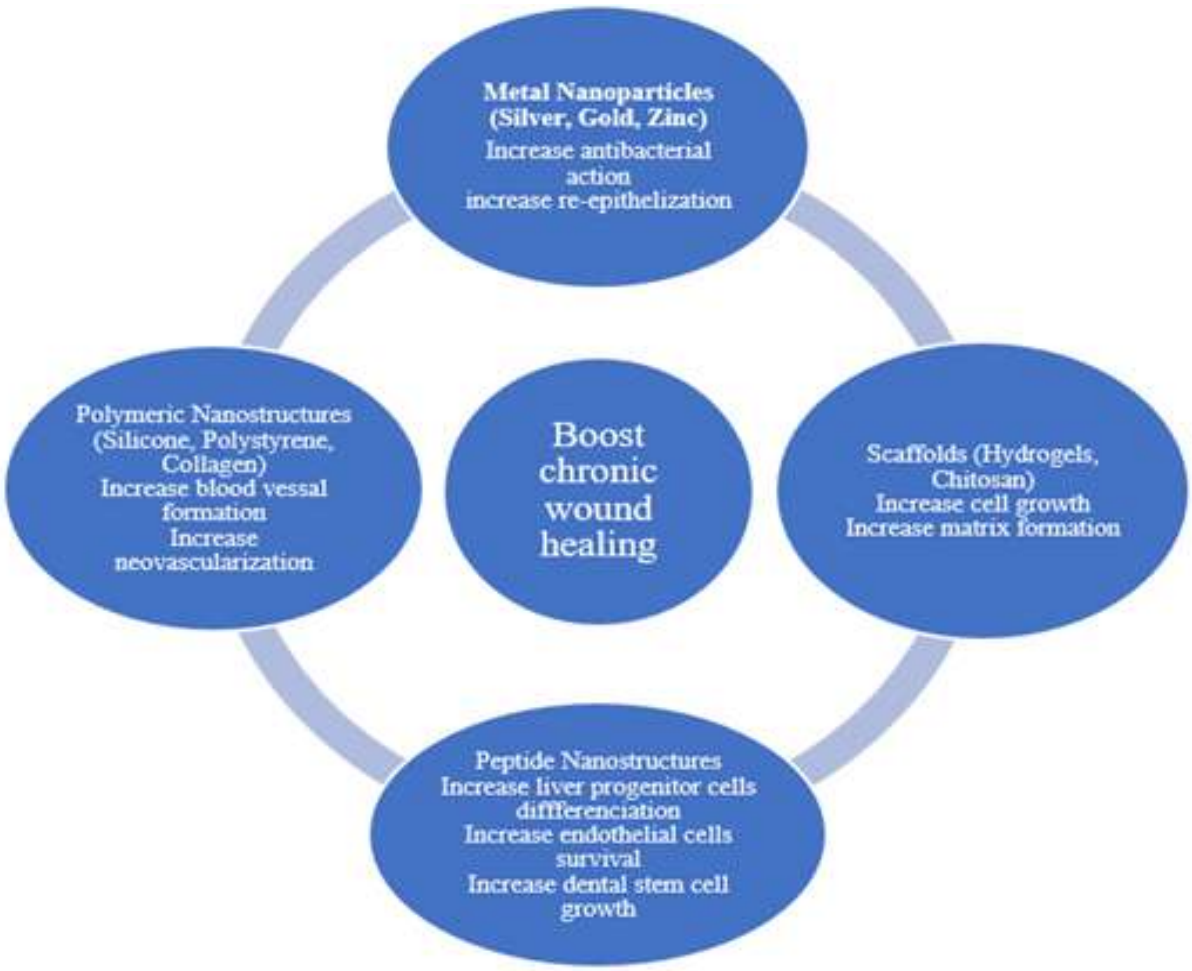


**Fig. 1:** Potential role of nanoparticles in accelerating wound healing

Metal nanoparticles like silver, gold, and zinc possess remarkable properties, including low in vivo toxicity, as well as bacteriostatic and bactericidal activities. Typically measuring 10^-9 meters (1 nm) in size, nanomaterials represent a rapidly expanding field in modern medicine. This domain involves the development of molecules and materials at the nanometer scale or molecular level. Reduction in material size to the nanoscale dramatically increases surface area and surface area-to-volume ratios, resulting in enhanced physicochemical properties as shown in Fig.1 (Rajendran et al., 2018).

**Silver**

Silver is widely recognized for its bactericidal properties and is frequently employed in the treatment of burns, wound infections, and various ulcers. For instance, Silver Nitrate continues to be utilized in the management of chronic non-healing wounds. Presently, various forms of silver coatings in wound dressings are accessible, facilitating efficient drug distribution and playing a pivotal role in the management of chronic wounds (Zhang et al., 2016). Silver nanoparticles (AgNPs) based dressings are not hindered by prolonged use. Combining AgNPs with collagen results in strong antibacterial activity, making it a suitable component for wound dressings (Sarhan et al., 2016).

The mechanism of action of AgNPs involves their large surface area, which contributes to efficient antimicrobial activity. Initially, AgNPs attach to the bacterial cell membrane and penetrate the bacteria. Within the bacterial cell, they interact with proteins containing sulfur and phosphorus groups, as well as with DNA (Sondi et al., 2007). Once inside bacterial cells, AgNPs transform into a low molecular weight (MW) region at the center, shielding cellular DNA from silver ions and preventing DNA damage. Subsequently, the nanoparticles release silver ions within the bacterial cells, augmenting their bactericidal activity. AgNPs target and disrupt the respiratory chain, as well as interfere with cellular division, ultimately resulting in cell death (Marambio-Jones et al., 2010).

**Gold**

Gold nanoparticles (AuNPs) are biocompatible and find extensive use in tissue regeneration, targeted drug delivery, and wound healing. Unlike AgNPs, AuNPs do not possess inherent antimicrobial activity as a standalone material. Therefore, AuNPs must be combined with other biomolecules to effectively utilize them in biomedical applications. When cross-linked with collagen, AuNPs can easily integrate with other biomolecules such as polysaccharides, growth factors, peptides, and cell adhesion molecules by binding to the gold surface without altering the collagen structure (Akturk et al., 2016). These modified AuNPs exhibit properties like biocompatibility and biodegradability, making them suitable for widespread use in wound healing. Similarly, gelatin and chitosan can also be easily incorporated with AuNPs, showing safe and beneficial effects in enhancing wound healing (Jayakumar et al., 2011).

**Zinc Oxide**

Zinc oxide (ZnO) functions as an inorganic antibacterial agent, offering superior stability compared to organic alternatives. Zinc, an essential element with prolonged activity within living cells, plays a vital role in wound healing, particularly in cases of delayed healing and burns. Topical application of zinc has demonstrated effectiveness in reducing inflammation, promoting re-epithelialization, and inhibiting bacterial growth in chronic wounds. As a cofactor for metalloproteinases, zinc significantly contributes to the regeneration of the extracellular matrix (ECM). ZnO nanoparticles possess antibacterial, anti-1inflammatory, and antiseptic properties, leading to their widespread use in cosmetics, skin creams, and ointments (Pati et al., 2014; Garg et al., 2015).

**Combination of Natural Products and Nanoparticles in Wound Healing**

Natural products exhibit a wide array of pharmacological activities, including anti-tumor, anti-inflammatory, and antioxidant properties, making them valuable sources for drug development to prevent and treat various complications. However, the inherent limitations of natural products in physiological environments, such as poor solubility, stability, and short biological half-life, hinder their clinical application (Zhu et al., 2022).

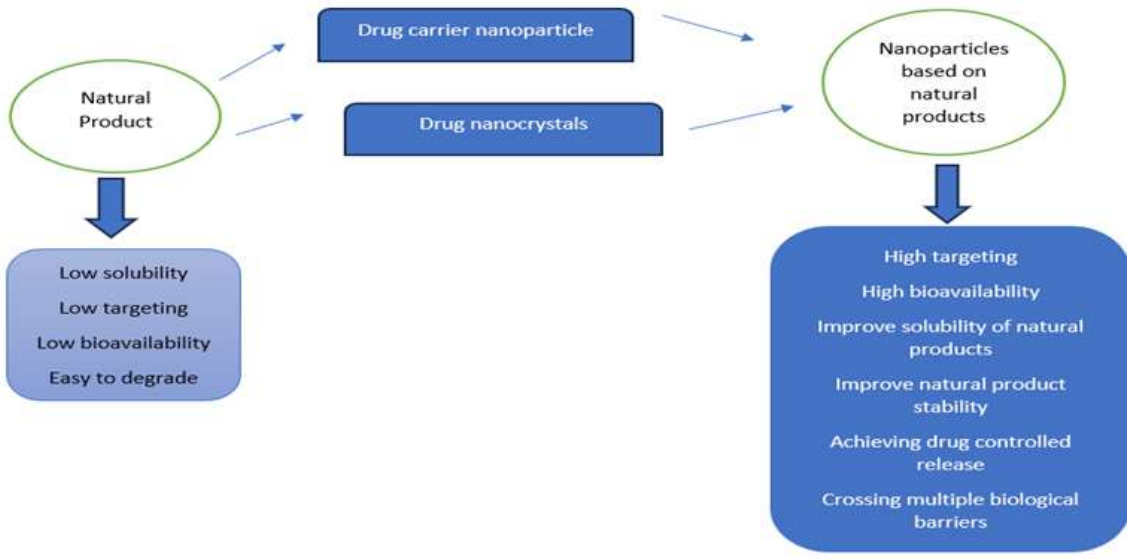


**Fig. 2:** Inherent defects of natural products and advantages of natural product nanoparticles

Nonetheless, a considerable body of in vitro and in vivo research has demonstrated the efficacy and safety of nanomedicine based on natural products in preclinical models of various diseases. Combining nanoparticles with

natural products has shown promise in the treatment of various wounds, proving to be both efficacious and beneficial as shown in Fig. 2.

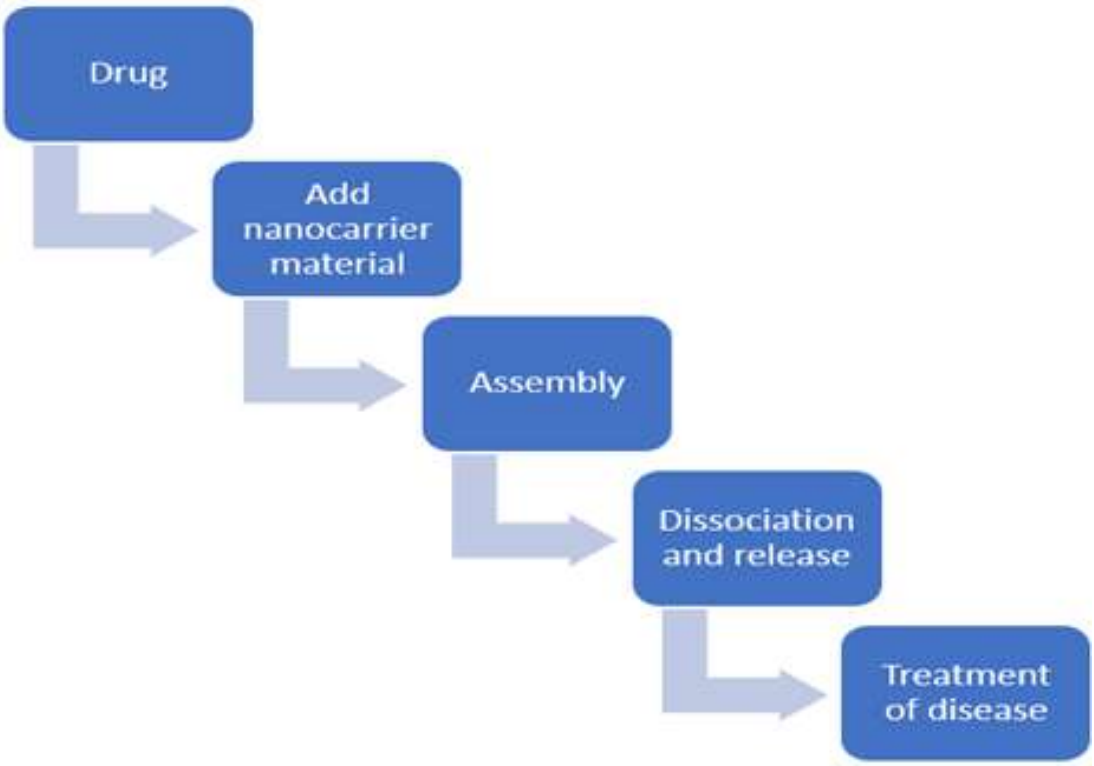


**Fig. 3:** Nanomedicine therapy strategies based on natural products.

Nanometer-sized particles exhibit unique properties and functions, as evidenced by clinical experience. The application of nanoparticles has been shown to increase the accumulation of active ingredients at lesion sites and wounds, mitigate the systemic toxicity of drugs, enhance the solubility of insoluble drugs, improve drug stability in vivo, and reduce drug resistance (Zahedi et al., 2012). Nano drugs typically have a size of less than 1000 nm. One technique involves drug loading by nanoparticles, wherein pharmacologically active compounds are either physically enclosed within nanoparticles or chemically attached to their surface as shown in Fig.3. Nanocarriers primarily include lipid nanoparticles, Nano-emulsions, polymer nanoparticles, inorganic nanoparticles, and biological nanoparticles (Agrahari et al., 2019).

**Curcumin Loaded Lignin Nanoparticles**

Lignin nanoparticle, recognized as the most abundant biomass material, holds significant importance as a biopolymer globally (Tang et al., 2020). It serves as a notable source of natural antioxidants and demonstrates a wide array of beneficial properties, including anti-inflammatory, antibacterial, antifungal, antiparasitic, antigenotoxic, anticarcinogenic, and antimutagenic activities (Vinardell et al., 2017).

Curcumin, being a natural product, derived from the rhizome of *Curcuma longa*, a member of the *Zingiberaceae* family, is a traditional herbal medicine renowned for its antibacterial, anti-inflammatory, and antioxidant properties. It serves as a novel therapeutic agent for wound healing, as supported by research (Nguyen et al., 2013; Gunes et al., 2016). In numerous in vitro and animal studies, curcumin has been shown to promote wound healing (Kulac et al., 2013). It exerts its effects by reducing oxidative stress through interaction with free radicals, also known as reactive oxygen species (ROS) (Tang et al., 2020)**,** and acts as a scavenger for free radicals. By doing so, curcumin mitigates oxidation through both enzymatic and non-enzymatic mechanisms, consequently dampening wound inflammation.

Curcumin has been found to enhance extracellular matrix (ECM) biosynthesis and promote the expression of transforming growth factor beta (TGF)-β in wounds, facilitating wound contraction (Thangapazham et al., 2013). Furthermore, it inhibits DNA breakage and lipid peroxidation, contributing to its beneficial effects on wound healing. Curcumin accelerates the wound-healing process by reducing inflammation through increased pro-inflammatory activity, which occurs downstream of the inhibition of nuclear factor kappa B signaling (Sandur et al., 2007). Additionally, curcumin facilitates a swift transition from the inflammatory phase to the proliferative phase of healing. This transition is marked by increased neovascularization, accelerated re-epithelialization, heightened collagen deposition, and enhanced tissue formation (Panchatcharam et al., 2006).

In general, topical application of curcumin is favored for cutaneous wound healing over oral or systemic administration, as it allows direct access of the agent to the wound site. However, curcumin's therapeutic utility has been

limited due to its poor solubility in aqueous media, minimal skin permeability, rapid metabolism, and low stability, making it unsuitable for wound healing treatment (Flora et al., 2013).

Recently, the application of curcumin for wound healing has been enhanced through the design of delivery systems aimed at improving its solubility, stability, and sustained release in the wound area. One effective approach to enhancing curcumin's delivery across skin layers is by encapsulating it in nanoparticles (Bhawana et al., 2011; Krausz et al., 2015).

**Efficacy of Silver Nanoparticles from Azadirachta indica**

Silver stands out among various metals in therapeutic applications due to its inherent antimicrobial properties. AgNPs, synthesized from plants abundant in secondary metabolites, have been extensively studied for their intrinsic antibacterial properties against foodborne pathogens and antibiotic-resistant bacteria (Jain et al., 2017; Loo et al., 2018).

Among different drug delivery systems, thermo-sensitive hydrogels are preferred for tissue engineering and wound healing applications due to their capacity to hold water, uniform dispersion of therapeutic agents, and controlled release (Huang et al., 2019). Thermo-reversible Pluronic F-127, which undergoes sol-gel transition at body temperatures, has been shown to enhance topical wound healing (Arafa et al., 2018).

*Azadirachta indica* (AI), commonly known as neem, is a tropical tree native to the Indian subcontinent, but its distribution has spread worldwide. The United Nations has recognized the importance of this tree and bestowed upon it the title of "Tree of the 21st Century"

AgNPs have emerged as key players in disease management due to their unique properties, including small dimensions, large surface area, mechanical and thermal stability, chemical inertness, electrical conductivity, biosensing capabilities, and antimicrobial activity (Rai et al., 2014).

Untreated wounds are vulnerable to infections caused by bacteria such as *Staphylococcus aureus*. The broad-spectrum antimicrobial properties of AgNPs have spurred the development of AgNPs-based dressings for wound healing. Tian et al. (2007) found that AgNPs could effectively treat inflammation by modulating cytokine levels and promoting wound healing while reducing scar formation (Franková et al., 2016). Further studies have demonstrated the potential of Azadirachta indica (AI) - AgNPs in disrupting bacterial cells. Combined with its free-radical scavenging ability, AI-AgNPs hold promise for the development of wound dressings. Reactive oxygen species (ROS) play a crucial role in wound healing, as they help combat invading microbes when present in low concentrations. However, an imbalance in the oxidative-antioxidant respiratory system can lead to excessive ROS production (Cano Sanchez et al., 2018), which can be detrimental to wound healing when accumulated excessively within cells.

Topical application of AgNPs has been shown to stimulate the wound healing process, including remodeling, re-epithelialization, and wound contraction. According to Diniz et al. 2020, wounds treated with chemically synthesized AgNPs loaded on gelatin hydrogel took more than 14 days to heal completely. In contrast, another study demonstrated that AI-AgNPs in PF127 hydrogel led to a more effective healing effect in just 10 days. This was attributed to the continuous release of AI-AgNPs from the hydrogel, allowing them to timely enter the physiological system and interact with inflammatory cells present at the wound sites. This slow release ensured minimal damage to normal cells while prolonging the wound-healing effect.

**Alginate-Based Materials Loaded with Nanoparticles**

Alginate is a polysaccharide widely acknowledged for its safety, biocompatibility, and biodegradability. It finds applications in various fields, including food manufacturing (Bi et al., 2022), drug delivery systems (Jadach et al., 2022), the cosmetic industry (Kozlowska et al., 2019), tissue engineering (Sahoo et al., 2021), and the production of materials used in wound management. Indeed, the application of alginate in wound dressings is not a recent concept. Alginate-based dressings have been effectively marketed and utilized in wound management since the 1980s.

Alginate dressings are versatile and can be used to treat a range of wounds, including superficial wounds, lacerations, and cavities often found in the development of pressure ulcers. Due to their strong absorbent properties, alginate dressings are particularly recommended for managing wounds with moderate to high levels of exudate. Furthermore, available alginate products may vary in composition and properties, as they can be combined with other polymers or silver for added benefits. This diversity allows healthcare providers to choose the most suitable product based on the specific needs of the wound being treated as described in given Table 2 (Froelich et al., 2023).

Enriching alginate dressings with nanoparticles as carriers for therapeutic agents is a fascinating and extensively explored approach. This method allows for significant modification of the properties of the original dressing, resulting in the creation of novel materials with interesting therapeutic features depending on the type of nanoparticles used.

Currently, several silver-enhanced dressings with antibacterial properties have been introduced to the pharmaceutical market, some of which are alginate-based materials. However, most commercially available products contain silver in an ionic form (Rybka et al., 2022). Only a few dressings incorporate nanocrystalline silver, such as the collagen-based Acticoat®. Materials for wound healing that incorporate nanoparticles offer several important advantages. One such advantage is the ability to prolong the release of drugs, which can result in a reduced frequency of dressing changes.

**Table 2:** Commercially available alginate-based products (Froelich et al., 2023)

| Brand Names | Manufacturer | Composition | Applications |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Sorbalgon® | Hartmann (Heidenheim, Germany) | Calcium alginate | Moderate to heavily exuding wounds. |
| | | | Moderate to heavily exuding wounds, infected wounds |
| Sorbalgon® Ag | | Calcium alginate, silver | |
| Tegaderm™ Alginate | 3M™ (Saint Paul, MN, USA | Calcium alginate | Moderate to heavily exuding wounds |
| CarboFlex® | | Five-layer dressing with alginate absorbent layer, internal charcoal layer, and water-resistant top. | Malodorous wounds |
| | | | Moderate to heavily exuding wounds |
| Kaltostat® | ConvaTec (Reading, UK) | Calcium sodium alginate | |
| Comfeel® Plus Ulcer | | CMC (1 Carboxymethyl cellulose) particles, alginate (as an additive), polyurethane semipermeable film. | Low to moderately exuding wounds. |
| | Coloplast (Humlebaek, | | |
| Biatain® Alginate Ag | Denmar) | | |
| | | Alginate, CMC, ionic silver complex | Moderate to heavily exuding wounds, infected wounds |
| Biatain® Alginate | | Alginate (85%), CMC 1 | Alginate (85%), CMC 1 |

Additionally, for active agents prone to degradation, encapsulation within nanoparticles may enhance stability. However, the development of nanomedicines presents challenges, and concerns regarding the safety and potential accumulation of nanoparticles in the body are frequently raised. Despite these challenges, dressings embedded with therapeutic nanoparticles represent one of the most promising approaches for more efficient wound management. This is exemplified by the commercial success of dressing materials containing nanocrystalline silver (Carpa et al., 2022).

Metal and metal oxide nanoparticles have attracted growing attention in recent years as antimicrobial agents for various biomedical applications, particularly in preventing wound infections. Given the increasing concern about antibiotic resistance, nanoparticles such as silver, zinc, copper, gold, titanium, magnesium, and cerium oxides are viewed as potential tools to address this problem (Pelgrift et al., 2013). These nanoparticles have been incorporated into various biopolymeric hydrogel matrices, including alginate materials, for wound healing purposes (Wahid et al., 2017).

**Silver Nanoparticles Phyto fabricated through Azadirachta indica**

Natural products like *Azadirachta indica* and *Syzygium aromaticum* (clove) have been studied for their antimicrobial effects against *Enterococcus faecalis* (Thosar et al., 2013; Shah et al., 2014). Additionally, AgNPs have opened up numerous novel avenues in nano-biotechnological protocols. Their larger surface area-to-volume ratios contribute to their marked reactivity, and their extremely small size allows AgNPs to penetrate cells, interact with organelles, and exert distinct biological effects.

AgNPs have been shown to promote wound healing through several mechanisms. They can impede biofilm formation and disrupt microbial membrane structures, leading to the destruction of pathogens such as *Staphylococcus spp., Enterococcus spp., V. cholerae, and B. subtilis* (Bowler et al., 2012). Moreover, AgNPs exhibit cytotoxicity against these pathogens.

Novel collagen and chitosan scaffolds containing AgNPs at a concentration of 10 µg/mL have demonstrated the ability to promote wound healing through cellular migration (You et al., 2017). Additionally, AgNPs have been found to possess anti-inflammatory properties, further contributing to their effectiveness in wound healing.

AgNPs, along with their composites, assemblies, and complexes, are widely employed for antimicrobial and wound-healing purposes. Incorporating metals, metal oxide nanoparticles, and silver-containing compounds into various forms such as gels (Orlowski et al., 2018), hydrogels or gelling fibers (Kwan et al., 2018), and mesh or polymeric membranes has been highlighted as a natural solution for developing unique bandages (Gao et al., 2019).

Nanogels and nanomeshes functionalized with AgNPs, growth hormones, antibiotics, or enzymes have been proposed as wound-dressing systems to enhance wound healing, reduce inflammatory responses, and bolster immune responses (Shi et al., 2018). AgNPs incorporated into wound-dressing polymers such as alginate, cotton fabrics, cellulose, or chitosan have been shown to promote wound healing and control the growth of multidrug-resistant microbes (Paladini et al., 2015; Ballottin et al., 2017). Ag+ ions have the ability to create pores in bacterial cell walls by binding to sulfur- and phosphorus-containing proteins in the cell wall and membrane (Kim et al., 2016).

Ag+ ions have been shown to disrupt cellular permeability (Jena et al., 2012), induce oxidative stress, and lead to cell death (Hindi et al., 2009). These factors contribute to the antibacterial effects of AgNPs by causing protein leakage, compromising cellular wall integrity, and inactivating lactate dehydrogenase (LDH) through reactive oxygen species (ROS) production.

**Phyto-Engineered Gold Nanoparticles**

AuNPs have garnered significant attention in antibacterial, antioxidant, and wound-healing applications, especially when combined with natural phytoconstituents (Kuppusamy et al., 2016; Abdel-Raouf et al., 2017; Boomi et al., 2019). Insulin plant *Chamaecostus cuspidatus*-mediated AuNPs have been shown to restore normal blood glucose, glycogen, and insulin levels, demonstrating their potential for in vivo wound-healing activity (Ponnanikajamideen et al., 2019).

Arafa et al. formulated two transdermal formulations containing AuNPs and evaluated their in-vitro antibacterial activity against *Staphylococcus aureus*, commonly associated with burn infections. Using a model of burn-induced infected wounds in mice, the developed formulations showed antibacterial activity along with promising wound-healing properties as demonstrated by in vivo and histopathological studies (Arafa et al., 2018). AuNPs have been employed to enhance wound healing by stimulating various phases of the healing process.

AuNPs have been shown to accelerate the resolution of the inflammatory stage, promote blood vessel formation, and facilitate remodeling of the collagen matrix, thereby contributing to faster skin regeneration and improved wound healing (Krychowiak et al., 2014; Shankar et al., 2015; Hajialyani et al., 2018;). Additionally, AuNPs possess inherent antibacterial properties that aid in reducing wound infection during the inflammatory and hemostasis phases (Nethi et al., 2019). The mechanisms underlying the wound-healing activity of AuNPs may involve pathways such as angiogenesis, alteration of membrane potential, inhibition of ATP synthase enzyme, and modulation of intracellular ROS levels, ultimately leading to enhanced energy metabolism and wound healing.

**Future Directions**

The future trajectory of wound management utilizing nanoparticles could center on several pivotal domains. Further investigation is imperative to evaluate the prolonged safety and biocompatibility of nanoparticle-infused wound dressings. Understanding their potential toxicity and impact on the human organism is fundamental for eventual clinical implementation.

Ongoing endeavors should be channeled towards refining nanoparticle formulations to bolster their effectiveness in wound healing. This encompasses exploring novel synthesis techniques, enhancing drug loading and release kinetics, and fine-tuning the physicochemical attributes of nanoparticles tailored to specific wound types. Exploring the synergistic effects of pairing nanoparticles with other therapeutic agents, such as natural products or growth factors, holds promise for augmenting wound healing outcomes. Combinatorial strategies may offer heightened efficacy and address various facets of the wound healing process concurrently.

Innovating targeted delivery mechanisms to precisely transport nanoparticles to the wound site could amplify their therapeutic potency while mitigating off-target effects. This may entail leveraging smart materials or stimuli-responsive nanoparticles that release their cargo in response to specific cues within the wound microenvironment.

Progressing towards clinical application necessitates rigorous preclinical investigations to establish the safety and efficacy of nanoparticle-based wound dressings in relevant animal models. Furthermore, conducting well-designed clinical trials to assess their effectiveness in human subjects is imperative for widespread adoption in clinical settings.

**Conclusion**

In summary, incorporating nanoparticles into wound-healing materials holds great promise for advancing wound management. AgNPs and AuNPs have shown impressive antimicrobial, anti-inflammatory, and wound-healing properties, effectively combating infections and promoting tissue regeneration. Furthermore, integrating natural products like *Azadirachta indica* (neem) and *Curcuma longa* (turmeric) enhances the therapeutic potential of these materials. Alginate-based dressings enriched with nanoparticles offer prolonged drug release and enhanced stability, making them attractive for clinical use. However, challenges such as nanoparticle safety and accumulation in the body remain, underscoring the need for ongoing research to refine nanoparticle-based wound dressings and improve patient outcomes.

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Chapter 17

# Innovations in Synthetic Techniques, Characterization, and Pharmaceutical Applications of Nanoparticles

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**ABSTRACT**

Nanoscience and nanotechnology have emerged as the fastest-growing scientific fields in the past ten years. Metallic nanoparticles (NPs) are critical in various scientific domains, including mechanics, physics, medicine, and pharmaceutics. Researchers have discovered that metal nanoparticle manufacturing and applications have a lot of potential and even employed several spectroscopic or microscopic characterization techniques. Metallic NPs are the focus of this chapter, with their advantages and use in pharmaceutics being the main subject of discussion. It covers a variety of topics, namely the different methodologies for the synthesis of metallic NPs with physical, chemical, and biological approaches; as well as related characterization methods, such as spectroscopic, microscopic, and physiochemical techniques, and the numerous ways that the metal NPs are used in tissue engineering, bio-medicines, enzymology, surface coating, biosensing devices, diagnostics, and theragnostic, before concluding with the potential uses of metal NPs in the future.

**KEYWORDS**
Nanoparticle, Biological Techniques, Chemical Techniques, Physical Techniques.

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## INTRODUCTION

Nanotechnology includes the synthesis and commercialization of various nanomaterials. The study of nanotechnology makes it possible to synthesize various NPs with distinctive characteristics (Adil et al., 2023). NPs comprise intricate material particles having a diameter ranging from 1 to 100 nm. The nanoscale dimensions of these particles determine their chemical, optical, physical, and electrical characteristics (Bhattacharjee and Bose, 2021). The synthesis, development, and promotional efforts of these nanomaterials are all of the aspects of nanotechnology. Beside this, nanotechnology facilitates the development of many NPs with distinctive characteristics. The main nanoparticle, or core, is surrounded by a shell made of several layered materials and has a functionalized surface upon which it resides (Bolokang et al., 2015). The massive area-to-volume ratio, interfacial layer, affinity to solvents, type of coating, quantum mechanical effects, rate of diffusion, and mechanical, and ferromagnetic aspects distinguish the properties of materials in their bulk form from those in their nanoparticle form (Mehdaoui et al., 2011).

This chapter addresses the potential benefits of using metal NPs in drug delivery systems as an alternative application in pharmaceutical systems. It also discusses the various physical, chemical, and biological approaches that can be used to synthesize different metal NPs, as well as related characterization techniques, such as spectroscopic, microscopic, and physiochemical techniques. Furthermore, the applications of metal NPs in medical technology, tissue engineering, and the various ways in which they are employed in biomedicine, tissue engineering, and diagnostic methods culminate in the potential applications of metal NPs in the future.

### Classification of NPs

NPs may be artificial, engineered, metallic, non-metallic, organic, or inorganic. Non-metallic NPs consist of silica and carbon nanotubes, whereas metallic NPs include copper, magnesium, zinc, gold, titanium and silver etc (Khan et al., 2022). Anthropogenic NPs are by-products of industrial production, whereas designed NPs are directly obtained from manufacturing processes (Barhoum et al., 2022). Some of the NPs and their features (Khan et al., 2019; Khan and Hossain,

2022) have been summarized in Table 1.

**Table 1:** Some NPs and their Respective Features

| NPs | Features | References |
|---|---|---|
| Silver | Extremely potent, excellent antibacterial activity, wide use | Khan et al., 2022 |
| Gold | Effective for diagnosing cancer and other microorganisms, good at detecting protein interactions, helpful in tracking fingerprints, and able to detect antibiotics and malignant cells. | Khan and Hossain, 2022 |
| Iron | Suitable for medication administration, gene analysis, cancer treatment, and stem cell sorting, biocompatible | (Harish et al., 2022) |
| Quantum dot | less than 10 nm in diameter, semiconducting, and size-dependent | Khan and Hossain, 2022 |
| Carbon nanotubes | Strong electron bonds, excellent electrical conductivity, sp2 hybridized carbon atoms, and effective catalysts. | (Harish et al., 2022) |
| Copper | Good-quality NPs are produced by a broad absorption spectrum and unique optical characteristics. | Khan et al., 2022 |
| Ceramics | Inorganic amorphous solids are widely used in photocatalysis, imaging devices, and other fields. They can be polycrystalline, porous, amorphous, or dense. | Khan et al., 2019 |
| Semiconductor | Excellent for electronic equipment and water splitting due to its large and adjustable band gap nature. | Khan and Hossain, 2022 |
| Polymeric | Mostly organic materials that can readily be functionalized | Khan et al., 2019 |
| Lipid-based | Include lipid components and employ surfactants as central stabilizers. | Khan and Hossain, 2022 |

**Approach for NP Synthesis**

**Top-Down Approach**

The top-down destructive procedure for the synthesis of NPs reduces large molecules to tiny fragments before transforming them into suitable NPs (Priyadarshana et al., 2016). This Technique employs a variety of breakdown techniques, including physical vapor deposition (PVD), grinding, and chemical vapor deposition (CVD) (Abid et al., 2022). Milling is used to extract NPs from coconut shells as the crystallite size reduces over time. This procedure has been used to manufacture cobalt(III) oxide, carbon, iron oxide, and dichalcogenide NPs (Harish et al., 2022).

**Bottom-up Approach**

The technique involves the stepwise production of NPs from basic components. It is practical, affordable, less harmful, and environmentally friendly (Modan and PlĂIaȘU, 2020). Spin coating, green synthesis, biochemical, sol-gel, and other reduction and sedimentation methods are commonly utilized. This approach has been used to generate titanium dioxide, gold, and bismuth NPs (Ambre et al., 2023). The synthesis of NPs may also involve biological or chemical processes. Chemical synthesis procedures for NPs include sol-gel, wet chemical synthesis, hydrothermal, thermal decomposition, microwave, etc. In contrast, biological approaches employ enzymes, bacteria, plant extracts, and fungi (Hachem et al., 2022).

**Techniques for NP Synthesis**

**Chemical Method**

The following chemical processes are used to synthesize NPs such as sol-gel, precipitation, hydrothermal, thermal breakdown, solvothermal, vapor synthesis, etc (Rane et al., 2018).

**Wet Chemical Methods**

**Precipitation Technique**

This is a rapid and cost-effective method for producing NPs. It involves dissolving precursor salts in a suitable solvent. The reaction of the solutions induces the formation of solid NPs (nanoprecipitates) through mechanisms like ionic exchange or double displacement and separating the NPs from the solution using techniques like centrifugation or filtration. After that, washing and drying the NPs. This technique is suitable for a wide range of materials and offers good control over particle size through adjustments in precursor concentration, reaction temperature, and the use of additives. Precipitation technique is used to synthesize metal oxides (e.g., iron oxide, copper oxide), metal sulfides (e.g., cadmium sulfide, lead sulfide), and metal carbonates (e.g., calcium carbonate) (Darr et al., 2017).

**Hydrothermal Synthesis**

This technique utilizes high pressure and temperature in aqueous environments to promote reactions and crystal growth. It offers the control over nanoparticle morphology and crystallinity. Applicability to various materials, including oxides, sulfides, and phosphates. Hydrothermal synthesis technique is used to synthesize metal oxides (e.g., titania, zirconia), zeolites, and metal chalcogenides (e.g., molybdenum sulfide) (Darr et al., 2017).

**Sol-Gel Technique**

The simple sol-gel technique is conducive to nanostructures of the NPs with well-defined structures. It involves

such as dissolving precursors (metal salts, alkoxides) in a solvent to form a solution. Transformation of the sol into a gel through a gelation process, often involving condensation reactions. Drying and heat treatment of the gel to generate the final NPs. As a part of the process, the solvent is removed by gelation technique, so preliminary sol-gel is formed. Sol-Gel technique is used to synthesize metal oxides (e.g., silica, alumina), ceramic materials, and doped NPs. (Bokov et al., 2021).

**Solvothermal Synthesis**

Similar to hydrothermal synthesis, this technique uses elevated pressure and temperature, but with organic solvents instead of water. It allows for the synthesis of materials not readily achievable in water, and tailoring of nanoparticle properties through solvent selection. The solvothermal synthesis process can be utilized with solvents like metal, semiconductor, and polymer under the pressure range of medium to high to produce materials (Sasikala et al., 2017). Using regulated temperatures and shipments, the new and steady NPs resulted in the process. Several surfactant stabilizers are considered for optimal growth of nanodots from cationic source (Li et al., 2021). This process can be used to produce zinc oxide, zinc selenide, and cadmium selenide, which are used in the biotech and magnetic industries (Balakrishnan and Kadam, 2021).

**Vapour Phase Synthesis**

This method involves the reaction of gaseous precursors to form NPs. It includes techniques such as

**Chemical Vapor Deposition (CVD)**

This technique involves the decomposition of gaseous precursors on a heated substrate to form a thin film or NPs. The precursor molecules react and decompose on the hot surface, leaving behind the desired material as NPs. CVD offers high purity and good control over film thickness and nanoparticle uniformity. It's commonly used in microelectronics fabrication. CVD is used to synthesize silicon, silicon dioxide, metal nitrides NPs (e.g., silicon nitride, titanium nitride) (Danielson et al., 2020).

**Flame Spray Pyrolysis**

This technique involves introducing precursor solutions or suspensions into a hot flame, where they rapidly decompose and form NPs. The high temperature of the flame promotes rapid reaction and particle formation. Flame spray pyrolysis is a relatively inexpensive and scalable method for producing large quantities of NPs. However, precise control over particle size and morphology can be challenging. Flame Spray Pyrolysis is used to synthesize NPs such as Metal oxides (e.g., titania, alumina), carbon black, and ceramic materials. (Danielson et al., 2020).

**Laser Ablation**

This technique utilizes a pulsed laser beam to vaporize a target material, creating a plume of vapor that condenses into NPs. The high energy of the laser pulse allows for ablation of various materials (Laser ablation offers high purity and good control over nanoparticle size distribution. However, it can be expensive and requires specialized equipment. The laser ablation technique is used to synthesize metals NPs (e.g., gold, silver), metal oxides, and complex materials. Agglomerated particles are created, however, the particles produced by converting gases in furnace reactors or hot walls are typically quite clean (Zhao et al., 2024).

**Electrostatic Deposition (Electrospray)**

This technique utilizes an electric field to deposit charged NPs onto a substrate. The NPs are suspended in a liquid and sprayed as an aerosol. The electric field accelerates the charged particles toward the grounded substrate, allowing for controlled deposition. Electrostatic deposition is a versatile technique for creating patterned nanoparticle films. It is used for various types of NPs depending on the precursor solution, often used for depositing pre-synthesized NPs (Danielson et al., 2020).

**Microwave-Assisted Synthesis**

Microwaves can be used to accelerate reactions and promote nanoparticle formation. Microwaves interact with polar molecules in the reaction mixture, leading to rapid and uniform heating. This can enhance reaction rates and improve nanoparticle yield and uniformity. Microwave-assisted synthesis offers faster processing times and potentially higher energy efficiency compared to conventional heating methods. It is used for various types of materials similar to conventional wet chemical methods (John and Tricoli, 2022).

**Photochemical Reduction**

Light irradiation can be used to induce the reduction of precursor materials into NPs. Light energy excites electrons in the precursor molecules, facilitating their reduction to form the desired NPs. Photochemical reduction is a clean and environmentally friendly method for synthesizing NPs. However, it often requires specific light sources and careful control of reaction conditions. It is used for metal NPs (e.g., silver, gold), semiconductors (e.g., titanium dioxide) (Rane et al., 2018). The advantages and disadvantages of chemical methods for the synthesis of NPs are

discussed in Table 2.

**Table 2:** Chemical Methods for Nanoparticle Synthesis

| Method | Description | Advantages | Disadvantages | Examples of Materials | Ref. |
|---|---|---|---|---|---|
| a). Wet Chemical Methods | | | | | |
| Precipitation Technique | Rapid and cost-effective method using precursor salts. NPs form through reactions like ionic exchange or double displacement. | Simple, rapid, cost-effective | Limited control over size and morphology | Metal oxides (iron oxide, copper oxide), metal sulfides (cadmium sulfide), metal carbonates (calcium carbonate) | (Rane et al., 2018). |
| Hydrothermal Synthesis | Utilizes high pressure and temperature in water to promote reactions and crystal growth. Offers control over morphology and crystallinity. | High-quality NPs, good control over morphology | Requires specialized equipment, high pressure, and temperature | Metal oxides (titania, zirconia), zeolites, metal chalcogenides (molybdenum sulfide) | |
| Sol-Gel Technique | Well-suited for nanostructures with defined structures. Involves dissolving precursors in a solvent, forming a gel, drying, and heat treatment. | High purity, well-defined structures, precise control over properties | A multi-step process can be time-consuming | Metal oxides (silica, alumina), ceramic materials, doped NPs | (Bokov et al., 2021) |
| b) Solvothermal Synthesis | Similar to hydrothermal synthesis but uses organic solvents instead of water. Allows for materials not achievable in water and tailorable properties. | Synthesis of materials not achievable in water, tailorable properties | Requires specialized equipment, high pressure, and temperature | Zinc oxide, zinc selenide, cadmium selenide | (Balakrishnan and Kadam, 2021) |
| c) Vapor Phase Methods | | | | | |
| Involves reactions of gaseous precursors to form NPs. | | | | | |
| Chemical Vapor Deposition (CVD) | Decomposition of gaseous precursors on a heated substrate to form thin films or NPs. | High purity, excellent control over film thickness and uniformity | Expensive, requires specialized equipment, limited to certain materials | Silicon, silicon dioxide, metal nitrides (silicon nitride, titanium nitride) | (Danielson et al., 2020) |
| Flame Spray Pyrolysis | Precursor solutions/suspensions are introduced into a hot flame for rapid decomposition and nanoparticle formation. | Scalable, inexpensive | Limited control over size and morphology, potential for agglomeration | Metal oxides (titania, alumina), carbon black, ceramic materials | (Danielson et al., 2020) |
| Laser Ablation | A pulsed laser beam vaporizes a target material, creating a plume of vapor that condenses into NPs. | High purity, ability to ablate various materials | Expensive, requires specialized equipment, limited control over size distribution | Metals (gold, silver), metal oxides, complex materials | (Zhao et al., 2024) |
| d) Electrostatic Deposition (Electrospray) | Utilizes an electric field to deposit charged NPs onto a substrate. | Versatile for creating patterned films | Requires control over nanoparticle charge and solution properties | Various types of NPs depend on the precursor solution | (Danielson et al., 2020) |
| e) Microwave-Assisted Synthesis | Microwaves accelerate reactions and promote nanoparticle formation through rapid and uniform heating. | Faster processing times, potentially higher energy efficiency | May require optimization of reaction conditions for specific materials | Various materials similar to conventional wet chemical methods | (John and Tricoli, 2022) |
| f) Photochemical Reduction | Light irradiation reduces precursor materials into NPs by exciting electrons. | Clean, environmentally friendly | Requires specific light sources, careful control of reaction conditions | Metal NPs (silver, gold), semiconductors (titanium dioxide | (Rane et al., 2018). |

**Physical Methods**

Physical methods for nanoparticle synthesis involve breaking down bulk materials into NPs through various mechanical processes. These methods offer advantages like scalability and control over particle size and morphology

(Danielson et al., 2020).

**Mechanical Milling**

Mechanical milling encompasses techniques like ball milling and high-energy ball milling to produce NPs. The media particles applied are slamming against the chamber, or shearing of particles occurs from the motion of the grinding media. This difficulty-expanding particle processing mechanism eventually produces very minuscule particles often on the nanoscale order of magnitude. Its advantages are scalability, and control over the size and morphology of NPs. The challenges of mechanical milling are contamination, broad size distribution and heat generation (Iqbal et al., 2016).

**Mechanochemical Processing**

Mechanochemical processing combines mechanical milling with chemical reactions. The milling process facilitates intimate contact between reactant materials, promoting chemical reactions at relatively low temperatures compared to conventional methods. This approach allows for the Synthesis of New Materials, Reduced Agglomeration (Yadav et al., 2012). Some other Physical methods are discussed in Table 3

**Table 3:** Physical and Chemical Methods for Nanoparticle Synthesis

| Method | Category | Description | Advantages | Disadvantages | Examples of Materials | Ref. |
|---|---|---|---|---|---|---|
| Physical Vapor Deposition (PVD) | Physical | Vaporization of a solid or liquid material followed by deposition onto a substrate. | High purity, good control over film thickness and uniformity | Expensive, requires specialized equipment, limited to certain materials | Silicon, silicon dioxide, metal nitrides | (Chan et al., 2022) |
| Laser Ablation | Physical | A pulsed laser beam vaporizes a target material, forming NPs from the condensed vapor. | High purity, ability to ablate various materials | Expensive, requires specialized equipment, limited control over size distribution | Metals (gold, silver), metal oxides, complex materials | (Ezealigo et al., 2021) |
| Sputtering | Physical | Energetic ions bombard a target material, ejecting atoms that condense into NPs on a substrate. | Precise control over film thickness and composition | Complex equipment can be slow | Metals, semiconductors, oxides | (Mughal et al., 2021) |
| Arc Discharge | Physical | Electric arc creates a high-temperature plasma that vaporizes the target material, leading to nanoparticle formation. | Scalable, can handle refractory materials | High temperatures can damage substrates, potential for contamination | Metals, carbides, nitrides | (Zhao et al., 2024) |

**Biological Methods**

Biological methods for nanoparticle synthesis offer a clean, eco-friendly, and sustainable alternative to chemical approaches. These methods utilize microorganisms, plants, or enzymes to produce NPs with unique properties (Ezealigo et al., 2021).

**Microorganism-Mediated Synthesis**

The Microorganism-Mediated Synthesis of NPs using microorganisms (bacteria and fungi) is a clear, uncontaminated, and environmentally-supported method. This methodology has been employed to make various NPs, such as iron oxide, silver, nickel oxide, copper oxide and zinc ferrite etc. Intracellular and extracellular synthesis of NPs depends on microorganisms that can synthesize NPs either inside their cells (intracellular) or outside (extracellular) Samrot et al., 2021).

**Intracellular synthesis of NPs OR Bioreduction**

Bioreduction is a type of intracellular synthesis where enzymes naturally present within microbes facilitate the reduction of metal ions into NPs. This process often results in well-defined NPs with smaller sizes. In manufacturing intracellular NPs, Metal ions are taken up by the microbes, and enzymes within the microbe act as catalysts, reducing the metal ions and promoting their transformation into NPs. The size and morphology of the NPs can be influenced by the specific enzymes involved and the cellular environment. The microorganisms used in intracellular synthesis of NPs or bioreduction are bacteria including *E. coli, Bacillus subtilis* and *Pseudomonas aeruginosa,* and Fungi including *Aspergillus fumigatus* and *Fusarium oxysporum* (Chan et al., 2022).

**Extracellular Synthesis**

Extracellular synthesis involves the production of NPs outside the microbial cell. This method typically utilizes fungi with secretory organs that can release enzymes and biomolecules into the surrounding environment. These enzymes and biomolecules then interact with metal ions in the solution to promote nanoparticle formation. As they are cost-effective, non-toxic, and in heavy metal detoxification, microorganisms are entrenched in the nanoparticle production (Ezealigo et

al., 2021).

**Plant-Mediated Synthesis (Phyto-nanotechnology)**

Despite not using toxic synthetic agents, phyto nanotechnology enables the production of plant-based NPs that are compatible with biological systems alongside the usage of raw materials that are abundant and stable. Plants offer another route for nanoparticle synthesis using plant extracts (Murtaza et al., 2024). The geometric features of plants, the amount of binding, as well as the concentration of the metal ions available for biosynthesis, are all altered by the pH levels. The different kinds of sources of particles, ways of synthesis, and areas of application of the NPs are explained in Fig 1. However, factors like plant species, pH levels, and the presence of metal ions can influence the size, shape, and properties of the resulting NPs (Jamkhande et al., 2019). This method is cost-effective and non-toxic, biocompatible, and abundant and renewable raw materials. Utilizing waste materials, bacteria, and fungi, biogenic methods of generating NPs are inexpensive and environmentally friendly (George et al., 2021).

**Enzymatic Synthesis**

This method utilizes enzymes to promote the formation of NPs. Enzymes act as catalysts, accelerating specific reactions and enabling control over nanoparticle size and morphology. Enzymatic synthesis can be intracellular such as enzymes naturally present within microorganisms can be leveraged for intracellular nanoparticle production. And in vitro such as Isolated and purified enzymes can be used in a controlled environment to synthesize NPs outside of living cells. The advantages of biological methods are environmental friendly, cost-effective and scalability (Mughal et al., 2021).

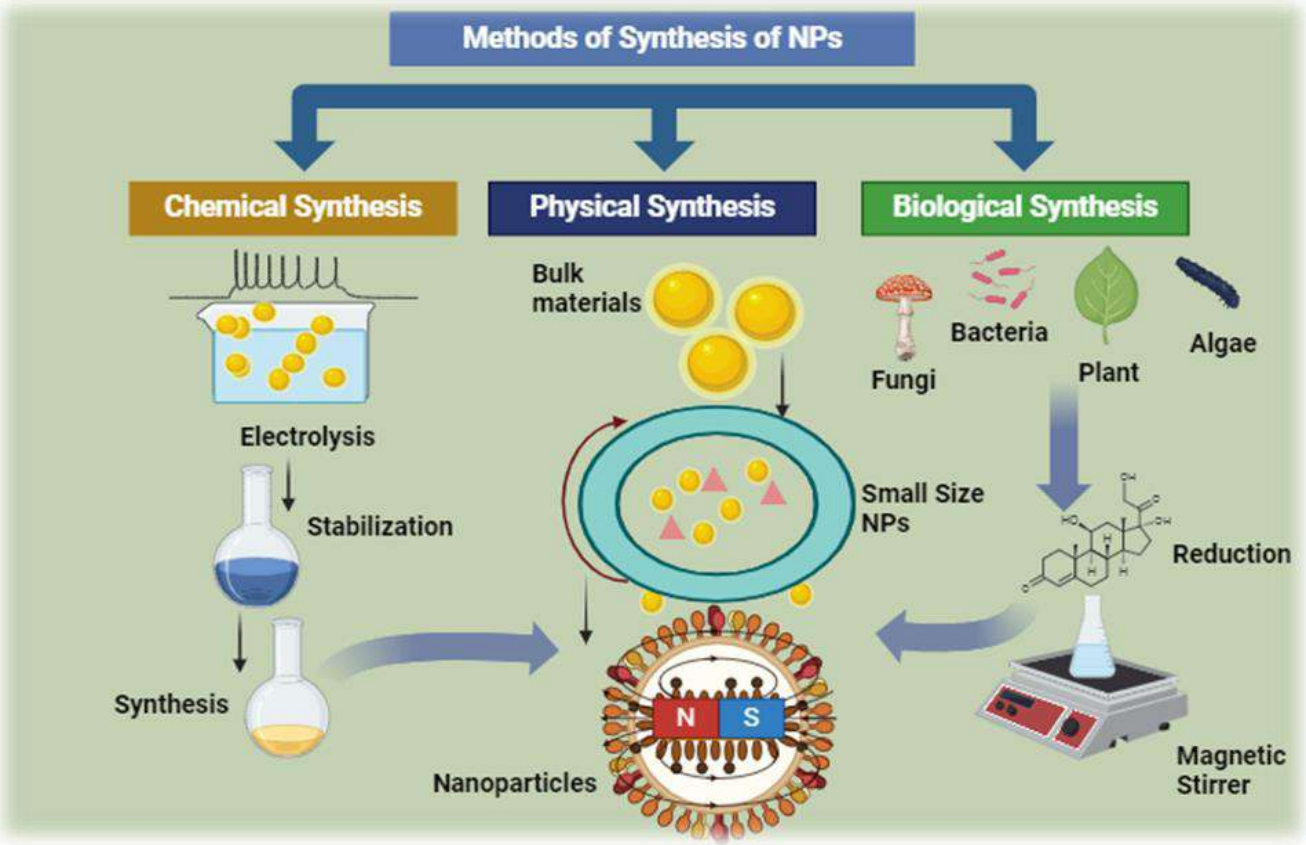


**Fig. 1:** Methods of Synthesis of NPs

**Characterization Methods for NPs**

It is necessary to thoroughly characterize NPs using pertinent characterization techniques to determine their shape, size, surface morphology, crystalline nature, light absorption, and other characteristics (Cuenya, 2010). Some of the methods used to characterize NPs include:

**Morphological Features**

An important factor influencing the characteristics that NPs exhibit is their shape. The dispersion and shape of NPs can be studied at the nanoscale and surface using a scanning electron microscope (SEM) (Tang et al., 2020). Utilizing electron transmittance, transmission electron microscopy (TEM) may obtain large amounts of data at both high and low magnifications (Zhang et al., 2020). Because NPs are smaller than the limit of light diffraction, the optical microscopic approach does not apply to them. Elemental studies would be possible with the combination of electron microscopes and

spectroscopic methods (Taghavi Fardood et al., 2020).

**Optical Studies**

The photochemical, luminescence, transmittance, and reflectance characteristics of NPs are found by using optical methods, which reveal these properties. Spectroscopy is a measurement that estimates the size, shape, and concentration of NPs by analyzing how they respond to electromagnetic waves and light (Gudkov et al., 2023). The use of NPs as a function of spectroscopy is of soaring importance and includes infrared, UV–VIS, photoluminescence (PL), DRS, and MRI (Wang et al., 2020; Mmelesi et al., 2024).

**Structural Analysis**

The distinctive qualities of the bulk material are projected through the crystal structure of the NPs, as the kind of an inter-atomic link is revealed. X-ray diffraction (XRD), infrared (IR), BET, and other electron microscopy-related techniques play key roles in the investigation of composition and structure of NPs. The powdered crystal X-ray diffraction patterns will provide information about the phases, sizes, types, and roentgen graphic nature of NPs (Khan et al., 2020).

**Table 4:** Characterization Methods for NPs

| Property | Technique | Description | Advantages | Disadvantages | Ref. |
|---|---|---|---|---|---|
| Morphological Features (Shape and Size) | Scanning Electron Microscopy (SEM) | Uses a focused electron beam to generate high-resolution images. | Excellent for studying dispersion and shape at the nanoscale. | The destructive technique requires conductive samples. | (Ngoi et al., 2021) |
| | Transmission Electron Microscopy (TEM) | Uses electrons to produce high-resolution images at high and low magnifications. | Provides detailed information on size, morphology, and crystal structure. | Expensive, complex sample preparation. | Zhang et al., 2020 |
| Optical Properties | Spectroscopy (UV-Vis, PL, DRS) | Analyzes the interaction of light with NPs. | Reveals information on size, shape, concentration, band gap energy, and luminescence. | Requires expertise in interpreting spectra. | (Wang et al., 2020) |
| Structural Analysis | X-ray Diffraction (XRD) | Uses X-rays to determine crystal structure and phases. | Provides information on crystallinity, phases, and sizes of NPs. | Limited to crystalline materials. | (Khan et al., 2020) |
| Elemental Composition | Energy-dispersive X-ray Spectroscopy (EDX) | Analyzes the elemental composition of a sample. | Provides information on elemental identity and relative abundance. | Limited detection for lighter elements. | |
| | X-ray Photoelectron Spectroscopy (XPS) | Most sensitive spectroscopic technique for elemental analysis. | Provides detailed information on elemental composition, chemical state, and surface bonding. | Expensive, complex instrumentation. | (Castro and Zuazo, 2024) |
| | Raman Spectroscopy, FT-IR | Analyze vibrational modes of molecules in the sample. | Reveals information on functional groups and bonding on the nanoparticle surface. | Requires knowledge of specific material's spectra. | (Castro and Zuazo, 2024) |
| Size Estimation | SEM, TEM, AFM | Microscopy techniques are used to directly image NPs. | Provides high-resolution size measurements. | Destructive for some techniques (TEM). | (Hou et al., 2020) |
| Surface Area | Brunauer–Emmett–Teller (BET) | Measures gas adsorption/desorption to determine surface area. | Provides information on total surface area and pore characteristics. | Requires careful control of experimental conditions. | Mehmood et al., 2023) |
| Physicochemical Properties | Various Techniques | Techniques depend on specific properties (magnetism, thermal conductivity, etc.). | Provides insights into functional properties relevant to applications. | Techniques can be complex and specialized | (Roostaee and Sheikhshoaie, 2020) |

**Elemental Studies**

Energy-dispersive X-ray spectroscopy (EDX), Raman, FTIR, and other available techniques that assist in establishing the elemental composition of NPs can be used. EDX delivers information on elements belonging to the powder assemblage (Patil et al., 2022). By contrasting the created spectra from the model of a computer with the resulting spectrum, a better contrast is provided. The in-depth study of the exact composition of elemental constituents as well as finding out the

precise ratios among them can be achieved using XPS, this method being the most sensitive one among the spectroscopic techniques. The functionalization of the peaks and the information of particles are shown in the vibrational responses of Raman and FTIR techniques (Castro and Zuazo, 2024).

**Size Estimation**

Scanning Electron Microscopes, X-ray diffraction instruments, Transmission Electron Microscopy, and Atomic Force Microscopes can be used to measure the sizes of NPs (Hou et al., 2020; Selvan et al., 2021).

**Physiochemical Characteristics**

The physicochemical features of NPs which make them suited for industrial applications include their mechanical character to flexibility, and their optical activity to absorb sunlight (Kishore et al., 2023). The mechanical properties of NPs, such as stress, adhesiveness, friction, hardness, strain, and surface coatings, are important for understanding NPs and have a significant impact on surface quality (Mehmood et al., 2023). Several characterization methods for NPs are discussed in Table 4.

**Application Areas of NPs**

NPs have been used in a variety of fields due to their distinctive characteristics, including regenerative medicine, anticancer drugs, vaccinations, mechanical manufacturing, cell imaging, and delivery systems etc. (Aghebati-Maleki et al., 2020). NPs, in addition to serving as environmental sensors and material protectors, aid in the absorption of pollutants from surface water during water purification (Pooja et al., 2020). Some of the application areas of the NPs have been summarized in Table 5.

**Table 5:** Application areas of some NPs.

| NPs | Application areas | References |
|---|---|---|
| Nickel oxide | Dye-sensitized solar cells, supercapacitors, batteries, water treatment, catalytic systems, and gas-sensing devices | (Singh et al., 2021) |
| Carbon nanotubes | Integrated circuits, electronic components, textiles, construction, cosmetics, medicine | (Pooja et al., 2020) |
| Cerium oxide | Biomedical equipment, electronic appliances, energy devices | (Shrestha et al., 2020) |
| Titanium dioxide | Coatings, water purifiers, paints | (Ahmed et al., 2022) |
| Silver | Clothing, textile industries, food packaging, agriculture, automotive, electronics, medicine, fitness centers | (Dikshit et al., 2021) |
| Iron | Optical devices, water purifiers | (Dikshit et al., 2021) |
| Calcium | Agriculture, automotive, food | (Shrestha et al., 2020) |
| Zinc oxide | Agriculture, automotive, cosmetics, home appliances, food | (Singh et al., 2021) |
| Gold | Cosmetics, environmental products, food, medicine | (Dikshit et al., 2021) |

**Conclusion and Future Perspective**

Scientists are currently working to develop NPs because the industry is desperate for eco-friendly, or at least green materials that can be used in biological systems simultaneously. NPs are widely used at the nanoscale due to the possibility of stable, environmentally acceptable materials that can coexist with biological systems. This chapter begins with an overview of NPs, including classification, benefits, and drawbacks, before moving on to synthesis and characterization methods. There are several inexpensive and straightforward methods for synthesizing NPs, including chemical synthesis, biological synthesis, the top-down methodology, the bottom-up method, and mechanical approaches. Various methods for characterizing NPs are being developed to extract information about their shape, structure, optical features, mechanical qualities, and physiochemical properties. Every undesirable situation can be effectively controlled by machines. The most essential properties of NPs are determined by the synthesis and characterization techniques used. NPs are very beneficial in medical, drug delivery, cosmetics, optics, electronics, and solar energy devices.

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Chapter 18

# Nanotechnology: A Synergistic Approach for Enhanced Therapeutic Outcomes in Alternative Medicine

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## ABSTRACT

Nanoparticles (NPs), the materials ranging in size from 1 to 100 nm are highly advantageous in the medical industry since they can easily penetrate cells and have specific binding sites. Not only can they spread extensively across the body, but they can also enter organelles and disrupt their normal functioning. By employing these attributes, it has become possible to improve the visualization of tumors and other diseased tissues in the body, as well as organs, in distinctive manners. Nanotechnology has various advantages, including the precise and targeted delivery of medications, making it suitable for effectively managing chronic human diseases. Nanomaterials are presently employed in the management of various ailments and conditions, such as immunotherapeutic medications, chemotherapeutic agents, and biological agents. Scientists have been doing thorough evaluations to evaluate how much the use of nanomaterials has improved the effectiveness of newly developed and existing medications. Multiple treatment modalities with potential for use in clinical settings have been documented. The mechanisms encompassed in this context are molecular diagnosis, sickness detection, nanoscale immunotherapy, and the administration of anticancer medications. This chapter aims to demonstrate the application of conjugated nanoparticles as a therapeutic tool in the realm of alternative medicine.

**KEYWORDS**
Nanoparticles, Alternative medicine, Therapeutics, Imaging, Nanotechnology

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## INTRODUCTION

### Generation of Nanoparticles

Nanoparticles can be divided into three size ranges as, 1 and 100 nm, bigger than 500 nm, between 100 and 500 nm, (Akbari et al., 2011) even though the nanoscale is essentially defined as 1 to 100 nm. The notable changes in surface area properties, such as (surface-enhanced) Plasmon resonance and catalysis, are made possible by the high surface-area-to-volume ratio of nanoparticles. Nanoparticles can have one or more single-property characteristics, such as being dielectric, metallic, magnetic, semiconductor, or multifunctional, (Mourdikoudis et al., 2018) meaning they have many features from a single property to multiple depending on their structure and composition.

The creation of nanoparticles can be approached through three primary methods: biological, chemical, and physical approaches (Irvani et al., 2014). The chemical and biological strategy together are referred to as the bottom-up approach, while the physical approach is also known as the top-down approach (Thiruvengadathan et al., 2013). In bottom up method, atomically sized materials are nucleated into final nanoparticles. The gas phase synthesis, citrate reduction method, microbial synthesis, block copolymer synthesis, is some typical synthesis techniques. Top-down techniques, such as milling, spark ablation, and laser ablation involve disassembling a bulk material physically into smaller molecules (Casey et al., 2006). All the strategies are further divided into different kinds according to the techniques they use. Because bottom-up synthesis techniques require batches of chemicals and solvents, they are frequently named as the "wet" synthesis technique. To guarantee that the particles do not grow any larger than the fixed range on nanoscale, they frequently need to be capped or sterilized in solution (Gawande et al., 2016).

**Physical Approach**

**The Mechanical Milling**

The milling process utilizes spheres or beads inside vessels. This process can be done in a variety of mills, usually planetary and shaker mills (Damonte et al., 2004). One useful method for converting bulk materials into minute (nanoscale) material is mechanical milling. (Yadav et al., 2012) Mechanical processes can be used to manufacture a variety of nanocomposite materials, including oxide and carbide-enhanced aluminum alloys, and alloys (Altammar et al., 2023).

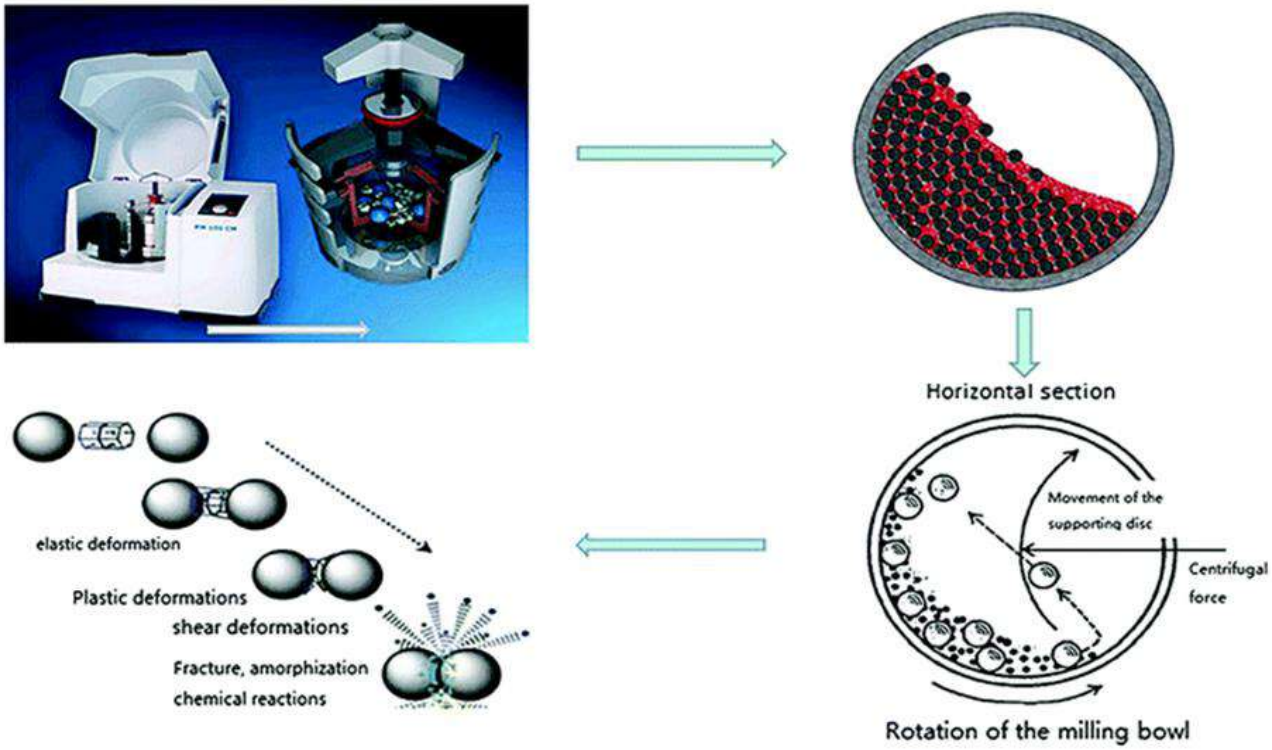


**Fig. 1:** The mechanical milling process for manufacturing nanomaterial (Baig et al., 2021).

**The Electrospinning Method**

It is typically used to turn a variety of materials—most frequently polymers—into nanofibers (Jayaraman et al., 2004). Charged threads up to a few hundred nanometers in diameter are drawn from polymer melts or solutions using an electrospinning process (Yarin et al., 2003). Coaxial electrospinning is an important development in the realm of electrospinning. Two coaxial capillaries comprise the spinneret in coaxial electrospinning. In these capillaries, two viscous liquids—one as the shell and the other as the core—can be used to build core-shell nano architectures. (Li et al., 2004)

**The Laser Ablation Method**

A single substance can be vaporized with a laser beam to create a micro feature. This method creates NPs by using a powerful beam of laser light to strike the target material. (Nowakowski et al., 2005) The target material vaporizes and evaporates as a result of the high intensity of laser radiation used in this process, producing NPs (Kim et al., 2017). Laser ablation method is an eco-friendly method of creating noble nanoparticles. (Altammar et al., 2023)

**Sputtering**

In this a method NPs are produced by hitting solid surfaces with high-energy particles such as plasma or gas. It is an effective process for creating thin films of nanomaterial. During this process, gaseous ions hit the target material, resulting in the physical ejection of tiny atom clusters (Sigdel et al., 2012). Sputtering can be done using a magnetron, a radio-frequency diode, or a direct current diode. This process is carried out in an evacuated chamber into which gas is injected. A high voltage is given to the cathode target, and free electrons clash with the gas to form gas ions.

**Chemical Approach**

**Chemical Reduction**

This method is very proficient when producing zero-valent nanoparticles from chemically reducing aqueous metal salts, such as AgNO3 in the case of synthesizing silver NPs (Naim et al., 2016. At least one reducing agent is needed to provide electrons for the metal ions, reducing them to zero valence. (Khanna et al., 2005) Reductants including ascorbate, citrate, and borohydride are frequently utilized. A stabilizing agent stabilizes reduced nanoparticles. Cetyltrimethylammonium

bromide [(C16H33)N(CH3)3Br; CTAB], which is frequently employed in the manufacture of gold nanoparticles, is an illustration of a stabilizing agent (Kang et al., 2018). When creating silver nanoparticles, the stabilizing agents might also be the reducing agents themselves, like sodium citrate.

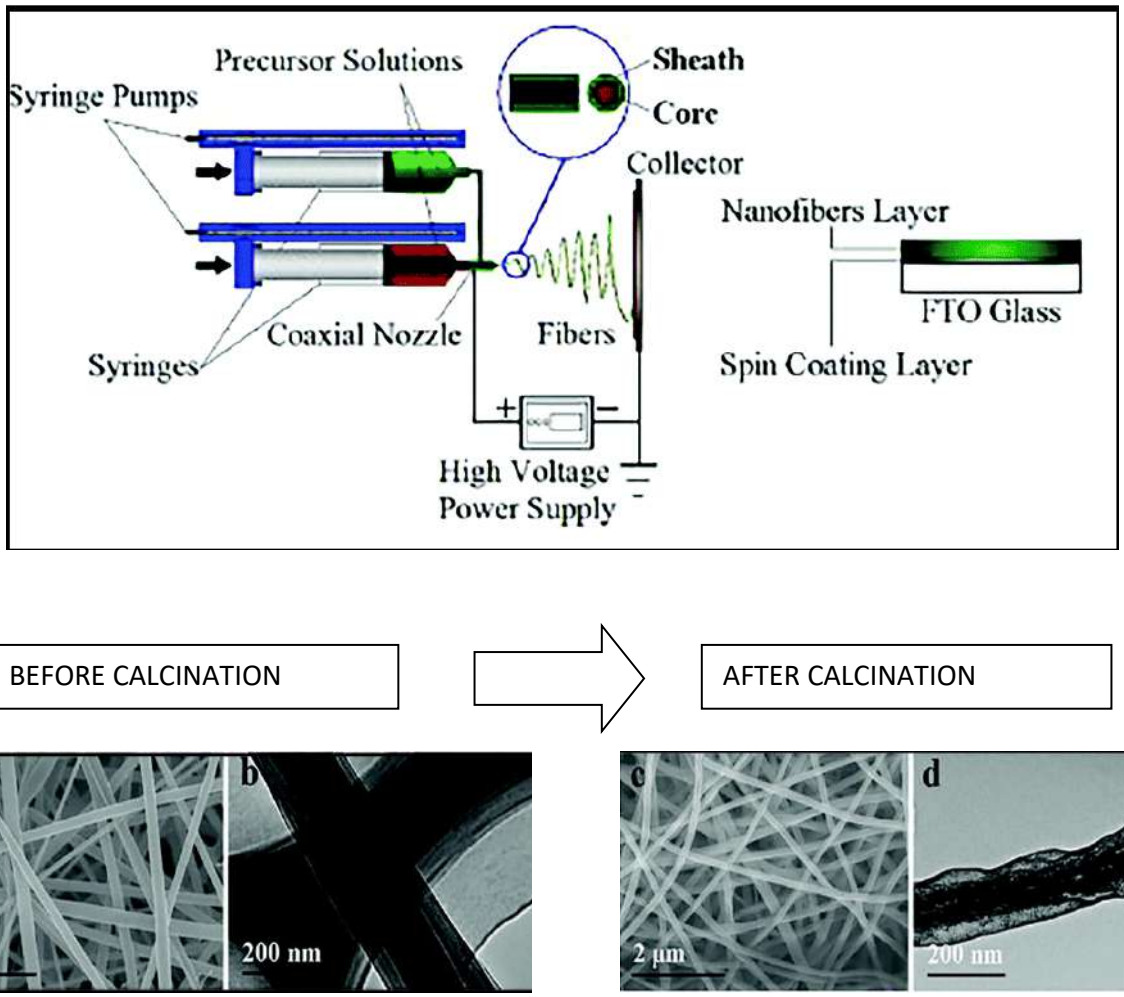


**Fig. 2:** Demonstration of electrospinning method for preparation of Nanomaterials (Baig et al., 2021).

**Coprecipitation**

The process is carried out by precipitation of soluble substance under certain conditions such that when concentration of substances reaches super saturation, (Taylor and Zhang, 2016) a nucleation suddenly appears in solution. Diffusion of this nucleated material will lead to the formation of nanoparticles (Thanh et al., 2014). Nucleation is proceeded slowly to get uniform particle size. Coprecipitation is one of the convenient methods to synthesize $Fe_3O_4$ nanoparticles.

**Microwave Assisted Synthesis**

The most recent methods of synthesizing the nanomaterial using microwaves combines the benefit of heating the precursor material uniformly with speed (Chikan and McLaurin, 2016). The fast crystal development takes place by homogenous nucleation of the preparatory material by the use of microwaves (Tsuji et al., 2005). The advantage of choosing the microwave-assisted production of NP's over other conventional methods is the homogeneous crystal formation and a short reaction time. This is because of the forces created by combining electric and magnetic fields of the microwaves, which cause friction and molecular collisions (Stuerga et al., 2006). Lead salt and sulfur sources are typically used in the microwave irradiation method of producing PbS nanoparticles (Onwudiwe et al., 2019).

**Hydrothermal Method**

In the hydrothermal process, those materials that are insoluble at normal pressure and temperature are converted into nanoparticle crystals through heterogeneous reactivity at high pressure and temperature. (Byrappa et al., 2007) The development of crystals is carried out in a steel pressure vessel that holds water and nutrients called as the autoclave. (Kharisov et al., 2012). The typical temperature range for hydrothermal synthesis is below 300°C. Metal oxide nanoparticles in supercritical water, metal nanoparticles, and semiconductor nanoparticles have all been created using this technique (Lane and Zimmerman, 2019).

**Sonoelectrode Position**

One helpful technique for creating nanoparticles is sonoelectrodeposition, which has been used to create metallic nanoparticles like FePt and CoPt (Lane and Zimmerman, 2019). The process of sonoelectrodeposition creates metallic nanoparticles by fusing the benefits of electrodeposition with the mechanical waves of ultrasonic waves (Hihn et al., 2012). Unexpectedly hazardous ions can be found in the finished products of traditional ways of synthesizing silver nanoparticles. (Xu et al., 2020) Nitrate and thiolsulfate, two of the silver precursor's ions, make up the majority of the product's hazardous ions. Although it is costly and challenging to work with in ambient circumstances, silver acetate is an excellent precursor to silver that can be employed (Zhang et al., 2018). A non-toxic solution made of silver nanoparticles using a modified sonoelectrodeposition method is well employed now.

**Sol Gel Method**

Sol gel method is one the most authenticated technique so far to synthesis of nanoparticles (Bokov et al., 2021). Sol gel approach is widely utilized for industrial purposes (Owens et al., 2016). This approach has the capability to produce high-quality NPs at an industrial level. NPs are produced by combining two or more metals or metal oxide precursors in specific ratios, thus allowing alloy products to be created in a single step (Lu et al., 2020).

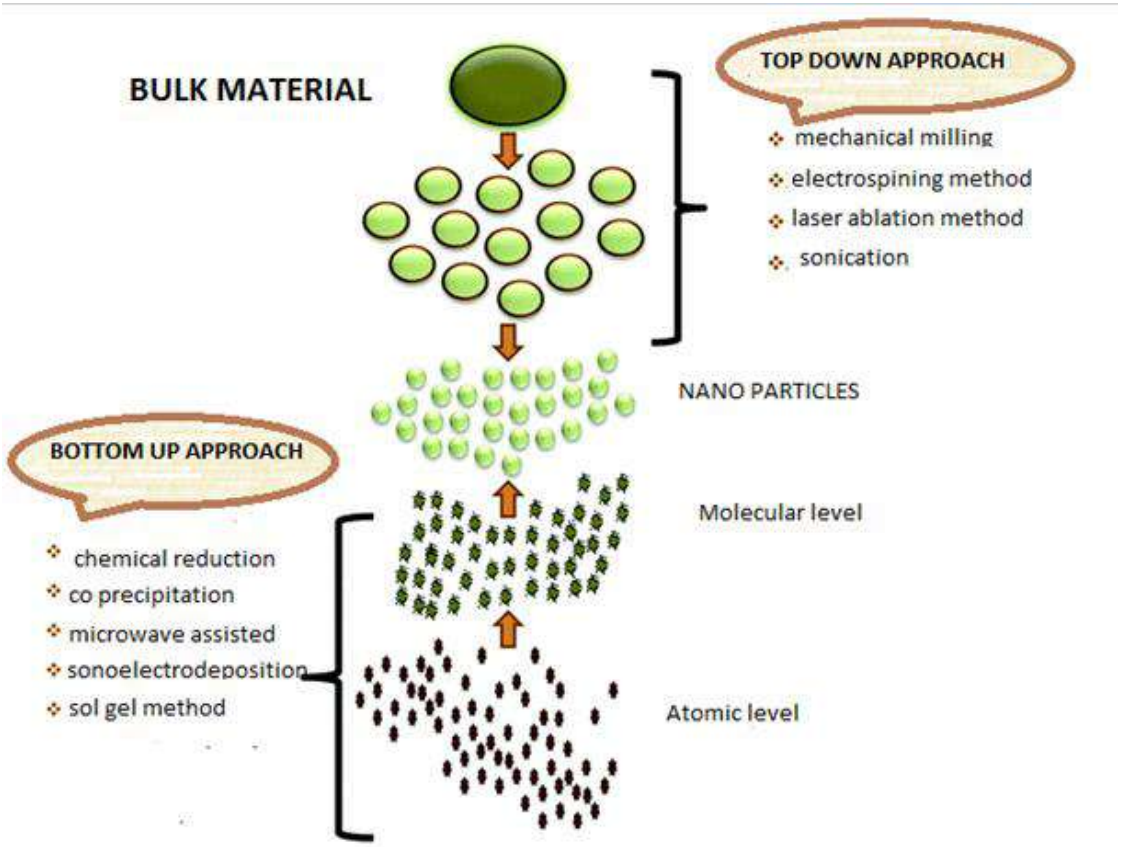


**Fig. 3:** Schematic illustration of methods used for synthesis of nanomaterials (Baig et al., 2021)

**Application of Nanotechnology in Medicine**

Nanomedicine refers to the use of nanotechnology for diagnosing and treating diseases. Various fields have utilized nanotechnology previously, but its application in medicine is a recent development. The primary goal of medicine is to promptly identify health concerns and offer appropriate treatment. Connecting nanotechnology with medicine can enhance the effectiveness of illness therapy (Qasim et al., 2014). Nanotechnology, including nano robots, microchips, and biosensors, is mostly utilized for diagnostic purposes (Jackson et al., 2017). Most current research involves linking antibodies with magnetic poly- D, L-lactide-co-glycolide nanoparticles containing doxorubicin (DOX). Magnetic nanoparticles and doxorubicin (DOX) were incorporated into the PLGA nanoparticles to target malignant cells affected by the disease (Liang et al., 2019). Nanoparticles can be utilized for diagnosing hepatitis. Gold nano-particles are better suitable for this purpose (Wilson et al., 2008). Gold nano-protein chips are created to identify antibodies specific to hepatitis. These chips are efficient for diagnostic purposes (Mughal et al., 2022).

**Application of Nanoparticles in Diagnosis for Imaging**

For a considerable time, non-invasive imaging methods have been a vital part of patient diagnosis. Ongoing attempts

are made to reduce adverse effects and enhance image quality (Doolub et al., 2023). Natural minerals (NMs) can be employed as a contrast agent in a variety of imaging techniques, depending on their peculiarities. Magnetic nanoparticles and inorganic NMs containing metals like gold, silver, or platinum can be employed as diagnostic instruments for tissue imaging (Nilghaz et al., 2021). Currently, a number of nanoparticle complexes and preparations are authorized for clinical usage as MRI contrast agents (Estelrich et al., 2015). Quantum dots – nanoparticles – emit light when exposed to UV radiation. When Quantum dots are directed towards the malignant cell inside, they begin to emit light, indicating the presence of the tumor. (Juzenas et al., 2008)

**Imaging Based on X-rays**

Compared to traditional iodine-based contrast agents commonly used in clinics, Au results in proficient absorption of X-ray radiation due to its higher electron density and atomic number (Ahn et al., 2013). Au NPs can also have longer circulation durations than traditional agents, which allows for cell monitoring, aiming particular cell types or ligands (Sibuyi et al., 2021), and extended imaging. Furthermore, precise control over Au NP payloads in biocompatible carriers is possible.

**Fluorescence Microscopy**

Bulk gold has a very weak fluorescence. Surprisingly, Au NPs show higher fluorescence when compared with a quantum bulk gold. Because of this, fluorescence can be set between 650 to 900 nm, (Kvaková et al., 2023) where light infiltrates tissues reasonably efficiently. It can be used in the biomedical area with ease.

**Surface-Enhanced Raman Imaging Spectroscopy**

One method for examining molecule rotations and vibrations is Raman spectroscopy. Raman spectra are extremely intricate and are utilized to determine the chemical makeup of a material by using them as "fingerprints" of individual molecules (Wang et al., 2017). Raman spectroscopy is very specific, but its poor sensitivity limits its applications. The intensity of Raman active molecules' vibrational spectra increases by several orders of magnitude as they absorb AuNP or other metal surfaces. Surface enhanced Raman spectroscopy (SERS) is a novel technology (Linke et al., 2019) that has emerged as a result of this finding. It is believed that this phenomenon, which is frequently observed and investigated with AuNPs, is caused by an electromagnetically generated field near the particle surface. Brain tumors were imaged using an AuNP based technology, which also provided enhanced tumor margin (Meola et al., 2018) delineation during surgery.

**Photoacoustic Imaging**

When a person is exposed to light during photoacoustic imaging, the light causes localized heating and a little expansion of tissue, which generates a sound wave (Tan and Technology, 2018).

Ultrasonic sound waves are produced when brief laser pulses are used. Since photoacoustic imaging uses nonionizing radiation and produces ultrasound as the output, (Hester et al., 2020). It has an advantage over some other imaging techniques. This is because ultrasound scatters less in tissue than optical approaches, allowing for higher spatial resolution. It can distinguish between different biological tissue types since different biological tissues (Pian et al., 2014) have distinct light absorption coefficients.

**Imaging via Optics**

Exceptional light scattering characteristics of Au NPs are not found in non-plasmonic NPs. AuNP morphology and size variations also affect scattering, offering a way to fine-tune the agent for the best possible light scattering results (Jin et al., 2014). For instance, larger nanoparticles scatter light more efficiently than smaller ones because of a rise in both the optical cross section and the reflection coefficient with increasing size (Jain et al.,2006). Additionally, Au NPs do not experience photo bleaching in contrast to fluorescent probes employed in optical imaging. Resonant light scattering from Au NPs was tracked during two cell cycles of cancer cells using dark field microscopy (Cao et al., 2020). The observation of Au NP entrance into the cell nucleus was made possible by the high scattering signals.

**Mononuclear Phagocyte System Imaging**

Nanoparticles are commonly used to scan the mononuclear phagocytic system, which includes the liver, spleen, and lymphatics. Most NPs are absorbed by mononuclear phagocytic system because of the abundance of macrophages in those organs (Weaver et al., 2017). Many nanoparticle imaging agents are proposed for various imaging techniques such as CT, MRI, SPECT, ultrasound, OI, and PAI. Only iron oxide NPs have been utilized for clinical mononuclear phagocytic system imaging, particularly for liver imaging. NPs are typically removed from the bloodstream through phagocytosis by macrophages, leading to their accumulation in the liver, spleen, and lymph nodes. If NPs primarily meant for imaging phagocytosing cells in tissues like lymph nodes, extended half-lives are necessary. This allows NPs to adequately infiltrate into the tissues and get absorbed by macrophages in those areas. This is achieved by reducing the size of NPs and ensuring they are uniform. Notable instances have shown the utility of iron oxide NPs ((U)SPIO)-enhanced MRI for identifying liver metastases, lymph node metastases, and characterizing inflammatory lesions such atherosclerotic plaques (Baetke et al., 2015).

**Tumor Angiogenesis Imaging**

To visualize tumor angiogenesis and vascularization is one of the key components for utilizing nanomaterials in a precise manner. Enhanced permeability and retention effect (EPR) are usually monitored by the non-targeted nano formations, while targeted nanoparticle formulations that bind to multiplying endothelial cells are employed to analyze tumor growth (Subhan et al., 2021. They also help evaluate alterations in tumor vascularization, such as maturation and widening of vessel during anti-angiogenic therapy or during radiotherapy. There are various approaches for utilizing NPs in molecular imaging techniques to evaluate tumor angiogenesis and the effects of anti-angiogenic therapy (de la Torre et al., 2020). Microbubbles are the largest particle molecular imaging agents utilized for ultrasound imaging.

**Therapeutic Application of Nano-particles**

In the field of cancer nanomedicine, the "targeting" standard has allowed for improved selectivity of anticancer medicines towards tumor cells and tissues. Alternative medications or nano-carriers are being explored by including specialized ligands that can selectively attach to distinct cell surface receptors, resulting in the internalization of cells by receptor-mediated endocytosis. These targets, primarily antigens, should be highly expressed or present in tumor cells or blood vessels. To achieve accurate target identification, NPs can be modified with tumor-targeting components such as monoclonal antibodies, proteins or peptides, nucleic acids, and various small compounds. These targeting ligands are anticipated to have both high affinity and accuracy towards target cells/tissues. Additionally, they are believed to play a crucial role in enhancing the uptake of NPs by cells through internalization processes (Shi et al., 2017).

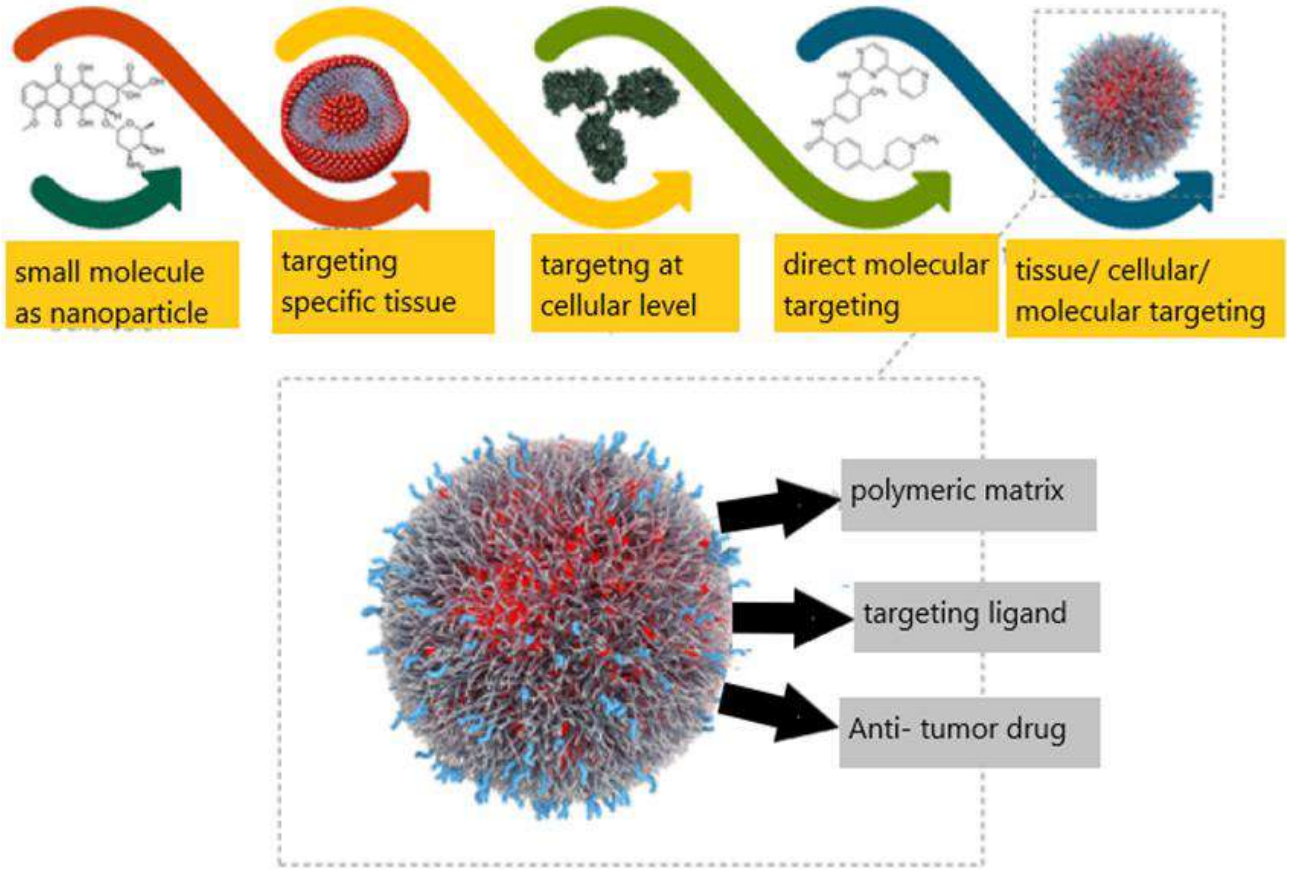


**Fig. 4:** Targeted action of nanoparticles (Tu et al., 2022)

Upon entering the bloodstream, nanoparticles have a tendency to aggregate and undergo protein opsonization, which involves proteins attaching to the surface of the nanoparticles, acting as a signal for detection by the immune system. The nanoparticles that have been coated with opsonins can be removed from the bloodstream through either phagocytosis or filtration in the liver, spleen, and kidney. The immune system's rapid and non-selective elimination of the drug results in a shorter time of its presence. So limiting its capacity to be absorbed by the body.

The retention duration of nanoparticles can be modified by coating their surface with polyethylene glycol (PEG), carbohydrates, acetyl groups, or protein components such as arginine-glycine-aspartate (RGD) peptide or albumin. Nevertheless, this surface alteration can also modify the recognition capability for targeted distribution. Therefore, it is crucial to carefully consider the cleanability and biodistribution of therapeutic nanoparticles during the design phase (Pelaz et al., 2017). The surface charge of therapeutic nanoparticles is crucial for their efficient removal from the body and precise delivery

to specific targets. Nanoparticles with a positive charge elicit a more robust immune response as compared to nanoparticles that are neutral or negatively charged. Furthermore, it has been demonstrated that nanoparticles with a surface potential ranging from -10 to +10 mV exhibit reduced vulnerability to phagocytosis and non-specific interactions. Nevertheless, the optimal range may vary depending on the specific nanoparticle material. The pH sensitivity of nanoparticles is directly linked to their surface charge. Nanoparticles can be engineered to selectively identify and localize within specific cellular compartments (Wilhelm et al., 2016).

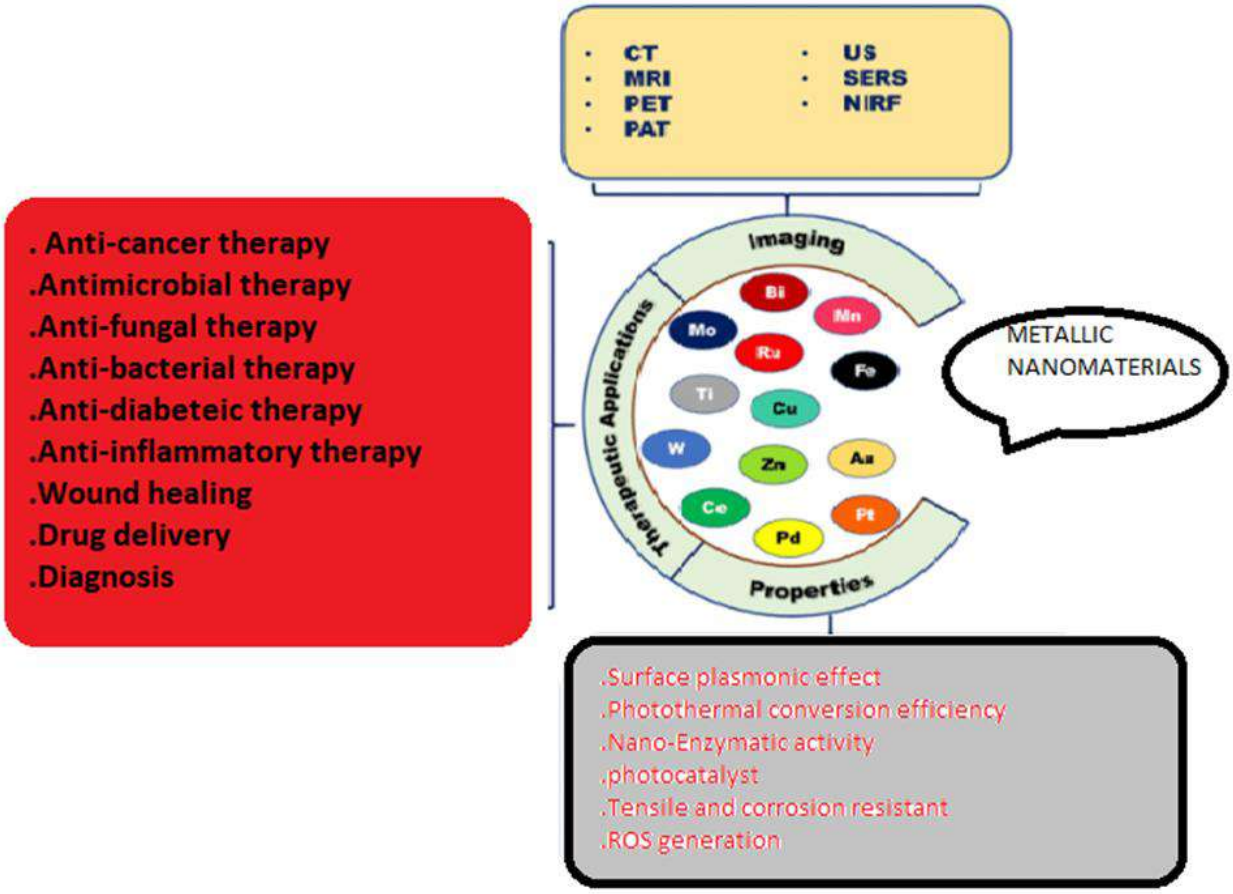


**Fig. 5:** Applications of nanoparticles in alternative medicine. (Utreja et al.,2020,)

**Examples of Nanoparticles Used for Enhanced Therapeutic Outcomes**

**ZnO-NPs**

In addition to producing reactive oxygen species, zinc oxide nanoparticles (ZnO NPs) can induce disequilibrium of zinc-dependent protein activity, which results in selective cytotoxicity towards cancer cells. ZnO NPs can destroy cancer cells by causing oxidative stress in the malignant cells. Additionally, zinc activates DNA repair, which stops both apoptosis and the proliferation of cancer cells, and is implicated in all cellular defense mechanisms against cancer (Alarifi et al., 2013).

Zinc channels regulate intracellular and free zinc movements to maintain a balance between cell death and survival. Additionally, zinc is essential for maintaining the activity of the tumors suppressor gene p53, which controls the process of apoptosis. Furthermore, zinc has the ability to aid in the activation of the apoptosis-causing caspase-6 enzyme. Consequently, a zinc deficit leads to p53 disruption and DNA damage, which compromises DNA integrity and may raise the risk of cancer. Zinc oxide nanoparticles are therefore thought to be a powerful treatment for many malignancies (Alarifi et al., 2013).

**CaO-NPs**

Calcium oxide nanoparticles (CaO-NPs) are inorganic and biocompatible materials that have strong antibacterial properties against *S. epidermidis, P. aeruginosa*, and *C. tropicalis*. Additionally, they have the capacity to neutralize endotoxins. CaO-NPs were determined to have lower efficacy compared to calcium hydroxide nanoparticles in eradicating bacteria within the dentinal tubules, according to a comparative investigation. Additionally, these particles were found to effectively reduce the levels of triglycerides and cholesterol in the bloodstream of mice (Alarifi et al., 2013).

**Ag-NPs**

Multiple researchers have documented that silver nanoparticles (AgNPs) have caused a harmful effect on leukemic cells. In a recent study, poly (N-vinyl-2-pyrrolidone) (PVP)-coated AgNPs demonstrated the capacity to reduce the viability of

acute myeloid leukemia (AML) cells, even at low concentrations. This finding suggests a promising new method for treating AML in the future (Wei et al., 2015).

Ag NPs have demonstrated cytotoxic activity in MCF-7 breast cancer cells in a dose-dependent manner by triggering apoptosis. The LD50 and LD100 dose of Ag NPs turned out to be 3.5 ng/mL and 14 ng/mL, respectively. Ag NPs causes cell death generating reactive oxygen (ROS) species, activating caspase 3, and fragmenting DNA. Ag NPs possess cytotoxic properties due to its apoptotic characteristics (Wei et al., 2015).

**CuO-NP**

The enormous specific surface area of copper nanoparticles allows for the conjugation with diverse biomolecules, making them suitable for use as anticancer treatments or efficient drug nano-carriers. Copper diethyldithiocarbamate (Cu(DDC)2) nanoparticles were developed to address the issue of resistance in prostate cancer treatment (Wei et al., 2015). According to in vivo experiments, Cu (DDC) 2 NPs not only had the capability to prevent the spread of cancer cells, but also demonstrated the ability to overcome drug resistance.

Studies have shown that the antibacterial and antifungal activities of copper nanoparticles, which were enhanced by the addition of plant extract, surpassed those of tested antibiotics. The antibacterial effects of CuO NPs are mostly attributed to their internalization within bacterial cells, release of copper ions, induction of oxidative stress by excessive reactive oxygen species (ROS) formation, and DNA destruction. Furthermore, *E. coli* growth was hindered in laboratory conditions by copper nanoparticles. Nevertheless, the antimicrobial characteristics were only detected when the quantity of copper nanoparticles exceeded 300 µg/mL. The increasing need for potent antibacterial medicines due to the rise of drug-resistant bacteria makes it clear that copper-based nanomaterials have significant potential in fighting microbial diseases (Esteban et al., 2009). Copper nanoparticles has the capability to produce reactive oxygen species (ROS), which renders them highly effective as an antiviral agent. The procedure involves the activation of key immune cells such as T helper cells, B cells, and macrophages, which then create antibodies targeted against certain infections. In addition, copper nanoparticles exhibit potent antiviral properties against a diverse range of viruses, including influenza, herpes simplex virus, and hepatitis B, hepatitis C, bronchitis virus, polio, human immunodeficiency virus (HIV-1), and potentially SARS-CoV-2. Therefore, copper nanomaterials present a significant opportunity for the advancement of comprehensive antiviral treatments (Woźniak-Budych et al., 2023).

**MgO-NPs**

The antimicrobial properties of MgO nanoparticles have been proven on Gram-negative as well as Gram-positive bacteria. The minimum inhibitory concentration for *P. aeruginosa* and *S. aureus* turned out to be 1.000 µg/mL, while for *E. coli* the minimum inhibitory concentration was 500 µg/mL. MgO nanoparticles have also shown bactericidal activity against *R. rhodochrous* and *B. subtilis, A. hydrophila*, *P. mirabilis*, *V. cholera*, *S. flexneri* and *S. typhi*.

MgO nanoparticles have superior fungicidal efficacy in comparison to other nanoparticles. Study shows that MgO nanoparticles suppresses the growth of *F. solani*, *A. niger* and *A. fumigates* (Alghuthaymi et al., 2021). These nanoparticles have also demonstrated the ability to inhibit the growth of *C. tropicalis* and *C. glabrata* fungus.

**Au-NPs**

Due to the permeable nature of underdeveloped blood vessels located at tumor locations, gold nanoparticles (GNPs) can aggregate passively at these sites. It is believed that they are absorbed into cells through non-specific receptor-mediated endocytosis. Although GNPs have the potential to deliver passively to tumor locations to a certain degree, their effectiveness is hindered by the varied vasculature found in different types of malignancies. Particles and ingestion by the reticuloendothelial system (RES) further hinder passive delivery (Anik et al., 2022). Due to their robust surface plasmon resonance (SPR), gold nanoparticles (GNPs) are consistently being evaluated for their potential use in photodynamic therapy (PDT). In PDT, the heat generated by light can be utilized to either deliver a chemical load or produce reactive oxygen species, consequently causing either cellular necrosis or apoptosis at targeted tumor spots (Daraee et al., 2016). A separate investigation involved the creation of gold nanoparticles that were combined with baicalin, an active flavonoid derived from *Scutellaria baicalensis*, known for its anti-cancer capabilities. The gold particles produced during the synthesis of baicalin had a detrimental effect on breast cancer cells by encouraging apoptosis (W. Chen et al., 2016).

**Conclusion**

Nanotechnology has revolutionized various scientific fields, offering groundbreaking solutions and significantly enhancing the efficiency of numerous applications. This article examines the manufacturing and many classifications of nanoparticles, with a specific emphasis on their vital role in improving treatment outcomes in alternative medicine.

The synthesis of nanoparticles can be accomplished through two primary approaches: top-down and bottom-up methodologies. Top-down techniques, such as lithography, mechanical milling, laser ablation, and thermal breakdown, involve breaking down bigger materials into nanoscale particles. On the other hand, bottom-up methods, such as chemical vapour deposition, sol-gel processes, spinning, pyrolysis, and biological synthesis, build nanoparticles by incrementally adding individual atoms or molecules. Every strategy offers distinct advantages, and the choice of methodology is usually based on the desired properties and applications of the nanoparticles. Nanoparticles exhibit unique chemical, physical, and biological properties at the nanoscale that are not present in bigger particles. Nanoparticles possess unique characteristics

that are used across various industries, particularly in medicine, to enhance the delivery and efficacy of therapeutic compounds. The nanoparticles commonly studied include metallic nanoparticles (such as gold and silver), metal oxide nanoparticles (such as zinc oxide and titanium dioxide), as well as other nanostructures like quantum dots and carbon-based nanoparticles.

Nanoparticles in alternative medicine offer a synergistic approach that significantly improves treatment outcomes. The small size and large surface area of these substances facilitate increased interaction with biological molecules, hence enhancing the absorption and efficacy of traditional herbal medicines and other natural products. In addition, nanoparticles can be engineered to specifically target specific cells or tissues, which reduces negative effects and improves the therapeutic effectiveness. For instance, silver nanoparticles exhibit potent antibacterial properties, rendering them advantageous for combating infections that are unresponsive to conventional medications.

In a nutshell nanotechnology offers a groundbreaking approach to enhance the efficacy of treatments in alternative medicine through the creation and use of diverse nanoparticles. The continuous exploration and development of therapeutics using nanoparticles offer significant potential for improving healthcare and overcoming the limitations of current treatments.

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Chapter 19

# A Paramount Study in Advancement of Acute and Chronic Wound Repairment using Inorganic Nanoparticles in Veterinary Sphere

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**ABSTRACT**

Advancements in the field of biomedicine have opened new paths like nanotechnology. Recent approaches in this area include drug delivery mechanisms, transportation of biomolecules like DNA, RNA by nanoparticles that can be useful in wound treatments. The use of nanoparticles has gained a lot of attention and recently it has provided an exceptional approach to repair the skin wound problems. Silver is considered as super metal with antibacterial characteristics since centuries. Silver based compounds with germicidal properties have widespread applications for wound dressings. The AgNPs play a substantial role in the wound healing due to its better effect, safety, less toxicity, and resistance to microbes on a broad spectrum. Moreover, the AgNPs possess good mechanical properties that remarkably increase the collagen deposition in the wound area and help improved healing process with excellent tensile strength. The gold nanoparticles (AuNPs) have unique features like higher stabilization, low toxicity, ease of detection and great functionality. These properties render them a great choice in biomedical fields. The AuNPs combined with epigallocatechin gallate and α-lipoic acids have significant antioxidant and anti-inflammatory properties, leading to effective wound healing.

**KEYWORDS**
Nanotechnology, Silver-nanoparticles, Gold-nanoparticles, Acute and chronic wounds, Wound healing

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## INTRODUCTION

Skin, the body's largest organ, is critical for protecting the viscera from injuries and harm from the outside world. However, in the event of trauma, burns, or certain endocrine disorders, such as diabetes, it becomes susceptible to infections (Boomi et al. 2020). Any break in the skin's integrity is called a wound. In most cases, the etiology of a wound determines its classification. External injuries, such as burns, gunshot wounds, surgical wounds, abrasive wounds, and so on, are typically the cause of acute wounds (Nandhini et al. 2024). A predisposing condition like diabetes, which interferes with physiological functions and causes epidermal and dermal tissue injury, is the main cause of chronic wounds (Volkova et al. 2016). Such persistent illnesses can lead to the development of chronic wounds such as pressure sores, bacterial infections that cause septic wounds, leg and foot ulcers, endocrine inflammation, tissue proliferation, and remodeling are three overlapping but separate stages of multiphase wound healing mechanisms. The hemostasis and inflammation are the defining characteristics of the initial stage of wound healing, referred to as the inflammatory phase (Asrar et al. 2023). The second stage of wound healing, referred to as proliferation, encompasses several key processes, namely epithelialization, angiogenesis, granulation tissue development, and collagen deposition. From a clinical perspective, it can be argued that the maturation and remodeling phase holds significant importance in the process of wound healing (Volkova et al. 2016). During this phase, the collagen forms an organized and well-mannered network. Ischemia, foreign bodies, edema, contamination, infection are few local factors that affect wound healing (Broughton et al. 2006).

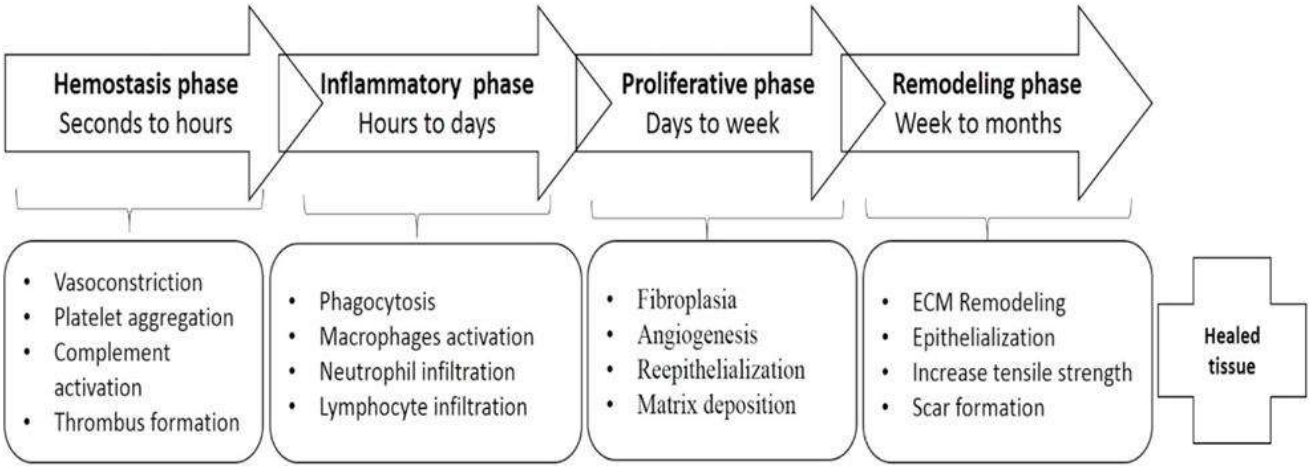


**Fig. 1.1:** Phases of normal wound healing (Rajendran et al. 2018)

The process of interacting between cells and molecules to repair wounds is known as wound healing, which includes several phases including hemostasis, inflammation, proliferation, maturation, epithelization, and scar tissue remodeling (Nour et al. 2019). The present approach to wound management mostly involves the application of dressings, infection prevention, excision of necrotic tissue, provision of adequate hydration, and drainage of surplus fluid (Donato et al. 2023). In majority of the times, stitches, staples, or surgical glue are used to join the borders of surgical wounds. The main goal of wound healing is this. However, secondary intention healing is a common method of treating wounds, leaving the wound exposed for the synthesis of new tissue to mend it. Rajendran et al in 2018 explained the phases of normal wound healing in above Fig. 1.1.

The presence of biofilm in chronic wounds can be a symptom of significant inflammation, which can hinder the healing process. This inflammation is caused by the overactive and prolonged release of nitric oxide, inflammatory cytokines, and free radicals. In addition to taking longer to heal, wounds caused by diabetes, burns, chronic conditions, and surgical procedures might sometimes not heal at all (Paladini and Pollini, 2019). The most common bacterial species identified were *Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa), Proteus mirabilis, Escherichia coli (E. coli)*, and Corynebacterium species. In addition, the interaction between *S. aureus and P. aeruginosa*, as well as the significant antibiotic resistance shown by Gram-negative bacteria, were associated with poly-microbial infections. The *S. aureus* infections commonly originate from infected wounds in both humans and animals. It is well-known that burn wounds exhibit exudate and, as a result, a significant prevalence of *Pseudomonas* species has been recorded (Paladini and Pollini, 2019).

The nanotechnology is an entirely novel field of technology that alters matter in the range of 1 to 100 nanometers. Various nanoscale materials from nanotechnology are being used in the biomedical applications to avoid diseases. Modifying the composition of proteins and ions in the growing media can modify the physical, chemical, and morphological structures of nanoparticles (Nandhini et al, 2024). The utilization of nanoparticles formed from polysaccharides, polymers, metals, and bioactive compounds derived from plants, in conjunction with active pharmaceuticals, has demonstrated significant efficacy in combating human infections, including bacteria and viruses. Moreover, these nanoparticles exhibit promising potential for the treatment of various clinical illnesses. (Rajendran et al. 2018).

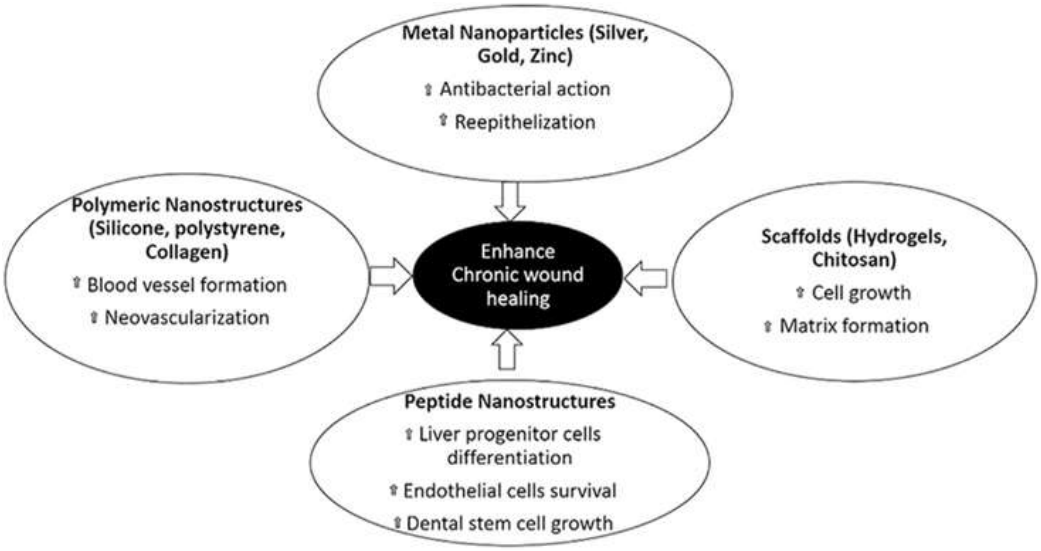


**Fig. 1.2:** Nanoparticles and chronic wound healing mechanism (Rajendran et al, 2018)

The silver products (such as silver nitrate and silver sulfadiazine) are commonly used in burn and chronic wound dressings because they produce silver ions, which have a significant antibacterial impact (Nam, 2015). The lysis of bacterial cells occurs when silver ions attach to thiol groups in peptidoglycans. Moreover, interference with respiratory enzyme pathways modifies the DNA of microorganisms. Additionally, silver compounds work well against bacterial biofilms and multi-resistant bacteria. Nevertheless, compounds generated from silver may be harmful to tissues. The nanoparticles used in wound healing are shown in figure 1.3 (Mihai et al. 2019). Rajendran et al, in 2018 explained the mechanism of Nanoparticles and chronic wound healing in above Fig. 1.2.

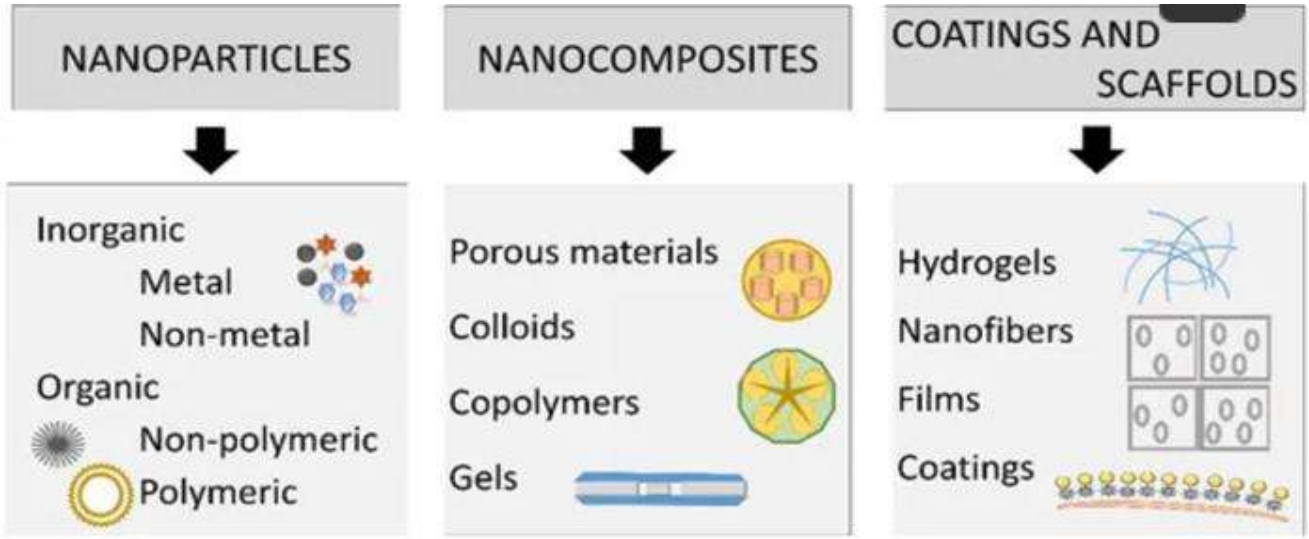


**Fig. 1.3:** Nanomaterial which can be used for wound healing (Mihai et al. 2019)

In addition to nanomaterials, nanoscale fibers can be used to make Nano-scaffold wound dressings. These dressings have a higher porosity, and their physical properties can be altered to provide the greatest absorbency and gas permeability for keeping the wounds moist and oxygenated (Nam, 2015). The structure of nanoscaffold wound dressings, which mimics the topography of endogenous extracellular matrix (ECM), can enhance the attachment and spreading of fibroblasts and keratinocytes, resulting in an increase in the collagen synthesis and re-epithelialization (Donato et al. 2023). Furthermore, when drugs are applied to the dressing, the nanoscaffold wound dressings' unusually high surface area-to-volume ratio maximizes their contact with the wound bed, allowing for better drug delivery. The physical nanoparticles, drug-releasing nanomaterials, and nanoscaffold wound dressings are promising therapeutic platforms to improve wound care. (Mordorski and Prow, 2016).

The most stable metallic NPs are inorganic nanoparticles like gold colloids, or AuNPs. A number of factors, including the microbial species and strains, the concentration, functionalization, and size of AuNPs, all have a role in the antimicrobial effect. The cytotoxicity and biocompatibility of NPs are directly impacted by their size, shape, dose, and surface functionalization. The production of AuNPs relies on finding equilibrium between their toxic effects and their antibacterial activity. For their many uses in fields as diverse as chemistry, food technology, water purification, medicine, pharmacology, microbiology, cell biology, parasitology, and home appliances, AgNPs rank high among the most studied metal and metal oxide compounds (Spirescu, 2021). As a result, investigations have shown that NPs with smaller diameters and bigger surface areas have more antimicrobial bioactivity reasons being; their concentration of ROS, necrotic factors, and apoptotic agents is higher. Coatings and surface characteristics of the NPs may also affect their antibacterial activities (Mordorski and Prow, 2016). Coating the surfaces with polymers or capping agents could offer anti-aggregating qualities, which is important because particle aggregation decreases bioactivity. Similarly, additional inorganic nanoparticles such as copper NPs, magnesium NPs, iron, and titanium NPs have the ability to resist bacterial infections and are thus implicated in wound healing (Spirescu, 2021). Inorganic NPs and their applications have explained by Spirescu in Table 1 below.

**Table 1:** Inorganic NPs and their applications (Spirescu, 2021)

| NPs type | Applications |
|---|---|
| AuNPs | Wound dressing, artificial skin, and tissue engineering and other biomedical applications |
| AgNPs | Endotracheal tube coating, urinary catheter coating |
| CuNPs | Biomedical applications |
| CuO NPs | Wound dressing |
| ZnO NPs | Antibiofilm platforms, medical device and implant coatings |
| MgO NPs | Wound dressing |

**Inorganic Nanoparticles**

The recent developments in nanotechnology have shown that the field of biomedicine can profit from new opportunities and promises. With this method, new avenues for treating chronic wounds have been opened up, including drug delivery mechanisms and the transport of biomolecules like DNA and RNA. Their smaller sizes and physicochemical characteristics aid in the delivery of medications and biomolecules into cells, shield particles from deterioration, and increase drug penetration at the wound site. The nanoparticles, which are smaller than 100 nm, are primarily the building components of nanotechnology (Yousaf et al. 2024). Because of their smaller size, the drug's bioavailability is increased and its harmful effect is reduced when it is released under control at the designated place. The use of nanoparticles in medicine is limited because they may contain harmful substances when produced utilizing various physical and chemical processes (Leu et al. 2012). Conversely, it has been shown that green synthetic nanoparticles made from specific plants are safer for the use in biological applications. Using naturally occurring biomolecules found in plants, fungi, or algae to reduce metals and create stabilized nanoparticles is known as biogenic synthesis. Typically, plant leaves, roots, fruits, or seeds are used to make extracts. Garlic is one of them for extracting metallic NPs. For many years, garlic (*Alium sativum*), a common vegetable in homes, has been utilized as a biological agent to heal infections and wounds. Plant extracts are a good source of proteins, carbohydrates, phenols, flavonoids, and certain enzymes. These phytochemicals are employed in the production of stabilized metallic nanoparticles and as a reducing agent for metals. Because there is such a wide range of phytochemicals in extract, the precise phenomena are still unknown. But it's evident that the main reducing agents are organic acids and polyphenols. These organic acids' and these phenols' inherent antibacterial qualities work in concert with NPs to provide a beneficial effect.

Inorganic Nanoparticles and their role in Wound healing

**Silver Nanoparticles**

The silver has been known for its antimicrobial action since ancient times. As an ion, silver was used to treat wounds in Egypt. The Egyptians used silver vessels to store water, and the Greeks and Romans used silver utensils to prevent food spoilage. Hippocrates also described the use of silver in the wound-healing process (Mendes, 2022). The silver compounds have been used for curing wounds since the 1970s. In the 20th century, silver nitrate was used for treating burns and wounds, and silver-impregnated dressings were also developed (Mendes, 2022). With the advancement in nanotechnology, silver nanoparticles (AgNPs) have been developed, which offer enhanced antimicrobial properties and improved wound healing outcomes. Silver nanoparticles are significant for wounds healing because of their chemical stability, catalytic activity, inexpensive cost, and broad-spectrum resistance to numerous infections (Gunasekaran, 2012). In the event of chronic wounds, silver is highly effective against multidrug-resistant microorganisms and biofilm-producing germs. Factors like size, shape, and dosage of AgNPs influence their antibacterial activity (Mendes, 2022). As the particle size is reduced their antibacterial activity is increased dramatically, which helps in the wound healing process.

The AgNPs have piqued scientists' interest in recent decades due to their chemical and biological capabilities, particularly in the biological and pharmaceutical industries. There are numerous reasons to use AgNPs in wound healing. The AgNPs can be synthesized on a wide scale using easy, safe, and cost-effective methods such as wet chemical, physical, and biological processes (Leu et al. 2012). Different shapes like spheres, rods, tubes, etc. can be synthesized by the reaction conditions selection. Because of the negative charge on their surface, the AgNPs are particularly reactive and may be modified using various biomolecules, which aids in drug administration. It is unclear how exactly the AgNPs counteract microorganisms (Gunasekaran, 2012). It is believed, however, that AgNPs impede bacterial growth by generating free radicals and rupturing bacterial cell membranes. These techniques kill bacteria by triggering the secretion of cell-deadening lipopolysaccharides and membrane proteins. When free radicals, such as reactive oxygen species (ROS), damage DNA and interrupt the mitochondrial respiratory chain, they halt the synthesis of ATP. Additionally, the AgNPs promote cell death by regulating genes such as p53. For these reasons, AgNPs are a promising alternative to conventional methods for wound healing in bacterial infections.

The silver nanoparticles (AgNPs) can influence the wound microenvironment by regulating the synthesis of inflammatory cytokines and proteins such as vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) (Gunasekaran, 2012). The silver nanoparticles (AgNPs) have been found to enhance cellular activity through the promotion of keratinocyte proliferation and migration, as well as the differentiation of fibroblasts into myofibroblasts, hence facilitating wound contraction. The silver nanoparticles facilitate the deposition and restructuring of collagen, hence accelerating the process of healing. Silver nanoparticles (AgNPs) have the ability to modulate the secretion of anti-inflammatory cytokines, leading to expedited wound healing while minimizing scar formation (Konop et al. 2016).. Silver nanoparticles (AgNPs) are employed in the treatment of wound infections, burns, ulcers, chronic wounds, and infection prevention. The silver nanoparticles (AgNPs) are used in wound healing through different methods, such as applying them topically (wound dressings, gels, or hydrogels), using nanocomposite materials (polymers or biopolymers), using nanofibers, and using semi-permeable film dressings.

**Gold Nanoparticles**

The gold nanoparticles (GNPs) are commonly used in the delivery of many bioactive substances because to their improved absorption and safety, which improves medication efficacy. GNPs are good carriers of medicines in therapeutic

purposes. The GNPs show excellent antioxidant and anti-inflammatory properties, which enable them to be utilized for tissue repair and wound healing (Leu et al. 2012). Antioxidant properties of GNPs mask the action of free radicals, including hydrogen peroxide ($H_2O_2$), hydroxyl (OH), and nitric oxide (NO), enhancing wound repair. Furthermore, the spherical GNPs have more surface area to interact with ROS, making them crucial for wound healing. They also act as catalysts in free radical deactivating processes.

The precise mechanism by which GNPs exert their effects remains incompletely elucidated. The binding of these entities to bacterial DNA is believed to impede the unravelling of the double helix structure during the processes of replication and transcription. Additionally, it is believed that they have the ability to penetrate bacterial cells, hence hindering energy utilization and altering membrane's potential. They can also block the ATP synthase enzyme to kill any remaining bacteria. The GNPs have been applied topically in rats to accelerate the healing processes (Leu et al. 2012). In addition to the expression of different cytokines and growth factors, they can also enhance collagen production, promoting tissue repair and remodeling. The GNPs also help in cell proliferation and migration, aiding in tissue regeneration and wound closure.

The gold nanoparticles can be applied in wound healing as wound dressings, hydrogel, and scaffolds. The gold nanoparticles can be utilized in photo-thermal therapy to get localized heat for wound sterilization and an accelerated healing process. While gold nanoparticles give great possibilities in wound healing, safety considerations are very crucial for their application. Factors such as nanoparticle size, shape, concentration, and surface modifications can affect their biocompatibility and potential toxicity. Proper characterization and testing are essential to ensure the safe use of gold nanoparticles in clinical settings and wound healing.

**Copper Nanoparticles**

Effective therapies are still required because wounds do not have a sterile environment. As a result, ongoing research seeks to develop more effective treatments for wound infections. A totally dissolvable, non-replaceable, or non-adherent wound dressing can improve therapeutic and pharmacological responses in the chronic wounds by accurately distributing medicines to the wound sites. In order to prevent infection and eliminate surplus fluid from the wound, dressings are typically applied and remained on the site for several days. When treating infections that require high concentrations of medicines, antimicrobial-embedded wound dressings can be helpful. Antibiotic abuse and misuse, however, has led to a significant rise of antibiotic-resistant bacteria, among other reasons. Since these infections are now causing issues, researchers have looked into non-antibiotic remedies for wound healing, such as essential oils and honey. These days, nanotechnology is an emerging therapy that treats wounds using materials that are only a few nanometers in size. It also has novel uses in regenerative medicine and the prevention of a number of diseases. The copper, silver, gold, and zinc, which are metal nanoparticles (NPs), are excellent options for incorporating into wound dressings due to their antibacterial characteristics and minimal toxicity. The copper (Cu) is classified as a bioactive nanoparticle (NP) due to its multifaceted impact on several cellular processes. It plays a crucial role in all stages of the wound healing process and influences the actions of various cytokines and growth factors.

The copper, a crucial element, is required in minimal quantities for a multitude of metabolic processes. Under appropriate regulated circumstances, copper has been observed to induce the production of extracellular matrix constituents, including fibrinogen, collagen synthesis, and integrin. These components play a crucial role in facilitating cell adhesion to the extracellular matrix. The excessive use of copper can have negative effects as it produces free radicals, which can result in cell death and lipid peroxidation. For example, only 3% of breast epithelial cells cultured with 10 mg of copper in nanofibers survived, demonstrating that the levels of copper produced by the nanofibers are particularly toxic to tissue culture cells. Nevertheless, other researches have demonstrated that applying copper concentrations at this level to human skin does not result in any negative side effects.

Under physiological settings, absorption, transport, and excretion of free copper regulate its level within cells. The primary mediator of this absorption is the CTR1 copper importer (Marcato et al. 2015). Copper can enter the cell through the CTR1 pathway and thereafter traverse the Atox1 copper chaperone, ultimately entering different cellular compartments facilitated by the ATP7A copper trafficking ATPase (Yulizar et al, 2017). The Cu-transporting ATPases, such as ATP7A and ATP7B, regulate copper excretion through the colon, liver, and mammary glands. On the other hand, a considerable copper surplus in certain tissues and a decreased outflow of copper from cells is associated with deactivation of their transport activity.

The transporter known as ATPase, the involvement of ATP7A in the regulation of secretory enzymes and intracellular copper levels is of significant importance. In typical circumstances, ATP7A is situated inside the trans-Golgi network (TGN), where it facilitates the transportation of copper to pro-enzyme of lysyl oxidase (Pro-LOX) and extracellular superoxide dismutase (SOD), which are responsible for secreting copper (Marcato et al. 2015). The presence of these enzymes is essential for the functioning of lysyl oxidase (LOX), a catalyst that facilitates the development and spread of tumors. Moreover, ATP7A contributes to the process of angiogenesis that is triggered by VEGF and ischemia in endothelial cells (ECs). Under pathological circumstances characterized by elevated cellular copper levels, ATP7A is transported from the transgonial nucleus (TGN) to the plasma membrane in order to eliminate surplus copper.

In addition, it has been demonstrated that non-metal stimulants such insulin, N-methyl-D-aspartic acid or N-methyl-D-aspartate, platelet-derived growth factor, and hypoxia can induce ATP7A to relocalize in the transverse glomerular

nucleus (TGN). The antimicrobial copper-based wound dressings are becoming increasingly common. Because silver is toxic to cells, they are replacing the silver-containing bandages for wound healing on a massive scale. The Fenton-like reaction has been related to copper toxicity because it generates reactive oxygen species (ROS) in close proximity to copper ions (Yulizar et al. 2017). These ROS can harm proteins and lipids. Furthermore, anoxic conditions have been demonstrated to exhibit extended copper activity through a ROS-independent mechanism, which is sufficient to competitively block cytoplasmic iron-sulfur enzymes (such as intracellular dehydratases). However, glutathione, Cu/Zn superoxide dismutase, and cytoplasmic metallothioneins offer some protection to mammalian cells. Figure 1.4 depicts the involvement of copper sulfide nanoparticles (Cu NPs) in the wound healing.

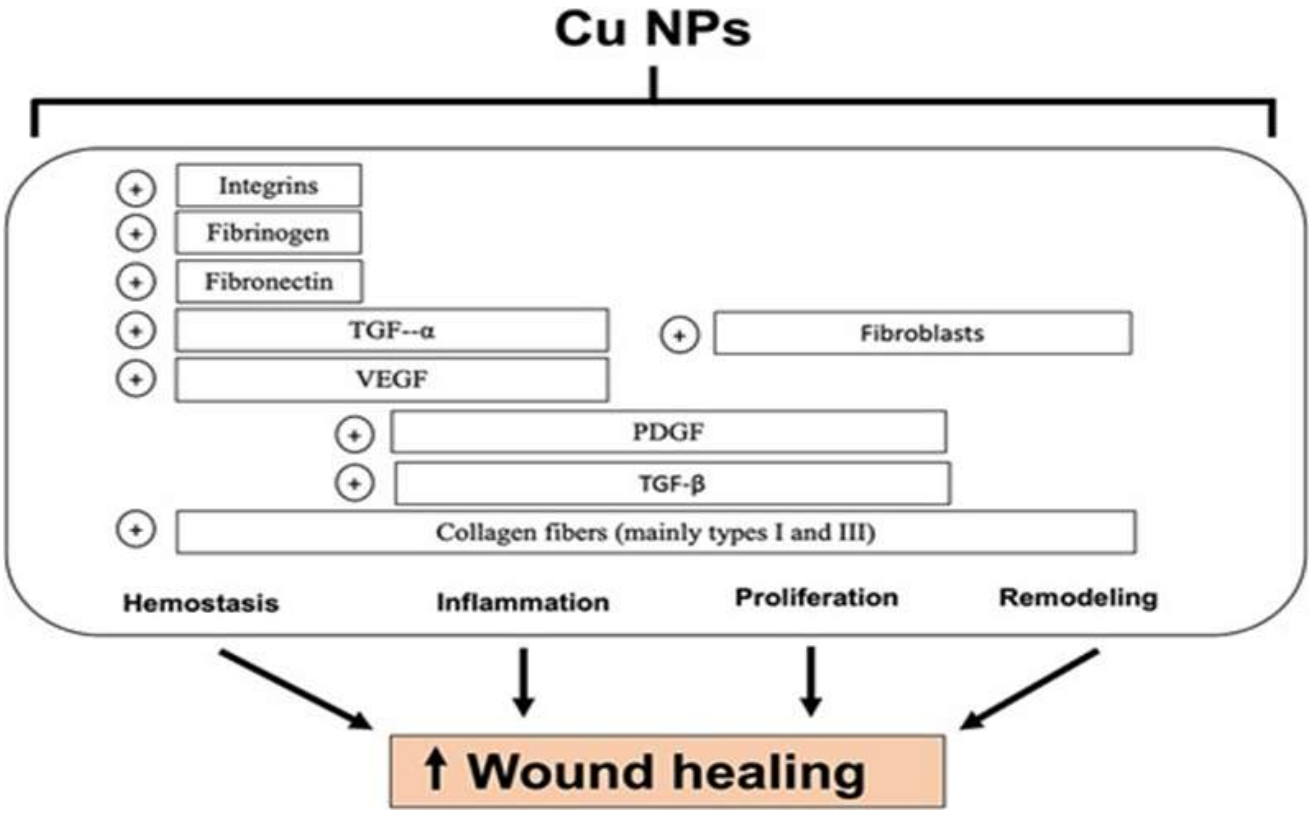


**Fig. 1.4:** Role of copper nanoparticles in wound healing (Yulizar et al. 2017)

The copper, in any of its forms, should be avoided at high concentrations. In human skin epidermal cells, Cu NPs were able to exert cytotoxic and genotoxic effects thanks to the activation of mitochondrial pathways by reactive oxygen species (ROS). At different doses (200, 100, 50, 20, and 10 µg/mL), copper nanoparticles (Cu NPs) did not cause any harm to the cells, but rather they facilitated their proliferation (Toczek, 2022). This may be attributed to the size of the Cu NPs, as the colon is responsible for removing the majority of particles that have not been absorbed. High copper contents in the feces strongly indicate that the liver and feces were the principal routes of elimination for absorbed or unabsorbed copper ions. Moreover, studies for cell viability have demonstrated a strong dependence on Cu NP content; for example, a higher NP concentration has been linked to a greater response to growth factor stimuli that encourage cell proliferation.

In addition, copper possesses significant biocidal qualities; nevertheless, in contrast to silver, it is easily digested by the human body unlike silver. The copper has a critical role in the creation of hypoxia-induced factor-1-alpha (HIF-1α), which is essential for the regeneration of skin and the formation of new blood vessels (Marcato et al. 2015). Through the promotion of VEGF and angiogenesis, it has been found to hasten the healing process in human and animal models. In the process of wound healing that is stimulated by copper, HIF-1 has been identified as a crucial helper factor (Konop et al. 2016). Due to the fact that low levels of copper at the wound site hinder people with reduced peripheral blood supply (diabetes, vascular illnesses, etc.) from healing, its action is crucial for the healing of wounds. Copper oxide-containing wound dressings have been shown in a number of case studies to enhance skin regeneration and wound healing, in addition to protecting the wound and dressing against microbial contamination (Nandhini et al. 2024). A number of these investigations have been reported. An important discovery has been made. In addition, when compared to sleeping on a conventional pillowcase, utilizing a pillowcase that has been treated with copper oxide has resulted in a considerable reduction in the appearance of wrinkles and crow's feet, as well as an improvement in the general looks of the face.

Copper has been found to increase MMP activity in fibroblasts at low doses (0.3-3 µM) and increase MMP expression at high concentrations (1-100 µM). However, excessive amounts of free metal can decrease MMP activity (Toczek, 2022). The copper has also been shown to upregulate MMP2 and MMP3. In the essence, copper ions may improve wound

healing by stimulating angiogenesis through VEGF release. A non-invasive, minimally invasive method of killing bacteria, photo-thermal therapy triggered by NIR light irradiation may also be possible with nano-formed copper, such as CuS NPs. The CuS NPs may thus provide angiogenesis and antibacterial action, both of which can help to speed up wound healing (Marcato et al. 2015). Additionally, *in-vitro* and *in-vivo* models demonstrated that NPs at a concentration of 200 µg/ml significantly increased the cell proliferation.

**Conclusions**

The therapeutic activity of inorganic nanoparticles has been attributed to its antibacterial activity and wound healing in humans and animals. Inorganic nanoparticles' promise shines brightly as a ray of hope for patients and clinicians alike as we stand at the forefront of innovation in wound healing treatments. Through the utilization of nanoparticles' distinct physicochemical characteristics and biological functions, we can explore novel approaches to expedite tissue healing, reduce problems, and enhance the quality of life for patients with both acute and chronic wounds. Let's embrace the revolutionary power of nanotechnology as we forge ahead into new territory and work towards a future where scars dissolve more softly, wounds heal more quickly, and lives blossom more brightly. It also helps in prevention of antimicrobial resistance.

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Chapter 20

# Nanoparticles as Novel Alternative Therapy in Veterinary Medicine

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**ABSTRACT**

Biotechnology and veterinary medicine are just a few fields in which nanoscience and nanotechnology are applicable in conducting research and finding applications. The application is rather novel in animal husbandry and veterinary care. Nanotechnology has enormous potential to affect not only how we live, but also how we practice veterinary treatment, increasing the safety of domestic animals, productivity, and farmer revenue through the use of nanomaterials. The current state and breakthroughs in nanotechnology are being used to improve animal growth promotion and output. To do this, nanoparticles are utilized as alternative antibacterial agents to combat the rising trend of antibiotic use and detecting harmful bacteria. In addition, nanoparticles are employed as medicine-delivery agents. Nanoparticles are also used as new drug and vaccine candidates exhibiting better functions and improved characteristics, diagnosis, therapy, feed additive, nutrient delivery, reproductive aid, production supplement, medicinal agents, and finally, a variety of functionalized nanoparticles, including liposomes, polymeric nanoparticles, dendrimers, micellar nanoparticles, and metal nanoparticles, will be used to improve food quality. It appears that nanotechnology is excellent for veterinary applications in terms of cost and revenue availability. The primary goal of this study is to discuss some of the most relevant current and future elements of nanotechnology's use in veterinary medicine.

**KEYWORDS**
Veterinary medicine, Nanoparticles, Animal production, Antivirals

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## INTRODUCTION

Nanotechnology, a fast-rising subject dating back to 1974, has resulted in the invention of several unique nanoparticles with typical sizes ranging from 1 to 100 nanometers (nm) (Mourdikoudis et al., 2017). The prefix nano is derived from the Latin word "nanus," which means "extremely little," because 1 nm equates to $10^{-9}$m (Pangestika and Ernawati 2017) Currently, nanotechnology is being employed in several industries, including agriculture and infection control (Rima et al., 2019), and biomedicine (Mandal et al., 2013). Nanoparticles exhibit numerous physical and biological properties and are used accordingly, for instance, area of interaction, reaction kinetics, size and volume ratio, mode of action, way of target, and delivery protocols (Timilsena et al., 2019). Furthermore, nanoparticles can permeate cells, tissues, and organs, making them efficient medication delivery methods (Rai et al., 2016). Various pharmaceutical compounds can be attached to the surface of nanoparticles (Prasad et al., 2014). To address challenging challenges, established therapies may not be adequate, and alternative ways must be investigated, which can shape future results and criteria for current problems (Saeed et al., 2019). Numerous nations' economy relies on animal-based sectors, and as many viral illnesses arise, improved disease management and preventive regimes are urgently needed (Espinosa et al., 2020). Nanotechnology has demonstrated enormous potential for improving the delivery of medications and vaccinations in the realm of veterinary care (Sharma et al., 2023). The expanding nanoparticle sector will result in the creation of novel therapies to treat viral or bacterial infections, as well as to improve the healing of severe wounds. Furthermore, these newly designed nanoparticles might successfully transmit drugs into different cells to cure ailments (Hajipour et al., 2012). Another incredible advancement in nanotechnology is nano-theranostics, a medical procedure that combines medications and diagnostics to

increase the efficacy of existing drugs (Liu Z and Liang 2012). Furthermore, this connection gives a significant chance to develop and design these agents, allowing therapeutic administration as well as a way of detection before and throughout the treatment process (Gobbo et al., 2015). Nano-pharmaceutical products are one of the most hopeful and favorable industries of nanotechnology, with various advantages in veterinary care (Underwood and Van Eps 2012; Kalia et al 2014). Because of the relevance of nanoparticles, this review will focus on them as innovative and potential therapies in veterinary medicine.

**Types of Nanoparticles**

Nanoparticles are classified according to their use in biomedical applications i.e., veterinary medicine, their origin, and shape. As their origin is concerned, they are classified into organic, inorganic, and hybrid nanoparticles. In classifying nanoparticles according to their shape, they are divided into spheres, tubes, and liquid drops. According to the use of nanoparticles in veterinary medicine, nanoparticles are classified in various types that are given in (Table 1):

**Table 1:** Classification of Nanoparticles

| Name | Definition | Uses | Reference |
|---|---|---|---|
| Polymeric Nanoparticles | These nanoparticles are composed of polymer (macromolecule consists of repeating units) having active molecule adsorbed on its surface. These are similar to dendrimers except the branches originated from a core having various numbers of branching points. | These can be used for the delivery of drugs, oligonucleotides, DNA, and protein. These can also be used for drug loading. | (Bai et al., 2018; Meena et al., 2018) |
| Liposomes | These are synthetic vesicles consisting of an aqueous core encased with phospholipid bilayers. | These can be used for vaccines, targeted drugs, imaging agents, gene delivery, and cancer therapy. | (Meena et al., 2018) |
| Buckyballs (fullerenes) and Bucky-tubes (nanotubes) | Fullerenes are carbon-based nanoparticles having the shape of small balls. Nanotubes are also carbon-based nanoparticles having cylindrical or tube-like shapes. | Fullerenes can link easily with cells, proteins, or pathogens. Nanotubes can be used as biosensors i.e. for detecting glucose, ethanol, and immunoglobulins or for electro-chemical hybridization of DNA. | (El-Sayed et al., 2020) |
| Nano-shells | These are round and composed of dielectric core e.g., glass or silica, and a very thin coating of metal i.e., gold. | They are used for cancer diagnosis by the penetration of IR radiation. They also have therapeutic applications in tumors by irradiation of infrared laser with them. | (El-Sayed et al., 2020) |
| Solid lipid Nanoparticles | They are made up of lipids that are solid at room temperature. Their stabilization takes place with surfactants and they are suspended in aqueous solution. | They are used in the formation of controlled-release formulations that last for several weeks. They increase the absorption of orally administered drugs due to the capability of attachment with mucosal surfaces. They are used in the delivery of drugs in the brain system. | (Meena et al., 2018) |
| Micelles polymeric | They are supramolecular structures i.e., composed of self-attachment of amphiphilic molecules. They ate a multiphasic nano-emulsion. They are unique as they allow the materials soluble in water or oil and produce a stable nano-system consisting of sizes from 300 nm to 1000 nm. | They are used to deliver drugs topically or trans-dermally. | (Prasad et al., 2021a) |
| Dendrimers | They are hyperbranched nanomaterials. They are composed of polymers that are smaller than body cells. They seem to be a tree of three-dimensional expanded atoms. | The fundamental usage of dendrimers is in the treatment of cancer. They are also used in medication used for imaging. Nanocomposites filled with dendrimers are effective antimicrobial agents against *Pseudomonas aeruginosa*, *E-coli,* and *Staphylococcus aureus*. | (Youssef et al., 2019) |
| Metallic nanomaterials | The most commonly used metals are gold, copper, and silver. Other metals i.e., gadolinium, manganese, and platinum. Their metallic cores are coated with shells with capsules. | They are in bio-sensing, bio-imaging, and cancer thermotherapy. They are also used for targeted drug delivery. | (Meena et al., 2018; El-Sayed et al., 2020) |

| | | | |
|---|---|---|---|
| Magnetic iron oxide nanoparticles | These nanoparticles are mainly composed of iron oxide. | They are used as biosensors for imaging and drug delivery. They are also used for heat therapy. They have clinical applications as well. | (Meena et al., 2018) |
| Ceramics | They are composed of materials like alumina, silica, and titania. They are easy to design and are completely inert. They can be changed into different forms, sizes, and porosity. | They give protection to the adsorbed particles against extreme pH and temperature. | (Meena et al., 2018) |
| Quantum dots | They are nanocrystals about 2-10nm in size. They are composed of an inorganic shell and core having an organic coating with which conjugated biomolecules are attached. | They are used for diagnosis and in clinical uses. They are also used as imaging agents. | (Woldeamanuel et al., 2021) |
| Nano-emulsion | These are dispersions of oil and water. These are composed of nano oil drops which are coated with a thin film of surfactant and co-surfactant. The coating of thin film is to stabilize the oil droplets physically. | Nano-emulsion does not affect the structures of cells which is why it is ideal for usage in humans and animals. In the future, they may be used as bactericidal and veridical for topical use in humans and animals. They are used for drug delivery. | (Meena et al., 2018) |
| Respirocytes and Microbivores | Respirocytes are nano-devices that act like the red blood cells but they deliver 236 times more oxygen per unit volume than natural red blood cells.<br>Microbivores are hypothetical white blood cells that are designed to capture circulating microbes. | Respirocytes have sensors that regulate the intake and output of oxygen and carbon dioxide. Microbivore circulation in human and animal blood clears the blood theoretically in septicemia (bacteria enter the bloodstream and cause blood poisoning) at a greater speed than the natural defense system. | (Meena et al., 2018) |
| Nano/micro-robots | They are programmed and computerized robots. | They are used to repair particular diseased cells. They function like antibodies that work in our natural healing. They shield of body from the presence of malignant cells. | (Meena et al., 2018; El-Sayed et al., 2020) |
| Aluminosilicate Nanoparticles | These are short-chain polyphosphates coupled with silica nanoparticles. | They increase the clotting mechanism naturally. This causes a decrease in bleeding. | (Youssef et al., 2019) |
| Nano-bubbles | The bubbles remain stable at room temperature but when they get heated i.e., in exposure to ultrasonic waves, they form aggregation to form micro-bubbles. | They are usually used for drug delivery selectively into tumor tissues. They are also used in gene therapy. | (El-Sayed et al., 2020) |

**Nanoparticles in Veterinary Medicine**

In several areas of veterinary medicine, including medicines, diagnostics, tissue engineering, vaccine production, and disinfectants, nanotechnology has a big impact (El-Sayed and Kamel, 2020). Applications of nanotechnology are now being utilized in the fields of animal welfare, raising, procreation, and nutrition. Using incredibly tiny doses that gradually reduce pharmaceutical build-ups and withdrawal times in homestead animals allows the treatment to be delivered directly into the target cells. The manufacture of medications, vitamins, probiotics, and nutritional supplements is one possible application of nanotechnology in animal husbandry (Prasad et al., 2022). Another is the non-surgical identification and removal of infection-causing pathogens through the use of nanoparticles. However, even if employing nanotechnology limits the range of antibiotics utilized due to their nano-size, the regular use of antibiotics in animal production might leave a residue that affects the end consumer (Haben Fesseha, 2020).

Several studies have been carried out to search out the potential efficacy of nanoparticles over antimicrobials and traditional medicine. Animal production must benefit from this time by using nano-fertilizers, which distribute vitamins in the necessary amounts to specific locations inside the forages (Gelaye, 2024).

The manufacturing of animal feed benefits greatly from the low cost and reduced usage of nano minerals, which also function as immune-stimulating and growth-promoting agents. In a similar vein, they can aid in controlling feed pathogens and enhancing rumen fermentation (Michalak et al., 2022). One of the most promising nano-minerals for enhancing immunological function, growth rate, and problems affecting livestock reproduction is nano-zinc oxide as shown in (Figure 1).

Feed components are microencapsulated to prevent light-induced oxidation and degradation, as well as protease-induced lysis. This process also promotes stability over a range of pH values, improves the dispersion and mixing of lipophilic additives, and extends the duration of their activity. Humans and animals face a significant problem is mycotoxicosis (Ajeeshkumar et al., 2021). It is possible to find them in around 25% of animal feed. Relatively strong nano ant mycotoxin, nano silica, and nano magnesium oxide effectively bind to and deactivate aflatoxins (Rashiku, et al., 2023).

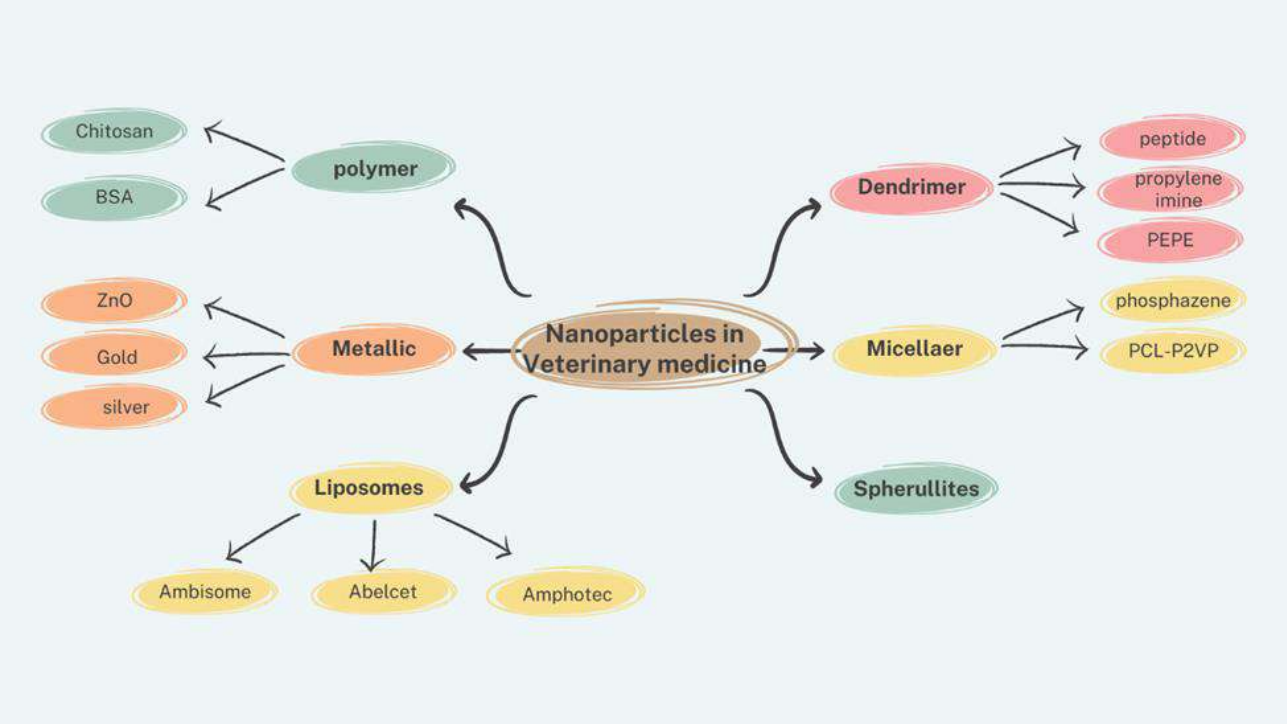


**Fig. 1:** Brief Classification of nanoparticles used in veterinary medicine

**Mode of Action of Nanoparticles**

The size, form, and surface reactivity of nanoparticles with the surrounding tissue determine their behavior. A particle's surface area to volume ratio, chemical reactivity, and biological activity increases with particle size. Reactive oxygen species are produced in larger quantities due to the higher chemical reactivity of nanomaterials (ROS) (Alghuthaymi et al., 2021) A wide variety of nanomaterials, including metal oxide nanoparticles and carbon nanotubes, have been reported to produce reactive oxygen species (ROS). One of the main processes that can lead to oxidative stress, inflammation, and the ensuing damage to proteins, membranes, and DNA is the generation of free radicals, or ROS. Reactive oxygen species have been demonstrated to be produced by nanosized particles of different chemistries (ROS). Nanoparticles as different as C60 fullerenes, SWNTs, quantum dots, and UFPs have been shown to produce ROS, particularly when exposed to light, UV radiation, or transition metals together as shown in (Figure 2). NSPs with different sizes and chemical compositions have been shown to preferentially migrate to mitochondria (Abbas et al., 2022). Since mitochondria are redox-active organelles, there's a chance that they'll change the amount of ROS produced, which might overburden or disrupt antioxidant defenses. Some of the antioxidant defense mechanisms that occur in mammals, and likely sites where NSPs may form oxyradicals. Although the precise process by which each of these various NPs produces ROS is still unclear, several theories include photoexcitation of SWNTs and fullerenes, which results in intersystem crossover and free electron creation; NP metabolism to produce redox active intermediates, particularly if cytochrome P450s are involved in the metabolism, In vivo inflammatory reactions that might lead to macrophages releasing free radicals (Woldeamanuel et al., 2021).

**Efficacy of Nanoparticles**

While the majority of nanoparticles are generally benign, some may also have harmful effects. For example, prolonged pulmonary exposure to carbon nanotubes may cause reproductive issues in pharmaceutical industry employees. Moreover, the formation of appealing iron oxide nanoparticles within the margin, or via damages accelerated by an unstable binding between the drug and the particles, which may also deliver the drug into solid tissues as opposed to the intended tissues (Krishnani et al., 2022). The direction's partial arrival away from the target tissue or organ will now result in toxicity to healthy tissue and the delivery of doses at the target component that are below the recommended therapeutic level. Their capacity to shift different biological barriers within the shell—such as the blood-brain barrier—has remarkable effects on the environment. For example, it increases the demand for radionuclides. Additionally, carbon nanofibers are implicated in depleting the ozone layer in the biological system (Citarasu et al., 2022).

**Comparison of Nanoparticle Treatment with Antibiotics**

Antibiotics were developed throughout the 20th century, which decreased the morbidity and death from microbial illnesses. Even with the significant advancements in antimicrobial therapy, many infectious diseases—particularly intracellular infections—remain challenging to treat. Microorganisms can survive continuous infections for months or even years. The development of antibiotic resistance can also lead to an inadequate therapeutic index (Fawzy et al., 2021). This is a major concern for pharmaceutical companies and researchers, who are working to develop new antibacterial capsules

and delivery systems at the same time to combat multidrug-resistant microorganisms. Side effects include nausea, vomiting, irritability, scaling, and a decrease in gut microflora. They upgrade remedial viability and symptoms of medications. They improve the dissolvability of inadequately water-solvent medications (Prasad et al., 2021b).

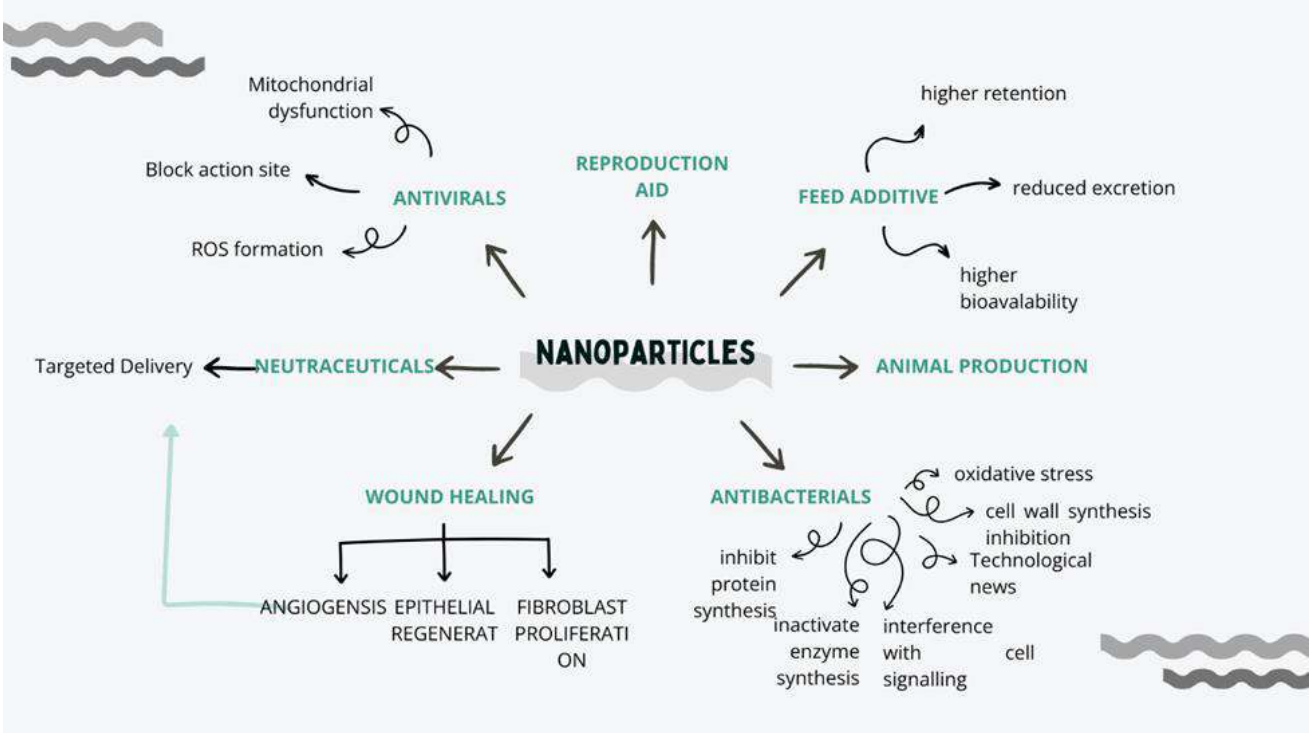


**Fig. 2:** Mode of action of nanoparticles

Antimicrobial medications are simpler to deliver thanks to nanoparticles' special qualities, which include their remarkably tiny, regulated size, vast surface area, and strong reactivity. When combined, they maximize therapeutic efficacy and minimize undesirable properties of pharmaceuticals. Additionally, they make drugs that are less soluble in water more soluble (Ali et al., 2021). They also increase the medicinal compounds' potency and selectivity towards the intended cell or tissue. They have extremely beneficial features, such as the ability to shorten the duration of a drug's systemic circulation, minimize sedative digestion, enhance absorption, and facilitate the administration of two or more treatments at once for mixed therapy. They are also utilized in the renovation of long-used, antiquated medicinal bases. They can reduce the adverse effects and toxicity of conventional therapeutic medicines (Spirescu et al., 2021). They can also minimize the hazards related to medicine residues since they leave fewer residues in animal products. The application of nanoparticles offers a notable advantage in that it reduces the necessary doses, frequency of dosing, and concentrations of the medication during the therapeutic process.

**Future Prospects**

Future achievements in the field of veterinary medicine include more advanced research in targeted therapies, nano-vaccine development against infectious diseases, and further study of gene delivery systems (Danchuk et al., 2023). Nano-scale technologies will start more yielding medical benefits within 10 years. Nanotechnology will become more widely used in the future for the production of animals. The nano-supplements to strengthen livestock feed will be used shortly. Uses of nanoparticles externally have been studied in animals i.e., antiseptic wound healing, and further uses are being studied (Hill et al. 2017). In the field of food safety, detection of diseases, cure, fabrication of vaccines, delivery systems, the technology used in reproduction, and the poultry industry nanoparticles are being used in the place of antibiotics (Abbas et al. 2022). Nanoparticles have advantages in genetic breeding and therapy of illness but the ways have not been explained yet. Nanoparticles provide uses in animal nutrition, reproduction, and welfare which give breeding business plans with better management and models (Wang et al. 2022).

**Conclusion**

Nanoparticles have a wide range of functions in animal production, health and reproduction this study aims to highlight these uses as well as suggest possible directions for further research. Nanoparticles are now on the market, and as they continue to be developed, their characteristics will be more precisely tailored for a greater range of uses, because of their shortened time of treatment, small doses, drug interaction and elevating antimicrobial resistance in previous years. Although research on nanotechnology in animal production is still in its early stages, promising outcomes from studies on

nutrition, biocidal, remedial, and reproductive applications are motivating more research. The challenge mostly faced during use of nanoparticles is its extraction from the body, metal toxicity and interaction with other body normal functions. Proper assays for metal toxicity and therapies for their release can enhance the efficacy and use of these nanoparticles in daily life, veterinary therapeutics and medicinal practices.

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Chapter 21

# Exploring Nanoparticles: Advancements and Applications in Complementary and Alternative Medicine

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**ABSTRACT**

The rise in popularity of Complementary and Alternative Medicine (CAM) can be attributed to its holistic approach and focus on the patient. In contrast, nanotechnology has led to significant progress in various industries, especially in healthcare, through the manipulation of materials at a very small scale. The recent integration of nanoparticles with CAM shows great promise in improving treatment effectiveness and overcoming current limitations. Different types of nanoparticles, such as metallic, polymeric, and lipid-based varieties, have unique physical and chemical properties that can be harnessed for precise drug delivery, increased bioavailability in the body, and controlled release of active ingredients in herbal treatments because of their mini-scale nature. Through the use of nanoparticles, the benefits of acupuncture can be enhanced by directly delivering therapeutic substances to the acupuncture points and improving acupuncture needles such as PANs, which ultimately improves the efficacy of treatments and enhances the healing outcomes and attaching Nanosensors to the needles can help in monitoring the drug targeting.

**KEYWORDS**
Complementary and alternative medicine, Nanoparticles, Herbal medicines, Acupuncture, Disease management

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## INTRODUCTION

A developing body of scholarly research has extensively probed the realm of Complementary and Alternative Medicine (CAM), which encompasses a vast array of medical procedures, treatments, and supplies that lie outside the boundaries of conventional medicine. This multifaceted and expansive domain incorporates a wide spectrum of techniques, including but not limited to mindfulness meditation, chiropractic adjustments, as well as herbal and acupuncture treatments. The growing worldwide fascination with complementary and alternative medicine (CAM) points to a substantial transition towards more customized and holistic strategies designed to enhance health and overall well-being among individuals. As the landscape of healthcare undergoes a continuous evolution in response to shifting needs and preferences, specific methodologies within complementary and alternative medicine (CAM) exhibit deep historical roots in various cultures spanning millennia, while others have surfaced more recently (Nejat et al., 2023)

A multitude of rigorous investigations have been undertaken to delve into the effectiveness, safety, and integration of complementary and alternative medicine, illuminating the escalating popularity of this field. Several research endeavors have meticulously examined the therapeutic potential of diverse complementary and alternative medicine (CAM) modalities in addressing conditions such as immunological disorders, anxiety, depression, and chronic pain. Additionally, the dominance of integrative medicine, which aims to synchronize evidence-based complementary and alternative medicine (CAM) practices with traditional medical interventions, represents a significant change in healthcare toward a patient-centered model covering a wide range of treatments and methodologies (Langhorst et al., 2015).

The application of nanoparticles in Complementary and Alternative Medicine (CAM) constitutes a revolutionary strategy aimed at boosting the specificity, effectiveness, and transport of both conventional and alternative medicinal agents. By exploiting the distinct attributes of nanoparticles, this amalgamation within CAM introduces an innovative approach that harbors substantial potential. Because of their microscopic dimensions, nanoparticles offer numerous

benefits in the realm of medicine, including an increased surface area to volume ratio, improved solubility, and the capacity to traverse biological barriers that are typically impassable by larger particles. These traits facilitate the precise and efficient delivery of active compounds present in herbal and natural remedies, potentially amplifying their bioavailability and diminishing undesired side effects. For example, the utilization of nanoparticles has been shown to enhance the efficacy of curcumin derived from turmeric, owing to improved solubility and absorption within the body, thereby augmenting its anti-inflammatory properties (Bitencourt, 2020; Shree et al., 2023).

**Nanoparticles**

Nanoparticles are ultrafine particles comprising 1 to 100 nanometers of thickness. They exhibit peculiar traits because of their extremely small dimensions, which give rise to exclusive properties. Their microscopic nature causes quantum effects to come into play, thus altering their optical, electrical, and magnetic attributes in fascinating ways. It is noteworthy to highlight that their highly exceptional surface area-to-volume ratio plays a significant role in magnifying their capacities in catalysis, adsorption, and reactivity processes. The realm of surface chemistry emerges as a critical aspect to consider due to its profound impact on factors such as stability, cellular compatibility, and environmental interactions (Zein et al., 2020). By utilizing surface modifications, nanoparticle customization becomes possible, which increases their suitability for particular applications like encompassing targeted drug delivery, catalytic processes, and advancements in nano-electronics. These exceptional features serve to emphasize the profound influence exerted by nanoparticles across a wide array of scientific and industrial sectors which holds the promise of introducing groundbreaking developments in fields like materials science, medicine, and technology (Suttiponparnit et al., 2011).

**Classification of Nanoparticles**

Nanoparticles (NPs) are divided into three classes according to their composition:

a. Organic.
b. Carbon-based.
c. Inorganic.

**Organic Nanoparticles**

The organic class of nanoparticles comprises proteins, carbohydrates, lipids, polymers, or other organic compounds. Notable examples in this class include micelles, liposomes, dendrimers, and protein complexes, such as ferritin. Organic nanoparticles can have a hollow core, like liposomes, and are frequently non-toxic and biodegradable. They show sensitivity to light and heat, as well as other forms of electromagnetic radiation. Furthermore, these nanoparticles are usually generated by non-covalent intermolecular interactions, which increases their reactivity and provides an avenue for the body to eliminate them. The composition, surface shape, stability, and carrying capacity of organic nanoparticles all influence their field of application. Nowadays, organic nanoparticles are widely used in the biomedical industry, especially for cancer therapy and targeted drug delivery (Ealia and Saravanakumar, 2017). Fig. 1 shows types of organic nanoparticles.

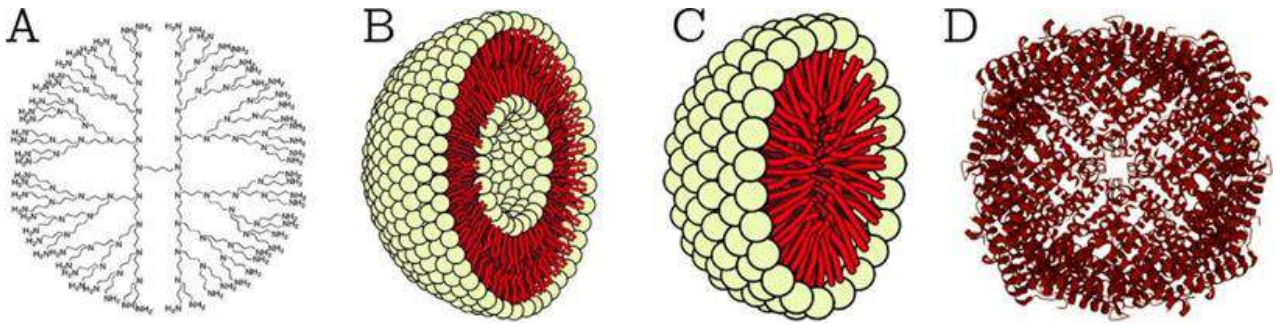


**Fig. 1:** Types of organic nanoparticles. A Dendrimers; B liposomes; C micelles; and D ferritin (Joudeh and Linke, 2022) Creative Commons CC BY

**Carbon-based Nanoparticles**

Carbon-based nanoparticles are a type of nanoparticle made entirely of carbon atoms. Fullerenes, carbon black nanoparticles, and carbon quantum dots are some notable examples. Fullerenes, such as C60, have a closed cage structure, whereas carbon black nanoparticles form grape-like aggregates. Carbon quantum dots are discrete carbon nanoparticles smaller than 10nm. These carbon-based nanoparticles have distinct features such as electrical conductivity, high strength, and optical and thermal properties, making them useful in a variety of applications, including medication delivery, energy storage, bio-imaging, and environmental sensing. Nano-diamonds and carbon nano onions, which are more complex carbon-based nanoparticles, are also used for drug administration and tissue engineering due to their low toxicity and biocompatibility. Overall, carbon-based nanoparticles have enormous potential for developing a variety of sectors (Joudeh and Linke, 2022). Fig. 2 shows carbon-based nanoparticles.

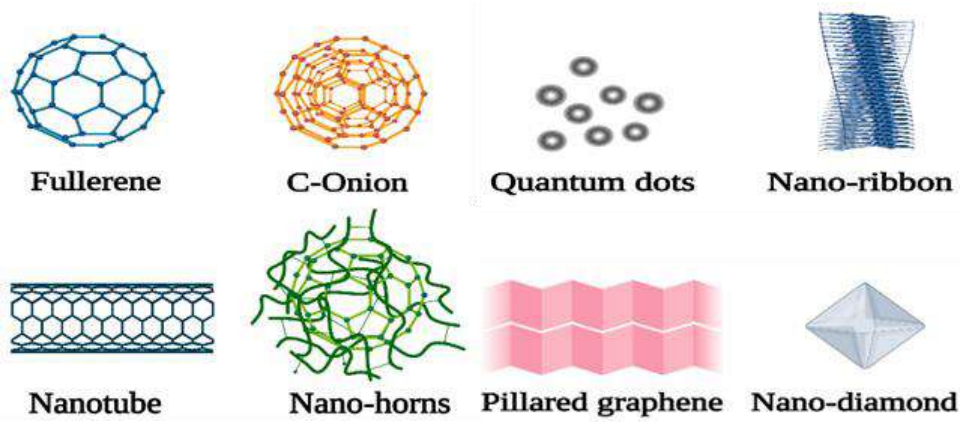


**Fig. 2:** Carbon-based nanoparticles (Singh et al., 2022) (https://creativecommons.org/licenses/by/4.0/).

**Inorganic Nanoparticles**

Metal, ceramic, and semiconductor nanoparticles are classified as inorganic. Metal nanoparticles consist entirely of metal precursors and might be monometallic, bimetallic or polymetallic. They have distinct optical, electrical, thermal, magnetic, and biological properties, making them indispensable for a variety of applications in physical, chemical, biological, biomedical, and pharmaceutical sciences (Mody et al., 2010). Semiconductor nanoparticles have large bandgaps so they can change their properties dramatically by bandgap tuning, which makes them useful in photocatalysis, electronics, and optics (Gupta and Tripathi, 2012). Ceramic nanoparticles are inorganic solids compiled of assorted metal and metalloid combinations. They are frequently employed in biomedical applications due to their excellent stability and load capacity. They are also used in catalysis, dye degradation, photonics, and optoelectronics. Understanding the properties and applications of these inorganic nanoparticles permits the creation of cutting-edge materials for a variety of fields (C Thomas et al., 2015). Inorganic nanoparticles are shown in the following fig. 3.

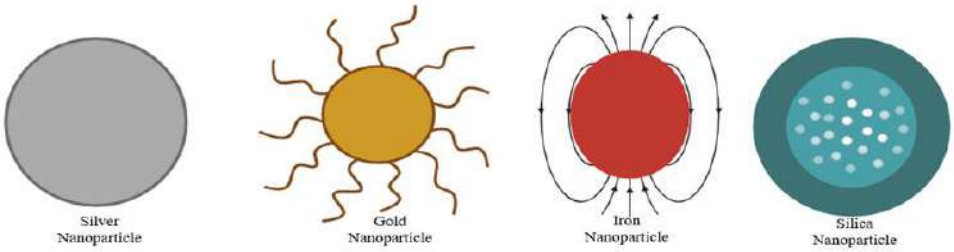


**Fig. 3:** Examples of inorganic nanoparticles

**Synthesis of Nanoparticles**

A wide range of methods are used in the synthesis of nanoparticles, all of which are designed to create materials with precisely controlled nanoscale features. Chemical synthesis is a widely used technique in which precursor molecules are subjected to controlled reactions to generate nanoparticles. This method frequently makes use of stabilizing agents, reducing agents, and solvents to control the content, size, and shape of the particles (Gudikandula and Charya Maringanti, 2016). Physical techniques like laser ablation or evaporation are a further option. These technologies use energy to directly convert bulk materials into nanoparticles (Iravani et al., 2014). Additionally, biological techniques use the ability of living things or proteins to create nanoparticles with very high accuracy. Every synthesis process has its advantages and difficulties, meeting the particular needs of a wide range of applications, from electronics to medicine. Interdisciplinary partnerships are advancing materials science and nanotechnology by pioneering new ways and refining current techniques as nanoparticle research progresses (Hasan, 2015).

**Functions of Nanoparticles**

There are numerous roles of nanoparticles, a few of them are mentioned below:

a) Drug Delivery: Nanoparticles can package drugs and transport them to specific locations within the body, thereby improving drug effectiveness, minimizing side effects, and enabling precise therapy.

b) Imaging: Engineered nanoparticles can serve as contrast agents in medical imaging methods like MRI, CT scans, and fluorescence imaging, facilitating clearer visualization of tissues and organs.

c) Catalysis: because of their high surface area-to-volume ratios, nanoparticles function as efficient catalysts for chemical reactions in domains such as environmental cleanup, fuel cell technology, and industrial processes.

d) Sensor Technology: Nanoparticles are applicable in sensor innovation for the detection of diverse substances such as gases, chemicals, biomolecules, and pathogens, offering high sensitivity and specificity.

e) Electronics: They possess distinguishing electrical and optical properties which include confined quantum effects. This makes them valuable components in electronic devices like transistors, LEDs, and solar cells.

f) Theranostics: By merging therapy and diagnostics, they can provide imaging contrast for monitoring treatment response during drug delivery.
g) Textiles and Coatings: Nanoparticles can introduce valuable features to textiles and coatings such as antimicrobial properties, UV shielding, stain resistance, and improved durability.
h) Energy Storage and Conversion: Nanoparticles are essential in enhancing the proficiency of energy storage tools like batteries and supercapacitors as well as in energy conversion technologies like photovoltaics and fuel cells.
i) Environmental Remediation: Nanoparticles are utilized to eliminate pollutants from air, water, and soil through various techniques like adsorption, catalysis, and filtration, contributing to environmental sustainability.
j) Biomedical Applications: In addition to theranostics and drug delivery, they are utilized in various biomedical applications such as bio-imaging, tissue engineering, biosensing, and cancer therapy, including hyperthermia and photodynamic therapy.
These illustrations show the wide range of functions that nanoparticles perform in contemporary science and technology, and how their unique characteristics and versatility continue to spur breakthroughs in several fields.

**Applications of Nanoparticles in Complementary and Alternative Medicine**

**Nanoparticles in Herbal Medicine**

The ancient use of plants for medicinal purposes dates back to ancient civilizations, as plants are comprised of a variety of valuable compounds that promote human well-being with minimal side effects and pervasive acceptance. (Ijaz et al., 2018; Martins-Gomes et al., 2022).

Phytochemicals also regarded as secondary metabolites, are organic compounds produced by plants that play a pivotal role in traditional medicine. These compounds have been widely studied for their various biological functions, providing a strong scientific basis for the use of plant-based remedies in traditional healing practices. Their pharmacological properties allow them to effectively address a variety of health issues, including bacterial and fungal infections as well as chronic degenerative diseases such as cancer and diabetes. (Hussein and El-Anssary, 2019).

Herbal medicines are becoming more popular globally for their potential in therapy and prevention due to their apparent safety and cost-effectiveness. However, limitations such as poor absorption, stability, and distribution hinder their effectiveness, demanding high doses and leading to reduced bioavailability and efficacy. These characteristics limit collectively and obstruct the clinical utility of herbal medicines (Ahmed et al., 2021; Dewi et al., 2022).

For example, puerarin which exhibits a wide range of pharmacological effects is obtained from the roots of *Pueraria lobata (Willd.) Ohwi*. Its applications span various medical conditions, such as diabetes, cardiovascular disease, endometriosis, osteonecrosis, Parkinson's disease, Alzheimer's disease, and cancer (Zhou et al., 2014). The solubility of puerarin in water is notably low, specifically at 0.46mg/mL. However, the highest solubility of this compound is detected in a phosphate buffer of pH 7.4, reaching 7.56mg/mL (Quan et al., 2007; Li et al., 2014). The partial solubility of puerarin poses limitations on its use in different applications. Consequently, recent years have witnessed advances in research efforts aimed at enhancing the bioavailability of puerarin. Among the various approaches explored for this purpose, solid lipid nanoparticle (SLN) carrier systems have emerged as a promising approach. When compared to traditional puerarin suspensions, SLN-puerarin formulations exhibit notably a faster absorption rate. Furthermore, the bioavailability of SLN-puerarin is shown to be more than three times higher than that of puerarin suspension ( Luo et al., 2011;Luo et al., 2013).

**Nanotechnology-based Delivery Systems**

Nanotechnology-based delivery systems play a fundamental role in acting as carriers for drugs, offering solutions to the abundant challenges faced by herbal medicines. These challenges include enhancing the bioavailability and bioactivity of phytochemicals present in herbal remedies. The utilization of nanotechnology presents itself as a potentially groundbreaking and innovative technological approach that can be effectively implemented to target phytochemical components, ultimately amplifying the efficiency of phytotherapy in herbal medicines.

A primary objective pursued by a multitude of researchers is the development of a delivery system for drugs that not only demonstrates high efficacy but also ensures the utmost safety for patients. Recent advancements in the realm of nanotechnology have sparked a resurgence of fascination and curiosity surrounding the formulation of herbal medicinal products. Various strategies have been put forth in the realm of delivery systems, encompassing methodologies like phytosomes, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), polymeric nanoparticles, and nano emulsions, among others. Through the utilization of nanoparticles, there exists a capacity to alter and enhance the pharmacokinetic properties of diverse drugs, thereby raising expectations for the nanotechnology approach to significantly enhance the bioavailability and bioactivity of herbal medicines (Ahmed et al., 2021).

According to the available literature, it has been reported that a significant portion, specifically 70%, of the active components derived from plants exhibit hydrophobic properties. Therefore, it is deemed appropriate that delivery systems possessing hydrophobic characteristics are utilized for drug delivery purposes. Such systems may include lipid-based delivery systems and polymer-based delivery systems. These systems have shown promise in enhancing the bioactivity of phytochemical compounds (Paroha et al., 2020).

**Lipid-based Nanoparticles**

Lipid-based nanoparticles, such as liposomes, solid lipid nanoparticles (SLN), and nanostructured lipid carriers (NLC), are extensively studied in drug discovery and cancer treatment due to their ability to carry both hydrophobic and hydrophilic molecules, minimal toxicity, prolonged drug action, controlled release, chemical modifications to evade immune detection, enhanced drug solubility, and pH-sensitive formulations for targeted drug release (Yingchoncharoen et al., 2016) (Fig. 4).

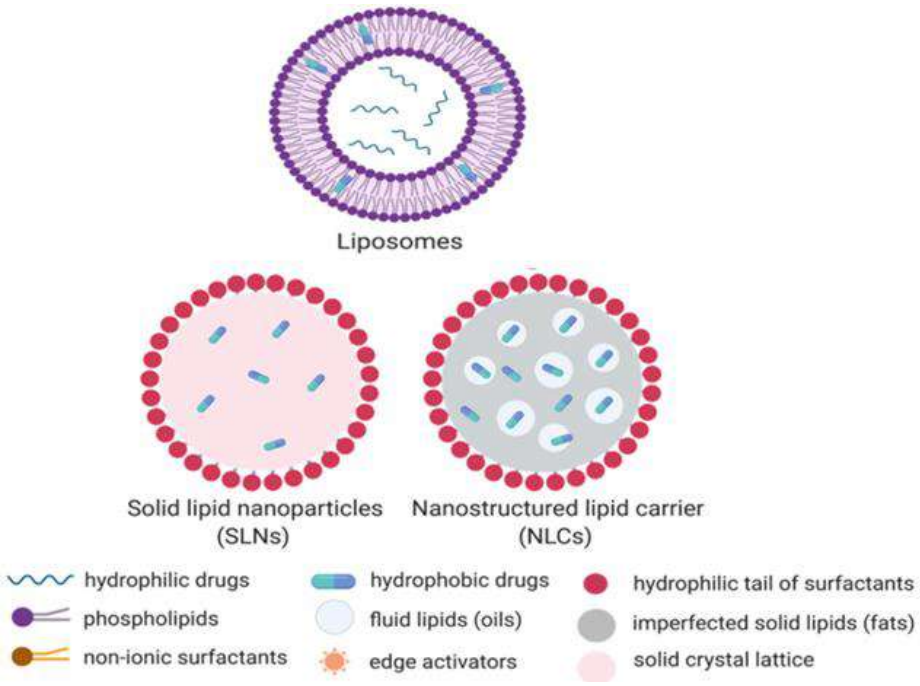


**Fig. 4:** Lipid-based nanoparticles. Adapted from (Thi et al., 2021) https://creativecommons.org/licenses/by/4.0/

**Polymer-based Nanoparticles**

Polymeric nanocarriers, such as solid polymeric nanoparticles and polymer conjugates, have garnered significant attention in drug delivery due to their unique physicochemical structures and sizes ranging from 1–1000nm. Examples include chitosan and PEG in PLA-based nanoparticles. These nanocarriers, fabricated by natural, synthetic, or semisynthetic polymers, offer advantages for controlled and targeted drug delivery, particularly when synthesized from biodegradable and biocompatible materials like natural polymers, known for their low toxicity and stability. Overall, polymeric nanoparticles provide benefits like encapsulation of diverse drugs, drug protection, and targeted delivery (Elmowafy et al., 2023) (Fig. 5).

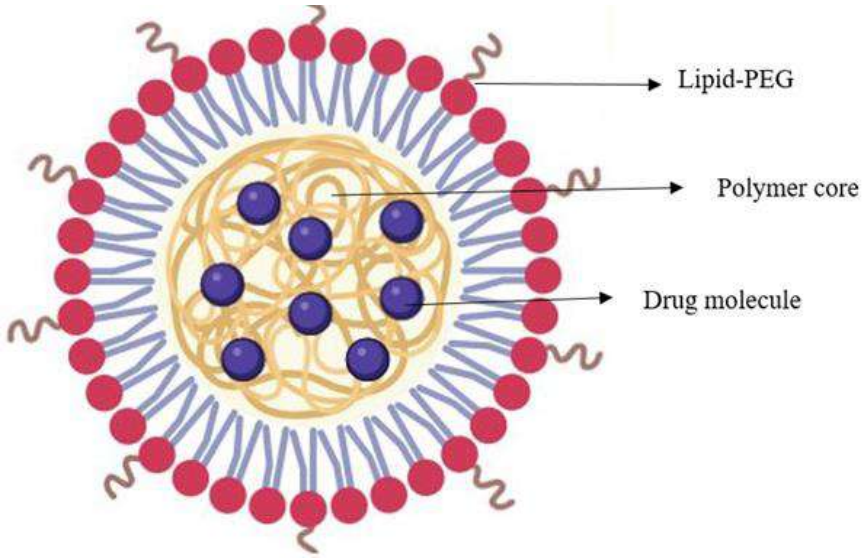


**Fig. 5:** Example of PEG-based polymeric nanoparticle (credit: Biorender)

**Significance of Herbal Nanoparticles**

Herbal nanoparticles have been chosen as a solution to address the limitations of traditional herbal medicines due to several factors like they can be utilized for targeted drug delivery to respective organs. Their unique property such as size, increases the efficient drug administration, solubility, selectivity, and safety. They passively target the disease site without needing specific ligands, reducing side effects.

**Herbal Nanoparticle Formulations**

Numerous natural bioactive compounds have been incorporated into polymeric and lipid-based nanoparticles for a variety of purposes, as outlined below. Among these compounds, bioflavonoids have garnered significant attention for their potential therapeutic effects and promotion of good health. These effects include immune stimulation, cytotoxicity, cardiovascular benefits, improved vision, and prevention of cell death related to amyloidosis. Furthermore, bioflavonoids exhibit effective anti-cancer properties through various mechanisms. They have been harnessed in the treatment of diverse conditions, such as oxidative stress, inflammation, skin ailments, cardiovascular issues, respiratory diseases, and diabetes (Elmowafy et al., 2023). Table 1 enlists several herbal compounds, along with their nano-formulations and their targeted diseases.

**Table 1:** Herbal nanoparticles used in various disease treatments

| Herbal Medicinal Compounds | Description | Nanoparticle Formulation | Targeted Diseases | References |
|---|---|---|---|---|
| Artemissia annua | Single-stemmed annual herb of family Asteraceae, active principle artemisinin, potent antimalarial action | Nano-coated artemisinin | Malaria | (Chen et al., 2009) |
| Berberine | Isoquinoline alkaloid from various medicinal plants, potent against physiological disorders and tumors | Single emulsion, multiple emulsion, ionic gelation | Physiological disorders, tumors | (Kuo et al., 2004; Sahibzada et al., 2024) |
| Centella asiatica | Herbaceous creeping plant, a wide range of pharmacological applications, physical instability addressed by nanoparticles | Chitosan-alginate nanoparticles | Anxiolytic, anti-anxiety, antioxidant, wound healing, cancer | (Okonogi et al., 2008) |
| Curcumin | Polyphenols from turmeric, have a wide range of biological activities, bioavailability enhanced through nanoparticle formulation | PEG blended PLGA nanoparticles | Anticancer, anti-inflammatory, antioxidant, antiviral, antibacterial | (Arya et al., 2018) |
| Danshen | Dried roots of *Salvia miltiorrhiza,* used for circulatory disorders and cancer, enhanced antioxidant properties in nanoparticle form | Nano-coated *Salvia miltiorrhiza* | Circulatory disorders, hyperlipidemia | (Jia et al., 2024; Mahalakshmi et al., 2021) |
| Dodder | Parasitic plant, flavonoids and lignans, anti-cancer and immune-stimulatory effects, poor solubility addressed by nanoparticles | Nano-precipitation method | Cancer, anti-aging, immune stimulation | (Patil et al., 2021) |
| Epigallocatechin-3-Gallate | Major constituent of green tea polyphenols, chemopreventive for various cancers, enhanced delivery through nanoparticles | PLGA, PLGA–PEG nanoparticles | Prostate cancer | (Sanna et al., 2017) |
| Genistein | Isoflavone from leguminous plants, phytoestrogen with anticancer properties, solubility, and bioavailability improved through nanoparticles | Nano-precipitation method | Cancer, osteoporosis, cardiovascular diseases | (Coutinho et al., 2023) |
| Kaempferol | Present in onions, green tea, and grapes, promising pharmacological actions, nanoparticle formulations for cancer prevention | Nonionic poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) and PLGA nanoparticles | Antitumor, antioxidant, anti-inflammatory, anti-allergic | (Elmowafy et al., 2023; Wang et al., 2018) |
| Murva | Controversial drug from various medicinal plants, potential against multiple diseases, solubility, and bioavailability enhanced through nanoparticles | Nanoparticles formulation | Anaemia, fever, diabetes, stomach disorders, cancer | (Shah et al., 2021) |
| Oridonin | Natural diterpenoid with therapeutic actions against cancerous cells, limited aqueous solubility addressed by nanoparticles | PLA nanoparticles | Cancer | (Fonseca et al., 2002) |
| Paclitaxel | Natural anticancer drugs with limited aqueous solubility. Various nanoparticle formulations enhance delivery and efficacy | PEGylated/PLGA, PLGA- magnetic nanoparticles, PCL, chitosan nanoparticles | Various cancers including ovarian, lung, and breast | (Elmowafy et al., 2023) |

| | | | | |
|---|---|---|---|---|
| Quercetin | Flavonoid from Sophora japonica, antioxidant and anticancer properties, bioavailability improved through nanoparticles | PLA nanoparticles, bovine serum albumin nanoparticles | Cancer, antioxidant | (Elmowafy et al., 2023) |
| Resveratrol | Natural polyphenolic substance found in various plants, cardioprotective, anticancer, antioxidant properties, enhanced delivery through nanoparticles | PEG-modified PLA nanoparticles | Cardiovascular diseases, cancer | (Yee et al., 2022) |
| Saponins | Group of plant glycosides, considerable cytotoxic activity, nanoparticles enhance delivery and selectivity against cancers | Chitosan nanoparticles | Prostate cancer, liver diseases, leishmaniasis | (Elmowafy et al., 2023) |
| Silymarin | Active extract from milk thistle seeds, hepatoprotective, chemopreventive against cancers, nanoparticles improve anticancer efficacy | PLGA nanoparticles | Liver diseases, cancers | (Azadpour et al., 2021) |

**Nanoparticles in Acupuncture**

**Acupuncture Overview**

Acupuncture has gained global popularity in recent years. It is a traditional form of healing with roots in Chinese medicine dating back over 3000 years. This therapy involves the insertion of thin needles into precise points known as acupoints on the body to balance the flow of energy, known as *chi or qi*. This method is grounded in the theory of regulating energy flow through pathways called meridians (White and Ernst, 2004).

Meridians in acupuncture are pathways that guide the flow of vital energy *Qi* in the body, similar to how blood circulates. Disturbances in this energy flow can lead to symptoms and illnesses. Eastern medicine, including acupuncture, aims to rebalance the body's energy flow by working with the meridian system. The body is divided into twelve main meridians linked to specific organs and functions and classified as *yin and yang* meridians. These meridians are interrelated and crucial for maintaining internal balance and harmony. Acupuncture treatment targets specific acupoints along with these meridians to enhance energy flow and support overall health (Longhurst, 2010).

The worldwide utilization of acupuncture has significantly increased, with numerous countries executing regulations and integrating it into their healthcare systems. From China to the United States, Europe, Australia, Japan, and South Korea, acupuncture has gained popularity and recognition, marking a global shift in traditional medicine practices. The World Health Organization recognized 63 diseases that can benefit from acupuncture, including migraines, arthritis, stroke, and mental health issues (Zhang et al., 2022).

Acupuncture needles evolve from stones in ancient China to bamboo, ceramic bone, and metals. Numerous modes of acupuncture have emerged globally including Japanese, Korean, Leamington Five-Element, and French energetic acupuncture, alongside specialized approaches like hand and foot, auricular, and scalp acupuncture with techniques like fire needle acupuncture utilizing materials such as gold-plated needles developed by generations of acupuncturists (Matos et al., 2021).

Both electricity and lasers are being applied to replace handheld needles in certain situations with electric acupuncture enhancing therapeutic results by improving electric current flow between needles, while laser acupuncture is suitable for specific patient groups due to its non-invasive nature. Regardless of the controversy, electrical and laser acupuncture therapies have exhibited similar or superior effects to traditional manual acupuncture, showcasing the evolution of acupuncture through multidisciplinary research and novel technologies to achieve enhanced efficiency and functions in clinical settings. Different types of acupuncture techniques are shown in Fig. 6.


**Fig. 6:** *Types of acupunctures* a). Chinese- target specific part, b). Japanese-based on whole body, c). Korean- hand is used mainly for acupoint, d). Auricular- based on ear, e). Scalp, f). Electroacupuncture, g). Capping, h). Moxibustion- heat therapy by burning dried mugwort.

**Advancements in Acupuncture using Nanotechnology**

Nanoparticles have a notable impact on the enhancement of conventional acupuncture through the provision of novel strategies and possible progressions within the discipline. Their utilization, as discussed below in diverse formats, serves to enhance the effectiveness of acupuncture therapy and its results.

**Acupuncture Needle Advancement**

To enhance therapeutic outcomes, relatively thick acupuncture needles have been commonly utilized in clinical settings to provide increased stimulation intensity. Nevertheless, the use of larger-diameter acupuncture needles is often met with discomfort, sparking interest in the development of more advanced acupuncture techniques that offer improved efficacy along with enhanced comfort. In response to this need, we have introduced a novel category of acupuncture needles known as porous acupuncture needles (PANs), featuring hierarchical micro/nano-scale conical pores on their surface (fig. 7). These PANs are created using a straightforward electrochemical process, boasting a surface area roughly twenty times larger than that of traditional acupuncture needles. Through the assessment of electrophysiological and behavioral responses during in vivo stimulation of Shenmen (HT7) points in Wistar rats, the efficacy of these high-surface-area PANs is demonstrated, showcasing their superior ability in regulating electrophysiological and behavioral reactions compared to conventional acupuncture needles. A comparative examination involving the use of PANs and thick acupuncture needles for cocaine-induced locomotor activity shows the enhanced performance of PANs instead of conventional needles, along with notable pain reduction. This study presents a novel approach to achieve a more comfortable and effective acupuncture therapy (In et al., 2016).

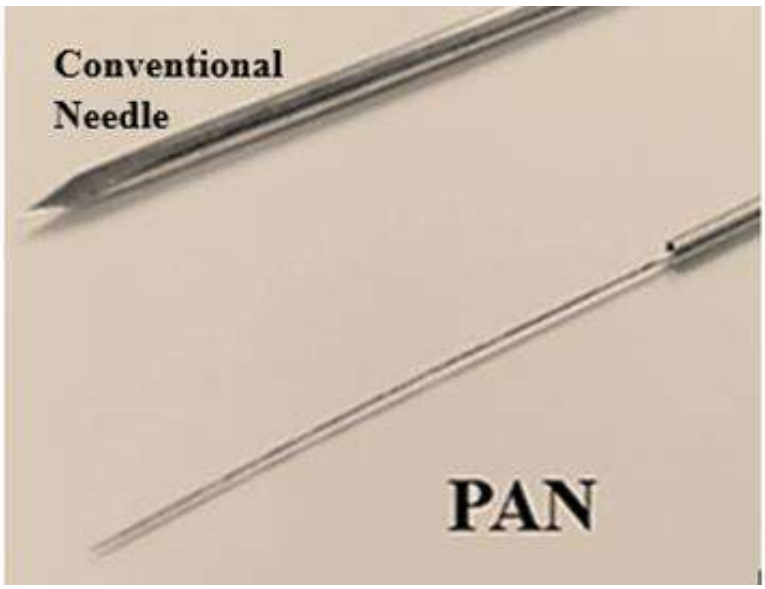


**Fig. 7:** Conventional vs PAN needles

**Nanochips and Nanosensors in Acupuncture**

The combination of nanochips and nanosensors in acupuncture needles offers several advantages in acupuncture therapy. By empowering the real-time identification of reactive molecules and the monitoring of physiological reactions, valuable insights are gained into how the body responds, leading to more precise and efficient treatment methods. Furthermore, the precise placement of needles ensures accurate targeting of acupoints, maximizing therapeutic results for individuals. The increased sensitivity and specificity of Nanosensors permit precise measurement and identification of biological substances, contributing to a more refined understanding and implementation of acupuncture therapy. Moreover, the use of carbon nanotubes for sensing purposes enhances the versatility and adaptability of these devices, allowing for localized responses and improving the accuracy of treatment approaches. In summary, the integration of Nanochips and Nanosensors within acupuncture needles represents a notable progression, enhancing the precision, efficacy, and comprehension of acupuncture therapy for both practitioners and patients (Zhang et al., 2019).

**Acupoint Nanocomposite Hydrogels**

Acupoint nanocomposite hydrogels symbolize an innovative therapeutic strategy combining acupuncture with drug-delivery systems for the treatment of rheumatoid arthritis (RA) and other ailments. These particular hydrogels mimic acupuncture by targeting specific acupoints to deliver drugs serving as a link between acupuncture and pharmaceuticals. Triptolide (TP), an active compound derived from the Chinese herb *Tripterygium wilfordii Hook F*, is one example of a drug that can be carried through acupoint nanocomposite hydrogels. While TP exhibits potent anti-inflammatory and immunosuppressive properties. Its clinical efficacy is hindered by multiorgan toxicity and poor solubility. Through the integration of TP with nanocarriers within well-regulated hydrogels, precise and sustained drug delivery can be achieved which potentially mitigates its adverse effects and enhances effectiveness. The incorporation of hydrogel acupoint

embedding may impersonate acupuncture effects, and synergistic studies have explored the combination of acupuncture with hydrogel technology. The use of acupoint nanocomposite hydrogels can diminish TP's multiorgan toxicity, making it safe for clinical applications. Apart from drug delivery, these hydrogels can also simulate acupuncture effects and target drug delivery against RA. The fusion of cryogel/hydrogel biomaterials with acupuncture may foster tissue regeneration, presenting a promising novel approach to accelerate the healing process (Chen et al., 2021; Ren et al., 2021). Fig. 8 illustrates the nanocomposite hydrogel for the treatment of RA.

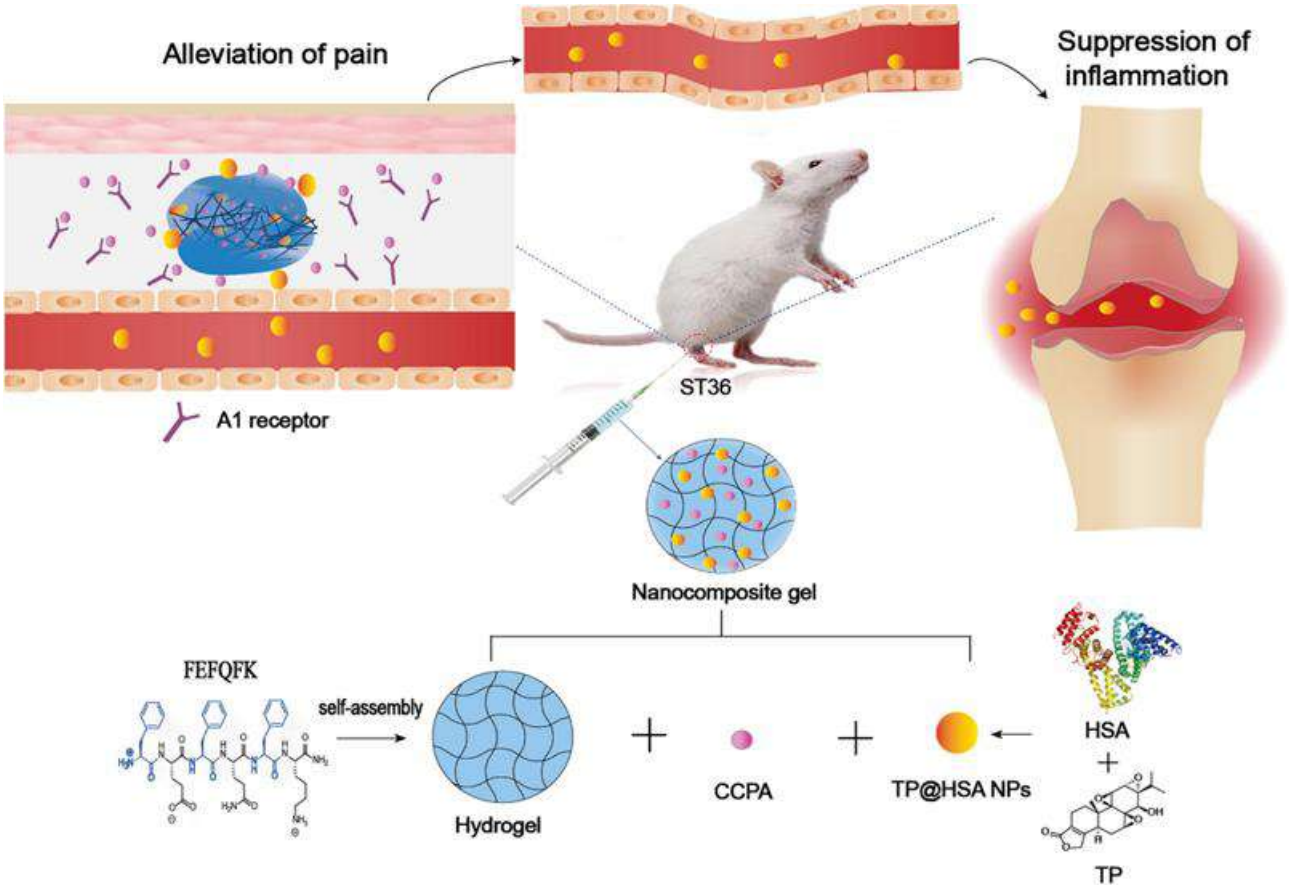


**Fig. 8:** Acupoint nanocomposite hydrogel for rheumatoid arthritis treatment (Ren et al., 2021) http://creativecommons.org/licenses/by/4.0/.

**Conclusion**

Nanotechnology harbors potential in augmenting complementary and alternative medicine (CAM) like herbal medicine and acupuncture by enhancing drug delivery systems and crafting porous acupuncture needles with increased surface area. The integration of nanotechnology with CAM therapies offers new possibilities for improving clinical outcomes and expanding research options for various disorders.

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Chapter 22

# Application of Nanoparticles against *Haemonchus contortus*

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**ABSTRACT**

*Haemonchus contortus* is a gastrointestinal nematode of sheep and goats. It is prevalent in almost all parts of the world. It causes an economic crisis because a host undergoes malnutrition, which ultimately leads to a decrease in production. Acute infections lead to sudden death. It is considered a model nematode to be studied because of its genetic diversity. It has become resistant to currently available antihelmintic agents. So, new techniques are under process for its prevention and control and the use of nanoparticles is one of them. Nanoparticles being smaller in size, easily cross the cell membranes of a parasite, leading to the death of a parasite. These nanoparticles also generate reactive oxygen species that induce oxidative stress within the parasite as a result of which the parasite will not be able to survive. They also disrupt the normal homeostasis of *Haemonchus contortus* as a result of which the worm will not be able to survive within a host. The most commonly used nanoparticles are silver, zinc oxide, and solid lipid nanoparticles. As these nanoparticles are derived from natural substances, their side effects are negligible. These nanoparticles will lead towards the solution of antihelmintic drug resistance.

**KEYWORDS**
*Haemonchus contortus*, Antihelmintic, Nanoparticles, Host, Parasite

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## INTRODUCTION

*Haemonchus contortus* is a parasitic nematode that is prevalent in subtropical and tropical regions of the world (Emery et al., 2016). It is a hematophagous organism, causing mortalities in the hosts (Besier et al., 2016). Its main hosts are sheep and goats (Besier et al., 2016). Cases of infection in cattle with *H. contortus* are also reported (Wang et al., 2017). *H. contortus* causes an economic crisis because it leads to loss of production and weight in the affected animals. The animals infected with *H. contortus* show anemia, hypoproteinemia, submandibular edema, ascites, and lethargy as the clinical signs (Saminathan et al., 2015). It affects the gastrointestinal tract of the animals (Salle et al., 2019), most commonly affecting the abomasum of small ruminants (Zarlenga et al., 2016). Its life cycle is direct. Eggs of a nematode are shed in the feces of the affected animals. These are immature eggs when passed. In the external environment, these eggs are converted into first-stage larvae (L1). These larvae are molted into second-stage larvae (L2) after feeding on the bacteria. They are then molted into the infective stage of larvae known as third-stage larvae (L3). Animals then take up these infected larvae while grazing, and then these larvae undergo further development into final-stage larvae (L5) which is its adult stage (Mohamed et al., 2023). This worm is of great significance, because of its short developmental period and high abundance (Rashid and Irshadullah, 2014). Because of the large population sizes, *H. contortus* is among these nematodes that have high genetic diversity (Gilleard and Redman, 2016). Because of its characteristic barber pole appearance, it is easily diagnosed on a post-mortem examination. As these worms are also found in the coolest regions of the world like Sweden, Canada, and the United Kingdom, it is no longer called a tropically adapted worm (Crilly et al., 2020). Because of its close resemblance with a model nematode *Caenorhabditis elegans*, it is considered a model gastrointestinal nematode to be studied (Jiao et al., 2020). The typical approach to treat haemonchosis involves taking anthelmintics often. However, uncontrolled anthelmintic usage in commercial and private farms results in parasites that are resistant to several drugs, which is currently a significant

problem in the production of small ruminants (Rashid and Irshadullah, 2018). *H. contortus* is currently resistant to different antihelmintic drugs, the most common include benzimidazoles, levamisole, milbemycins, and avermectin (George et al., 2021). Therefore, we have to focus on alternative control strategies because these control strategies are essential to decreasing the disease burden in animal populations. The most common control strategies include rotational grazing, nutritional supplementation, selective breeding, selective treatment, and the feeding of plants that contain natural antihelmintic components (Sahoo and Khan, 2016). Recently, a new technique has been used to develop new antihelmintic drugs against which parasites are not previously exposed. This technique involves the usage of nanoparticles. Nanoparticles are bringing a promising approach towards the solution of antihelmintic resistance. This chapter focuses on the importance of these nanoparticles against *H. contortus*, their mechanism of action, and their future perspectives.

**Application of Nanoparticles against *H. contortus***

**Introduction to Nanoparticles against *H.contortus***

Nanoparticles, being smaller in size and having remarkable surface reactivity, are becoming the potential candidates for the initiation of new antihelmintic agents (Adeyemi and Whiteley, 2013). They generate reactive oxygen species and can cross the cell membranes, as a result of which great reactivity occurs and ultimately the death of infectious agents occurs (Bhardwaj et al., 2012). Organic and inorganic nanoparticles are under processing for the development of antihelmintic agents including anti-hemonchotic drugs (Esmaeilnejad et al., 2018). Among the nanoparticles that are employed often are organic ones. Nanoparticles are very biocompatible and biodegradable, and they are easily made in huge quantities utilizing a variety of techniques. These nanoparticles are effective nanocarriers for the regulated and sustained release of medications because they can dissolve, absorb, and encapsulate a drug in a polymer matrix (Bhatia et al., 2016). To reduce the drug toxicity, drug dosage has to be decreased but it also decreases the efficacy of that drug. Nanoparticles, on the other hand, don't require this (Carvalho et al., 2020). The nanoparticles can enhance the immune response within the hosts against the *H. contortus* (Zhao et al., 2014). Delivery methods based on nanoparticles efficiently target immune cells in vivo. Because of their small particle size and broad bio-distribution characteristics, nanoparticles provide several unique advantages over conventional delivery methods when used in vaccines (Zupančič et al., 2017). The use of natural resources to treat a variety of infections is an ever-lasting practice (Chintamunnee and Mahomoodally, 2012). Using an aqueous extract, *Azadirachta indica*, silver nanoparticles against *H. contortus* have been developed (Tomar and Preet, 2017). Ethanol extracted from beeswax is used to develop albendazole-loaded nanoparticles against *H. contortus* (Buchwald et al., 2009). Similarly, ZnO nanoparticles have excellent results against *H. contortus* (Tomar and Preet, 2017). These nanoparticles are safer to use because they have no side effects on the host's health and are natural products (Tomar, 2018). The breakdown of the parasite cell membrane, reducing enzymatic activities, production of reactive oxygen species, stimulation of apoptosis, change of parasite metabolism, modification of parasite gene activity, immune system modulation, injury to the body, disturbance of the reproductive system, impairment of the nervous system, and so on are some examples of the general mechanisms of nanoparticles against *H. contortus* (Kaitaty et al., 2023).

**Application of Silver Nanoparticles against *H. contortus***

*H. contortus* has physiological and morphological changes after being exposed to silver nanoparticles. Silver nanoparticles completely dissolve the cuticle and shrink the body morphologically. Reactive oxygen and nitrogen species levels are markedly elevated physiologically, leading to oxidative stress and physical harm to the worm's tissues (Goel et al., 2020). Worm tissue shows a substantial rise in glutathione concentration and stress-responsive activity of enzymes such as catalase, superoxide dismutase, and glutathione peroxidase in response to oxidative stress (Ali et al., 2021). Furthermore, the amounts of protein, lipids, and glycogen in *H. contortus* are all reduced by silver nanoparticles. Parasites use glycogen, or stored carbohydrates, as fuel to carry out important metabolic functions (Preet and Tomar, 2017). The main structural and functional elements of nematode parasites are lipids. Lipids are a vital source of energy for the free-living stages of parasites, and damage or depletion of these ingredients can result in the parasite's death (Gutbrod et al., 2020). Lipids are found in plasma membranes and eggs. Thus, lipid biosynthesis inhibition may be a viable target for the creation of a medication that effectively treats haemonchosis. Similar to enzymes, proteins are essential for essential metabolic processes and optimal physiological functioning. Therefore, a lower protein level would interfere with the worms' regular physiological processes and could be the cause of death at greater concentrations. Changes in the morphology of eggs explain why *H. contortus* larvae disintegrate and shrink during development (Preet and Tomar, 2017). Silver nanoparticles derived from a natural product *Lansium parasiticum* alter the metabolic activity of *H. contortus* by increasing levels of reactive oxygen species and nitric oxide synthase. They not only kill the worms but also decrease the hatching capacity of the worms (Goel et al., 2020). 90% of the worms died within 12 hours of the treatment by these nanoparticles (Zhang et al., 2023). Silver nanoparticles developed from the leaves of *Melastoma malabathricum* kill *H. contortus* within an hour (Suteky, 2023). Silver nanoparticles derived from *Melia azedarach* and *Azadirachta indica* explained that their application causes the loss of the ability of *H. contortus* to hatch eggs (Batool et al., 2023). Silver nanoparticles also improve drug delivery. Silver nanoparticles biosynthesized from a fungus known as *Duddingtonia flagrans* have very promising results for the elimination of *H. contortus*'s infective larval stage which is L3 (Ferraz et al., 2022). Silver nanoparticles of *Moringa oleifera* show inhibitory effects on the hatching of eggs of *H. contortus* even in low doses (Ilavarashi et al., 2019). *Tinospora cordifolia* when used as a reducing agent for the biosynthesis of silver nanoparticles, then inhibits the egg-hatching ability of the mother nematode (Ramakrishnan et al., 2023). Thus, it seems that silver nanoparticles give the best results against the life cycle of *H. contortus* by inhibiting egg hatching from mother nematodes. The effects of silver nanoparticles are summarized in Fig. 1.

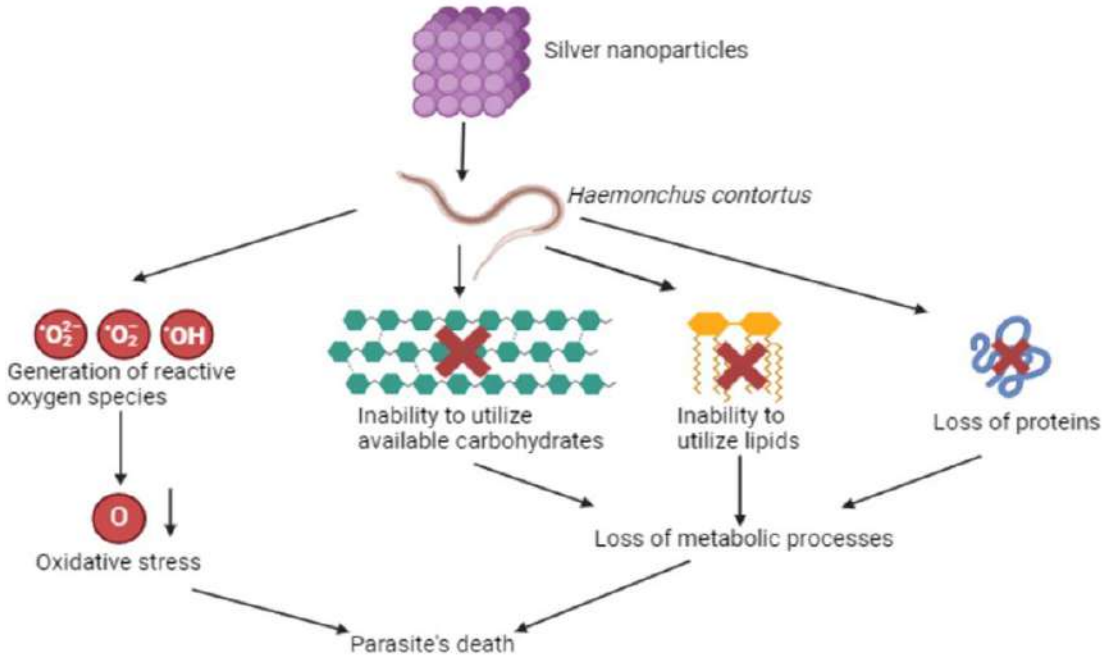


**Fig. 1:** Effects of silver nanoparticles on *H. contortus*

**Application of ZnO Nanoparticles against *H. contortus***

The parasites are entirely paralyzed by the ZnO nanoparticles. By causing extreme oxidative stress and denaturing the antioxidant enzymes, these nanoparticles can have a deleterious effect on *H. contortus*'s antioxidant systems. Different ZnO nanoparticle concentrations strongly changed the activity of antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase. The motility of adult parasites is either lost or the worms show very low motility (Esmaeilnejad et al., 2018). This increase in reactive oxygen species and oxidative stress can damage the DNA, proteins, carbohydrates and lipids of the parasites, ultimately causing the death of a parasite (Vasquez et al., 2021). ZnO nanoparticles disrupt the normal homeostasis of *H. contortus* (do Carmo Neto et al., 2022).

**Application of Solid Lipid Nanoparticles against *H. contortus***

Other than silver and ZnO nanoparticles, a variety of other nanoparticles are also available. One of the examples is the usage of solid lipid nanoparticles. Nanoparticles of essential oil from a naturally occurring tree known as *Melaleuca alternifolia* when applied on eggs of *H. contortus,* then it is concluded that eggs lost the ability to hatch. When applied on larval stages, the larvae undergo degeneration within 48 hours of application, and efficacy continues to increase with the increasing concentration of the nanoparticles (Grando et al., 2016). Albendazole-loaded solid lipid nanoparticles when orally administered to the animals, it has been observed that the potency of albendazole has been increased up to 50 times against the *H. contortus* (Nemati et al., 2024). Thus, these nanoparticles also improve the efficiency of currently available antihelmintics (Sharma et al., 2023). This formulation holds great promise as an anthelminthic drug delivery vehicle. By increasing the drug's bioavailability, it can not only lower the dosage but also minimize side effects caused by the medicine (Sharma et al., 2021). Different vehicles can be utilized for efficient delivery of antihelmintic agents like ivermectin. Solid lipid nanoparticles are being used as vehicles (Sharun et al., 2019).

**Application of Chitosan nanoparticles against *H. contortus***

Chitosan is formed from chitin. It can be used in the delivery of drugs and vaccines because it has excellent immunomodulatory mucoadhesive, biocompatible, and biodegradable effects (Wang et al., 2011). So, chitosan can be used as a delivery system for a vaccine of *H. contortus* as the efficiency of the vaccine increases this way (Wang et al., 2021). Chitosan nanoparticles are responsible for initiating effective immune responses within the host's body because it is evident that when chitosan is used in the delivery of a vaccine of *H. contortus*, then a significant number of antibodies are developed in the serum of a host against *H. contortus* (Hasan et al., 2020). Similarly, when chitosan nanoparticles are added with carvacrol and carvacrol acetate, then the death of the worms can occur (André et al., 2020). Whether it is the larval, hatching, or adult stage of a worm, chitosan-encapsulated bromelain nanoparticles destroy them (Hunduza et al., 2020). Similarly, the larval stages of *H. contortus* are eliminated by the chitosan-loaded *Eucalyptus staigeriana* nanoparticles (Ribeiro et al., 2015).

**Application of *Eucalyptus* Oil Nanoparticles against *H. contortus***

Nanoparticles lead to the development of nanoemulsions. One of the examples is *Eucalyptus globulus* nanoemulsion which has shown a good effect. *Eucalyptus globulus* nanoemulsion reduces the hatching capacity of a female worm. It also inhibits the development of larval stages, as a result of which infective larval stage ($L_3$) is not attained and the hosts are not

capable of developing infection (de Godoi et al., 2022). These nanoemulsions also improve the efficacy of the available antihelmintic agents against *H. contortus* (Riberio et al., 2017). Similarly, nanoemulsions of *Eucalyptus citriodora* almost reduce the hatching of eggs of female *H. contortus* by 100% (Riberio et al., 2014).

**Future Perspectives**

The world today is facing the problem of antihelmintic resistance. The entry of nanoparticles in the field of medicine will be very helpful in solving the problem of antihelmintics resistance. Still today, only a few nanoparticles have been developed against *H. contortus*. So, if we want to eradicate this parasite from the world, we need to develop further nanoparticles by utilizing the available natural resources. This will lead to improvement in the economy of the countries which are currently suffering from this parasite.

**Conclusion**

The field of nanoparticles has changed the perspective of currently available antihelmintic agents. Nanoparticles have brought our attention to the development of new drug candidates. As resistance against antihelmintic agents has been increasing in *H. contortus*, there is a need to develop new methods for the control, prevention, and treatment of haemonchosis and nanoparticles are performing their role. They are being used in the treatment of haemonchosis by increasing the efficacy of drugs by their better delivery. They are playing their role in the prevention of haemonchosis by effective delivery of vaccines. This is possible because they cause efficient activation of the immune system of the host. The haemonchosis is being controlled by their usage because some of these nanoparticles reduce the motility of the adult worms. Some of them inhibit the development of larval stages. Many of them cause the death of the worms. Most of them cause the loss of ability of female worms to lay eggs. Thus, they are disrupting the life cycle of the worm at any stage. A few nanoparticles against *H. contortus* have yet to be developed. The most commonly used among these nanoparticles are silver, ZnO, and solid lipid nanoparticles. However, the development of nanoparticles against *H. contortus* is still in the early stages. A lot of work has to be done in this field because the problem of antihelmintic resistance is increasing day by day and the population of nematodes is also increasing. If we can apply the nanoparticles against *H. contortus* in developing countries also, then it can lead to the elimination of this worm globally. Also then we can be able to prevent economic losses in the form of decreased production of retarded growth of animals affected by *H. contortus*. A lot of research is required to achieve the fascinating results.

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Chapter 23

# Nanoparticles and their Advances for Treating Salmonellosis

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## ABSTRACT

This chapter presents a comprehensive exploration of the potential applications of metal nanoparticles in combating salmonellosis, a pressing global health concern. The work encompasses an in-depth analysis of various types of nanoparticles, including silver, gold, and copper nanoparticles, highlighting their antimicrobial properties and potential for targeted drug delivery. Emphasis is placed on diverse synthesis methods, such as green synthesis using plant extracts and fungal-mediated synthesis, underscoring their eco-friendly nature and potential scalability. The findings underscore the promising role of metal nanoparticles in enhancing antimicrobial efficacy while minimizing adverse effects on mammalian cells. However, the chapter also addresses critical concerns regarding safety and toxicity, emphasizing the need for thorough evaluation and regulatory oversight. Additionally, future directions for research and development are outlined, focusing on optimizing targeted delivery systems, overcoming bacterial resistance, and exploring preventive strategies. Overall, the coherence of the findings underscores the transformative potential of metal nanoparticles in the fight against salmonellosis, while also highlighting the importance of addressing safety concerns and fostering interdisciplinary collaboration to realize their full therapeutic benefits.

**KEYWORDS**
Salmonella, gold, silver, animal, human, salmonellosis, nanoparticles

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## INTRODUCTION

Foodborne diseases (FBDs) pose a persistent threat to global health, affecting approximately one in ten people annually and resulting in an estimated 33 million deaths worldwide each year (WHO, 2022). Emerging and re-emerging of diseases pose significant challenges to animal and public health, and are aligning with global food security concerns (Ahmad et al., 2024). *Salmonella* serovars cause the disease salmonellosis, there are certain host-specific serotypes. Currently, there are more than 2,500 known *Salmonella* serovars and about 80 of these serovars are thought to be responsible for most diseases in humans and animals. The majority of human instances of salmonellosis are linked to the ingestion of tainted foods, including eggs, vegetables, juices, beef, hog, and poultry meat. Additionally, it can be connected to human-infected pet-animal contact (de Freitas Neto et al., 2010). Numerous ailments such as gastroenteritis, bacteremia, typhoid fever, and localized infections, can be caused by salmonellosis. The incubation period for gastroenteritis is six to seventy-two hours after consuming tainted food or water. A 38–39 °C fever is typical, although there might be a slight chill at first. Abdominal pain occurring frequently can vary from mild discomfort to severe agony. In uncomplicated cases, the acute phase usually resolves within 48 hours (Mani-López et al., 2012). Salmonella, intestinal bacteria, are a major cause of infectious diseases worldwide, affecting both humans and animals and often causing zoonotic infections. These bacteria belong to the Gram-negative, facultative anaerobic rod-shaped bacilli family Enterobacteriaceae (Dougan et al. 2011). Most Salmonella species are motile due to peritrichous flagella, though exceptions exist. Typically sized between 2-3 x 0.4-0.6

µm, Salmonella are chemoorganotrophs, capable of utilizing nutrients through both respiratory and fermentative pathways (Nwabor et al., 2015).

The World Health Organization (WHO) recently disclosed that antibiotic resistance poses a serious global concern. In particular, the WHO has classified 12 newly developing superbugs into three categories: critical, high, and medium, and has designated them as priority targets to be combated since they are resistant to several antibiotics (Lam et al., 2018). *Salmonella enterica*, a common zoonotic foodborne pathogen, presents a significant challenge to socioeconomic development, particularly in developing nations (Hussain et al., 2020). Infections caused by *S. enterica* remain a concern across both developed and developing countries, with a particularly high mortality rate observed in the latter. Notably, certain serovars of *S. enterica, such as S. enteritidis, S. typhimurium, S. typhi, and S. paratyphi*, contribute significantly to health issues in humans (Jaja et al., 2019). Animal-derived foods serve as primary carriers of *S. enterica* induced FBDs, with contamination occurring at various stages of meat processing, including skinning, slaughtering, transport, and butchering at local markets (Hussain et al., 2020).

Dating back to the 1960s, *Salmonella* displayed the first documented antibiotic (chloramphenicol) resistance. (Matthews et al., 2017). Subsequently, the escalation of Salmonella strains stands resistant to antimicrobial agents. Conventional first-line remedies for Salmonella infections, such as ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole, face resistance, leading to the emergence of multi-drug resistant (MDR) strains. Historically, MDR traits have been prevalent among *S. Typhi*, particularly in Africa and Asia. To combat this, fluoroquinolones and extended-spectrum cephalosporins have been introduced as alternative treatments. However, there is a growing concern over the rise in fluoroquinolone-resistant typhoid Salmonella cases. In regions with high MDR isolate rates, *S. Paratyphi* exhibits greater fluoroquinolone resistance compared to *S. Typhi*. Notably, resistance to nalidixic acid, an indicator of reduced susceptibility to ciprofloxacin and other fluoroquinolones, is widespread in isolates from countries such as Pakistan, India, and Vietnam, with incidence rates as high as 59%, 57%, and 44%, respectively (Eng et al., 2015).

Nanoparticles (NPs) find extensive applications across various fields, including agriculture and medicine. In medicine, NPs are continually refined for drug delivery, disease screening, tissue engineering, and more. Consequently, nanotechnology has become pivotal in catalysis, energy, environmental sustainability, agriculture, optics, sensors, computing, and beyond. The size of nanoparticles is a critical determinant governing their circulation and biodistribution in therapeutic applications. Particles smaller than 10 nm can be readily cleared by physiological systems like renal filtration, while those larger than 200 nm can be cleared by phagocytic cells of the reticuloendothelial system (RES). Consequently, therapeutic nanoparticles characterized by sizes below 100 nm benefit from extended circulation periods within the bloodstream. Studies indicate that nanoparticles ranging between 20 and 200 nm exhibit heightened accumulation rates in tumors, as they evade RES recognition and renal filtration. Targeted therapy involves administering a precise amount of medication to the affected body area over an extended period, necessitating the development of safer and more efficient therapeutic nanoparticles, a prime objective in nanomedicine. Upon entering the bloodstream, nanoparticles are susceptible to aggregation and protein opsonization, which marks them for immune system recognition (Yetisgin et al., 2020).

**Nanoparticle Formulations**

Nanoparticles are tiny materials made from various substances like copper, zinc, titanium, magnesium, gold, alginate, and silver. They have diverse applications, including medicine, energy storage (like solar and oxide fuel batteries), and integration into everyday products such as clothing and cosmetics (Hasan, 2015). Research into nanoparticle delivery systems is extensive, with many formulations and technologies already in clinical use. The FDA has approved several methods for distributing nanoparticles, including oral, local, topical, and systemic routes, depending on their intended use or target site (Anselmo et al., 2016). Nanoparticles can be broadly categorized into three types: organic, inorganic, and carbon-based. Organic nanoparticles, or polymers, include ferritin, liposomes, dendrimers, and micelles. Inorganic nanoparticles are typically made from metal or metal oxides, while carbon-based nanoparticles consist entirely of carbon, such as fullerenes, graphene, carbon nanotubes, carbon nanofibers, carbon black, and occasionally activated carbon in the nanoscale (Ealia et al. 2017).

**Types of Nanoparticles**

**Metallic Nanoparticles**

One significant and extensively researched class of materials with a wide range of applications is metallic nanoparticles. It has been demonstrated that both Gram-positive and Gram-negative bacteria can be effectively inhibited from growing by using nanoparticles like silver and gold (Pantidos and Horsfall, 2014). Single DNA strands can be nondestructively linked to metallic nanoparticles. This leads to the facilitation of applications within the realm of medical diagnostics. Nanoparticles exhibit the capacity to specifically localize within target organs via traversal of the vasculature. Consequently, novel opportunities may emerge in medicinal, imaging, and biological domains (Thakkar et al. 2010). Over the course of the year, nanoparticles such as iron oxide nanoparticles, gold nanoparticles, and silver nanoparticles as well as nanoshells and nanocages were continually developed and adapted to be used as diagnostic and therapeutic agents.

**Polymeric Nanoparticles**

These nanoparticles (NPs) are primarily made from natural materials such as polymers, lipids, carbohydrates, and

proteins. While they may not be as stable as inorganic nanoparticles under extreme conditions, they excel in being compatible with the body, stable, effective in targeting specific areas, and ideal for storing drugs that don't dissolve easily in water. (Aguilera-Correa et al., 2021). One reason PNPs have gained popularity recently is their tiny size and unique characteristics. They offer several benefits as carrier molecules, such as controlled drug release, the ability to combine therapy and imaging (theranostic), protection of the drug molecules, and precise targeting. These advantages enhance the effectiveness of treatments (Crucho et al., 2017). Polymeric nanoparticles (PNPs) work by encapsulating drugs within a polymer matrix, protecting them from degradation. They are designed to release the drug in a controlled manner, often targeting specific cells or tissues using ligands. This targeted delivery allows the drug to reach the desired site efficiently while minimizing side effects and enhancing therapeutic effectiveness. PNPs can also incorporate imaging agents for simultaneous disease treatment and monitoring (Kyriakides et al., 2021). Polymeric nanoparticles are designed based on their therapeutic application, target site (organs, tissues, cellular or subcellular organelles), and method of administration. Although intravenous injection is the primary method of administration for polymeric nanoparticles, there are also Non-invasive ways to administer them, such as dermal/transdermal, oral, and mucosal delivery (Elsabahy and Wooley, 2012). The challenges arise from the complexity of combining different physiological systems and the many factors to consider. Additionally, at the nanoscale level, cells react very differently, adding another layer of complexity (Banik et al., 2016).

Recent studies have demonstrated that polymeric nanoparticles, specifically those made from chitosan and polyanhydrides, show significant potential in reducing Salmonella colonization in poultry. Chitosan-based nanoparticles (CNP) have been particularly effective, enhancing immune responses by increasing iNOS mRNA expression and upregulating various Toll-like receptors (TLRs) and cytokines such as IL-1β, IL-4, IL-10, IFN-γ, and TGF-β. These CNP vaccines have been successful in significantly decreasing Salmonella colonization when administered through various methods, including oral gavage, drinking water, feed, and ovo delivery. Polyanhydride-based nanoparticles, synthesized from polymers like sebacic acid, adipic acid, and terephthalic acid, have also shown promise. For instance, poly (methyl vinyl ether-co-maleic anhydride) (PVM/MA) nanoparticles loaded with Salmonella antigens demonstrated robust immunogenicity, eliciting strong humoral and cellular immune responses, which are critical for combating intestinal pathogens like Salmonella. These nanoparticles have shown the ability to decrease Salmonella loads in internal organs and intestinal content, highlighting their potential as effective vaccine delivery systems to mitigate Salmonella infections in poultry (Acevedo-Villanueva et al., 2021).

**Lipid-based Nanoparticles**

The pharmaceutical industry has recognized lipid nanoparticles (LNPs) as a promising delivery system for a range of medicines. The use of LNPs has also been expanded into other industries, including agriculture, nutrition, medical imaging, cosmetics, and other cutting-edge domains like nanoreactors (Tenchov et al., 2021). LNPs were first introduced as a drug delivery system in the early 1990s. These nanoparticles have been used to encapsulate and deliver various types of therapeutic agents, including nucleic acids and small molecule drugs, to target cells more effectively (Kulkarni et al., 2018). Liposomes are small bubble-like structures formed when layers of synthetic or natural fats mix with water. Since their creation, their improved biocompatibility, effectiveness, and safety characteristics have mostly led to their usage for targeted distribution (Sivadasan et al., 2021). They can be used in less harmful therapeutic approaches because they are lipophilic, bio-acceptable, and biodegradable. lipid nanoparticles (LNPs) have been the most favored NPs to advance into clinical studies. LNP stimulates the immune system to fight the virus' spike proteins by acting as an adjuvant (Jagaran and Singh, 2022). Additionally, LNPs are being increasingly used in gene therapy, protein replacement therapy, and mRNA-based vaccines to combat infectious diseases and cancer (Aldosariet al., 2021). Conventional therapy for treating Salmonella infections is challenging because the bacteria build barriers to withstand the effects of antibiotics. LNPs have the potential for targeted treatments for salmonellosis due to their ability to encapsulate antibiotics and deliver them directly to infected cells, bypassing bacterial defense mechanisms and enhancing the effectiveness of the treatment. Liposomes, 6–12 microparticles, and nanoparticles are examples of existing carrier systems. These systems are limited in that they are not reliable enough to be given orally (Mudakavi et al., 2014). lipid–polymer hybrid nanoparticles, in which lipid layers are applied to the center of a polymeric nanoparticle loaded with antibiotics (Cheow et al., 2011).

**Selection Criteria for Nanoparticle Formulations**

**Antibacterial Efficacy**

Nanomaterials as nanocarriers are being used for the treatment of bacterial illnesses in addition to diagnostics and detection. The medications can be chemically conjugated, encapsulated, or adsorbed onto nanocarriers to deliver them to the desired location in an extremely focused way (Arshad et al., 2021). The discovery of new antibiotic compounds has significantly reduced the use of lipid nanoparticles in medicine. When its new form, silver nanoparticles (AgNPs), was discovered, it became once again a powerful antibacterial agent against a wide range of multidrug-resistant (MDR) microbes. AgNPs' antimicrobial and anti-inflammatory qualities are important in the medical domain (Husain et al. 2021). Nanoparticles have taken the place of antibiotics in the treatment of MDR-based diseases. The biological barrier can be crossed by their nano-formulations, which also overcome the drawbacks of traditional antibiotic treatments (limited penetration and retention in cells or biofilm). AgNPs are well-known for their ability to effectively combat numerous harmful microorganisms (Bano et al., 2023).

Nanoparticles with antibacterial properties function through various mechanisms of action (MoAs). These mechanisms

often involve the generation of reactive oxygen species (ROS), which can damage bacterial cell membranes, proteins, and DNA, leading to cell death. Additionally, nanoparticles can interfere with bacterial cell wall synthesis, inhibiting the formation of peptidoglycan cross-links, essential for cell wall integrity. Nanoparticles may also disrupt the bacterial cell membrane, altering its permeability and leading to cell leakage. Moreover, some nanoparticles can release metal ions, which can be toxic to bacteria by interfering with cellular processes and enzyme activity. Understanding these MoAs helps in designing more effective nanomaterials for antibacterial applications (Wang et al., 2017).

**Biocompatibility**

The section relates to the development of biocompatible nanoparticles for salmonellosis by providing background on the concept of biocompatibility and its importance in medical applications, particularly in relation to nanomaterials. Between 1940 and 1980, biocompatibility came to the attention of researchers about medical implants and their interactions both positive and negative with the body. This phrase hasn't been formally defined under its conceptual denotation rather than its practical description until the last twenty years. "A material's capacity to function with a suitable host response in a particular circumstance" (Naahidi et al., 2013). The biocompatibility of nanoparticles is influenced by their size, shape, surface chemistry, and composition. These factors affect how nanoparticles interact with cells and tissues, their distribution and clearance in the body, and their potential to provoke immune responses. To enhance nanoparticle biocompatibility, factors like optimal size and shape, surface chemistry modifications, use of biocompatible materials, surface coatings, and targeting ligands are considered. Ensuring high purity and consistent manufacturing also plays a crucial role. To address serious eye disorders like age-related macular degeneration and diabetes-related macular degeneration, researchers have tried medication and gene delivery via nanoparticles to the retina and other tissues of the eye (Karakoçak et al., 2016). The biocompatibility of many other nanomaterials is likewise significantly influenced by the size of the particles (Asefaet al., 2012). Understanding biocompatibility requires an understanding of in vivo investigations. The rest of the body is not taken into consideration by the cell-based models utilized in vitro, even if we believe that they faithfully mirror their counterparts inside the body. Even though a substance is not immediately cytotoxic, it can nevertheless cause a harmful effect (Kohane and Langer, 2010).

Nanoparticles develop biocompatibility for treating salmonellosis through their inherent properties and specific modifications that enhance their effectiveness and safety. Chitosan-based nanoparticles (CNP), made from the natural biopolymer chitosan, are non-toxic, biodegradable, and shown to increase immune response markers such as iNOS, TLRs, and cytokines without compromising poultry health. They effectively reduce Salmonella colonization when administered via various methods, acting as self-adjuvants that boost adaptive immunity. Polyanhydride-based nanoparticles, composed of biodegradable copolymers like sebacic acid and terephthalic acid, possess mucoadhesive properties that aid in stable antigen delivery and immune modulation. They can be tailored for enhanced hydrophilicity and controlled antigen release, ensuring consistent immunogenic responses. Both types of nanoparticles demonstrate significant potential in reducing Salmonella infections in poultry through their biocompatible, stable, and immunomodulatory characteristics (Acevedo-Villanueva et al., 2021).

**Stability**

Stability is one issue that needs to be addressed before implementing the delivery vehicles in a clinical context. This would entail determining the ideal storage conditions for the nanoparticles (Ball et al., 2017). The NPs' colloidal, chemical, and biofunctional stability can all be significantly impacted by the local intracellular microenvironment to which they are exposed. This can lead to NP disintegration, which can raise toxicity levels and impair NP functionality and characteristics (Soenen et al. 2015). The surface chemistry of nanomaterials greatly influences their interactions with living organisms, affecting both their chemical stability and toxicity. However, processes that take place at the solid/liquid interface are primarily responsible for the dispersion and chemical stabilities of nanoparticles in water (Labille and Brant, 2010).

**Targeting Strategies**

It is still a medical challenge to target intracellular infections such as Salmonella, Brucella, and Mycobacterium species (Dawood et al., 2023). These intracellular bacteria can stick around in infections despite inflammation, powerful antimicrobial treatments, and a robust immune response from the host. This persistence can lead to chronic infections. (Seleem et al., 2009). The release of the antimicrobial payload only at the target site, without damaging healthy cells, is a significant problem when developing NPs as antimicrobial delivery systems against bacterial infection. The objective is to decrease antibiotic dosages and treatment frequency, improve the selectivity and effectiveness of antimicrobials, and avoid unfavorable side effects related to non-specific drug delivery. Targeting can be done passively, actively, or by combining the two methods.

**Nanoparticles to combat Salmonellosis**

**Silver Nanoparticles**

The rise of drug-resistant bacteria is becoming a global concern, with antibiotic misuse being a significant factor, as highlighted by Wang et al. (2019). In South America, *Salmonella Typhimurium*, a common culprit in foodborne illnesses, has been found to resist beta-lactam antibiotics (Cordeiro et al., 2013). In Uruguay, studies by Casaux et al. (2019) revealed this bacterium as a prevalent strain in calves on dairy farms. The growing resistance to multiple antibiotics and recurrent

salmonellosis outbreaks are driving efforts to find new ways to combat these pathogens. One promising avenue is exploring nanotechnology for novel antimicrobial treatments, as suggested by Schäberle and Hack (2014). Utilizing nanoparticles for drug delivery can enhance effectiveness while minimizing side effects, making them a promising option for treating microbial infections (Youssef et al., 2019).

Silver nanoparticles (AgNPs) show great promise for various medical applications, particularly in developing novel antimicrobial treatments, as highlighted by Burdușel et al. (2018). Recent research, such as that by Porcaro et al. (2016), indicates their effectiveness against multiple drug-resistant ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter species*). These nanoparticles possess strong antimicrobial properties against bacteria, fungi, and viruses, while being relatively safe for mammalian cells due to their small size and diverse shapes (Franci et al., 2015). AgNPs work by triggering cell death through the generation of reactive oxygen species, as described by Gurunathan (2015). Their advantages include easy and cost-effective large-scale synthesis (Iravani et al., 2014), rare bacterial resistance, and the ability to modify their surface and shape (Khodashenasand Ghorbani., 2019). Notably, numerous studies have demonstrated AgNPs' effectiveness against various pathogens, including Salmonella enterica and mastitis-causing bacteria, as documented by El-Gohary et al. (2020) and Lange et al. (2021).

The development of silver nanoparticles (AgNPs) for the treatment of salmonellosis, particularly multidrug-resistant (MDR) strains, demonstrates significant potential. AgNPs exhibit broad-spectrum antimicrobial activity by interacting with bacterial cells through multiple molecular pathways. The nanoparticles adhere to the bacterial cell membrane, causing structural damage and increased permeability, leading to cell lysis. AgNPs also generate reactive oxygen species (ROS) that induce oxidative stress within bacterial cells, damaging proteins, lipids, and DNA. Additionally, silver ions released from AgNPs can bind to bacterial enzymes and proteins, disrupting essential cellular functions such as DNA replication and protein synthesis. This multifaceted mechanism of action not only inhibits bacterial growth but also prevents the development of resistance. In vivo studies show that AgNPs effectively reduce bacterial loads in infected animals and alleviate associated histopathological damage without significant toxicity, underscoring their therapeutic potential as an alternative to conventional antibiotics in treating MDR salmonellosis (Farouk et al., 2020).

**Gold Nanoparticles**

Combining gold nanoparticles (GNPs) with other nanomaterials has been shown to enhance their ability to fight bacteria. For instance, a blend of chitosan, gold, and silver nanoparticles displayed antimicrobial properties against *Escherichia coli* and *Staphylococcus aureus*, surpassing the effectiveness of individual nanoparticles alone, as observed in a study by Quek et al. (2022). Additionally, researchers found that GNPs coated with selenium effectively eliminated *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*, while causing minimal harm to human cells. These findings suggest that teaming up GNPs with other nanoparticles could offer a more potent antibacterial solution compared to using GNPs in isolation, as indicated by research like that conducted by Yougbare et al. (2019).

Gold nanoparticles (GNPs) have become a focus of interest due to their special properties, making them suitable for various applications in medicine. They are safe for use in the body and have been utilized in medical procedures such as imaging and photothermal therapy. GNPs offer a way to target and eliminate bacteria specifically, leaving healthy tissues unharmed. Their optical properties allow for the destruction of bacteria using near-infrared radiation. However, a study by Pelaz et al. found that GNPs didn't directly kill *Escherichia coli* and *Staphylococcus aureus* even at high concentrations. Yet, GNPs functionalized with amino groups were effective in disrupting bacterial biofilms, which are notorious for causing persistent infections. This discovery is significant as biofilms are hard to treat with regular antimicrobial methods, as noted by Okkeh et al. (2021).

One of the studies explores the innovative use of gold nanoparticles (AuNPs) conjugated with aptamers and antimicrobial peptides (AMPs) to develop an effective treatment for salmonellosis. Specifically, the AuNP-AptHis-A3-APOHis complex was tested for its ability to combat *Salmonella Typhimurium* infections in both in vitro and in vivo settings. The in vitro experiments showed that this complex significantly reduced the number of viable intracellular *S. Typhimurium* in infected HeLa cells by 30-50% compared to other treatments, demonstrating its enhanced bactericidal activity and improved stability and cell-penetrating ability. Further, in vivo tests in a mouse model of typhoid fever showed that the systemic delivery of AuNP-AptHis-A3-APOHis resulted in 100% survival of infected mice, effectively eliminating intracellular *S. Typhimurium* in vital organs. This approach not only enhanced the antimicrobial efficacy of A3-APOHis but also prolonged its stability, highlighting the potential of AuNP-based systems as a promising platform for treating intracellular bacterial infections like salmonellosis (Yeom et al., 2016).

**Copper Nanoparticle**

The FTIR technique is used to analyze the features of metal oxide or organic compounds present on the surface of copper nanoparticles or to identify the compounds applied to prevent the clumping or growth of these nanoparticles. When preparing particles through methods like decomposing metal acetylacetonates reducing salts in organic solvents, or using organic surfactants through thermal decomposition, they are coated with organic compounds to prevent them from clumping together due to van der Waals interactions or direct contact. This coating is crucial to maintain the particles' individual properties during processes like cooling, drying, or forming them into resin or molds. Agglomeration can ruin the unique characteristics of these ultrafine particles and hinder their effectiveness in sintering or film formation. In FTIR spectra,

the peak at 730 cm-1 indicates the presence of CuO2, while absorption bands around 2850-3000 cm-1 represent the stretching modes of -CH2- groups. Additionally, t-butanol, when present, shows absorption bands at 867 and 1359 cm-1. Similarly, copper nanoparticles coated with t-butanol exhibit absorption bands at 872 and 1342 cm-1, indicating the presence of t-butanol on the nanoparticles. UV-visible spectrophotometry is employed to confirm the formation of copper nanoparticles from an aqueous copper solution at 160°C, which acts as a precursor. The spectrum of copper nanoparticles typically shows minimal absorbance (0.01) and no distinct peak due to their small size. However, as the size of the nanoparticles increases beyond 10nm, their absorbance in the UV-visible region (250-800 nm) also increases, as demonstrated in studies like that of Sharma et al. (2022).

Recent advancements in utilizing copper nanoparticles (CuNPs) for the treatment of salmonellosis, an infection caused by *Salmonella* bacteria, leverage their potent antimicrobial properties at the molecular level. CuNPs release $Cu^{2+}$ ions that penetrate bacterial cells, inducing oxidative stress by generating reactive oxygen species (ROS) such as hydroxyl radicals (·OH), which damage DNA, proteins, and lipids, disrupting the cell membrane and denaturing essential enzymes. The Fenton-like reaction facilitated by $Cu^{2+}$ ions further amplifies ROS production, exacerbating cellular damage. Functionalization of CuNPs with polymers like chitosan enhances their stability and bioavailability, leading to a synergistic antimicrobial effect. Additionally, CuNPs inhibit biofilm formation, a key factor in *Salmonella* pathogenicity and antibiotic resistance, making the bacteria more susceptible to immune responses and treatments. In vitro studies have demonstrated significant reductions in bacterial viability and colony formation, suggesting that CuNPs could be integrated into various delivery systems such as medical device coatings, food packaging, and agricultural tools, providing a multifaceted approach to controlling salmonellosis (Ivanova et al., 2024; Subedi et al., 2024).

**Green Approach to Nanoparticle Synthesis**

Since the last decade, there has been significant exploration into the idea of "Green Chemistry" in pursuit of "Sustainable Development" (Hano and Abbasi., 2021). Sustainable development refers to a form of progress that meets present needs while also ensuring the ability of future generations to meet their requirements (Robert et al., 2005). Among the simplest methods to prepare silver nanoparticles (AgNPs) are those derived from plants (Khan et al., 2020). To create AgNPs using green synthesis, one needs a solution containing silver metal ions and a natural reducing agent. The most straightforward and cost-effective approach involves using a blend of biomolecules—such as polysaccharides, vitamins, amino acids, proteins, phenolics, saponins, alkaloids, and/or terpenes—to reduce and stabilize silver ions. Virtually all plants have the potential to be utilized for the production of AgNPs (Tolaymat et al., 2010). Plants contain various compounds—such as alkaloids, flavonoids, phenols, tannins, and alcohols—that possess the ability to reduce metallic ions to nanoparticles while maintaining their stability (Krestinin et al., 2015).

Plants offer a rich source of medicinal compounds capable of transforming complex metallic ions into simpler forms. Researchers have noticed the accumulation of metallic ions within plant cells, leading to the exploration of metal-reduction techniques for nano-sized materials. For instance, Alfalfa and Brown Mustard, when exposed to silver nitrate, accumulate significant amounts of silver, resulting in silver nanoparticles around 50 nm in size (Singh et al., 2023). Similarly, Alfalfa has been observed to produce 4 nm-sized gold icosahedra (Gardea-Torresdey et al., 2002), while Iris pseudacorus yielded approximately 2 nm-sized semi-spherical copper nanoparticles when grown in salt solutions. Recent advancements include using herbal extracts as reducing agents for synthesizing nano-sized materials in vitro (Das et al., 2012). Plant extracts from numerous species, along with various acids and salts of metals like copper, gold, silver, platinum, selenium, and iron, have been engaged in the green synthesis of nano-sized materials (Mondal et al., 2021; Singh et al., 2021). This green synthesis approach, devoid of bacteria or toxic chemicals, is favored over methods involving microbes or harmful substances due to its environmental friendliness and energy efficiency (Rai and Yadav, 2013). Various plant extracts from different parts of plants, such as stems, bark, roots, flowers, leaves, seeds, and fruits, have been successfully employed for the eco-friendly synthesis of nanoparticles (Masum et al., 2019).

**Table 1:** Plant-based production of Nanoparticles

| Plant source | Nanoparticle yield | References |
|---|---|---|
| Aloe vera | Silver nanoparticles (Ag-NPs) | Singh et al., 2014 |
| Green tea (Camellia sinensis) | Gold nanoparticles (Au-NPs) | Dwivedi et al., 2011 |
| Neem (Azadirachta indica) | Zinc oxide nanoparticles (ZnO-NPs) | Mittal et al., 2013 |
| Tulsi (Ocimum sanctum) | Copper nanoparticles (Cu-NPs) | Ahmad et al., 2016 |
| Ginger (Zingiber officinale) | Iron nanoparticles (Fe-NPs) | Shankar et al., 2016 |
| Turmeric (Curcuma longa) | Titanium dioxide nanoparticles (TiO2-NPs) | Singh et al., 2014 |
| Tea tree (Melaleuca alternifolia) | Silver nanoparticles (Ag-NPs) | Rónavári et al., 2017 |
| Spinach (Spinacia oleracea) | Iron oxide nanoparticles (Fe3O4-NPs) | Baskar et al., 2019 |
| Red cabbage (Brassica oleracea) | Cadmium sulfide nanoparticles (CdS-NPs) | Baruah et al., 2016 |
| Lemon (Citrus limon) | Selenium nanoparticles (Se-NPs) | Dhanalekshmi et al., 2018 |

**Fungal Approach to Nanoparticle Synthesis**

Using fungi to create silver nanoparticles typically involves cultivating the fungus on agar, transferring it to a liquid

medium, and then moving the resulting biomass to water to release the necessary compounds for synthesis. After filtering out the biomass, silver nitrate is added to the filtrate (Costa Silva et al., 2017). Fungi are preferred for this biogenic synthesis because they have a high tolerance to metals and release significant amounts of extracellular proteins that stabilize the nanoparticles (Netala et al., 2016). Compared to bacterial systems, fungal cultures offer advantages such as better biomass production and simplified filtration steps (Guilger-Casagrande and Lima, 2019). Additionally, fungal mycelial mass is more resilient to agitation and pressure, making it suitable for large-scale syntheses (Velusamy et al., 2016). By adjusting culture conditions like time, temperature, pH, and biomass quantity, it's possible to control fungal metabolism to obtain nanoparticles with specific characteristics (Zielonka et al., 2017).

Fungi hold great potential for producing diverse compounds with various applications. Microscopic filamentous fungi (ascomycetes and imperfect fungi) and other fungal species are known to produce around 6,400 bioactive substances. Fungi have great potential for producing diverse compounds with various applications and can serve as "nano factories" for producing nanoparticles with controlled properties (Guilger-Casagrande and Lima, 2019). In the process of extracellular synthesis, enzymes present in the fungal filtrate facilitate the reduction of silver ions, resulting in the formation of elemental silver at the nanoscale. Changes in the color of the filtrate indicate the occurrence of this reaction, which can be further confirmed using UV-visible spectroscopy to observe surface plasmon resonance bands, reflecting alterations in the material's optical properties (Basavaraja et al., 2008). The size of the nanoparticles depends on various synthesis conditions such as fungus species, temperature, pH, dispersion medium, and the presence of capping agents (Khandel and Shahi, 2018).

Various biomolecules, including those involved in complex pathways like electron transfer, can react with silver ions and take part in yielding (Thakkar et al., 2010). Among these, nicotinamide adenine dinucleotide (NADH) and NADH-dependent nitrate reductase enzymes are considered essential in the biogenic synthesis of metallic nanoparticles (Zomorodian et al., 2016).

**Table 2:** Fungi-based production of Nanoparticles

| Fungi Species (Scientific Name) | Nanoparticles Produced | Reference |
|---|---|---|
| *Aspergillus flavus* | Silver nanoparticles | (Vigneshwaran et al., 2007) |
| *Fusarium oxysporum* | Gold nanoparticles | (Ahmad et al., 2003) |
| *Trichoderma harzianum* | Copper nanoparticles | (Bindhu and Umadevi, 2014) |
| *Penicillium brevicompactum* | Zinc oxide nanoparticles | (Krishnamoorthy et al., 2012) |
| *Candida albicans* | Selenium nanoparticles | (Li et al., 2007) |
| *Rhizopus stolonifera* | Iron nanoparticles | (Anil Kumar et al., 2007) |
| *Phoma glomerata* | Titanium dioxide nanoparticles | (Ahmad et al., 2003) |
| *Mucor indicus* | Palladium nanoparticles | (Vigneshwaran et al., 2006) |
| *Pleurotus ostreatus* | Cadmium sulfide nanoparticles | (Sastry et al., 2003) |
| *Cunninghamella elegans* | Platinum nanoparticles | (Bansal et al.,2006) |

**Safety and Toxicity Concerns**

Nanoparticles offer great promise for treating diseases like salmonellosis, but ensuring their safety is crucial. Factors like size, shape, surface chemistry, and composition play key roles in their safety and toxicity. While nanoparticles can improve drug delivery and treatment effectiveness, they also have the potential to cause harm by triggering cytotoxicity and inflammation (Kennedy, 2006).

In the case of treating salmonellosis, researchers are investigating nanoparticles like silver nanoparticles, liposomes, and polymeric nanoparticles to deliver antimicrobial agents directly to the infection site. To assess their safety, it's important to consider factors like how well they interact with the body, whether they accumulate in important organs, and their potential long-term effects on cells and tissues (Pal and Tak, 2007; Naseem et al., 2023).

**Development of Targeted Nanoparticle Delivery Systems**

Targeted delivery systems using nanoparticles are designed to improve the effectiveness of drugs while reducing side effects. In the fight against salmonellosis, these systems can make treatments more successful by delivering antimicrobial agents specifically to infected tissues or cells. Methods include modifying the surface of nanoparticles with molecules that recognize bacterial antigens or creating nanoparticles that respond to changes like pH or temperature in the infection site to release the drug (Chaudhary et al., 2022).

Recent developments in nanotechnology have led to the creation of versatile nanoparticles that can effectively target Salmonella bacteria. These nanoparticles can carry different types of medication, such as antibiotics, antimicrobial peptides, or bacteriophages, directly to the inside of Salmonella cells, making the treatment more effective while reducing harm to the body (Turos et al., 2007).

**Combination Therapy with Nanoparticles**

Combining nanoparticles in therapy presents a powerful strategy to fight salmonellosis, addressing various aspects of bacterial infection at once. Nanoparticles can carry a mix of antimicrobial agents, like antibiotics, bacteriophages, or antimicrobial peptides, which work together to combat Salmonella effectively (Song et al., 2014).

Furthermore, engineered nanoparticles can overcome bacterial resistance mechanisms, such as efflux pumps or biofilm

formation, by delivering multiple drugs simultaneously or disrupting bacterial membranes. Additionally, nanoparticles improve the delivery of antimicrobial agents inside cells, enhancing their effectiveness against Salmonella infections (Rima et al., 2021).

**Potential for Nanoparticles in Salmonellosis Prevention**

Nanoparticles hold great potential for preventing salmonellosis through various means, including antimicrobial coatings, vaccine delivery, and detection systems. Modified nanoparticles can be integrated into food packaging materials to hinder the growth of Salmonella and other harmful pathogens, thereby lowering the risk of contamination during food handling and storage (Park et al., 2017).

Moreover, nanoparticles can act as carriers for oral vaccines, shielding vaccine antigens from degradation in the digestive system and improving their uptake by immune cells in the gut-associated lymphoid tissue. Additionally, nanoparticles equipped with specific antibodies or aptamers can be employed to swiftly and accurately detect Salmonella in food samples, allowing for prompt action to prevent outbreaks (Wang et al., 2019).

**Conclusion**

In conclusion, this review demonstrates the promising potential of nanoparticles in treating salmonellosis, showcasing their effectiveness as an alternative to traditional treatments. However, there are several gaps in the current review that need to be addressed. Many studies involve relatively small sample sizes, limiting the generalizability of the results. The short duration of most studies prevents a comprehensive understanding of the long-term effects of nanoparticle treatments. Methodological inconsistencies, such as variability in nanoparticle synthesis and characterization, also pose challenges. Furthermore, there is a potential bias in the selection of nanoparticle types and treatment conditions. Future research should aim to address these gaps by increasing sample sizes, extending study durations, standardizing methodologies, and minimizing selection biases. By overcoming these limitations, future studies can build on the current findings and advance the clinical application of nanoparticles for treating salmonellosis.

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Chapter 24

# Nanotechnology as an alternative option to combat Leishmaniasis: An updated overview

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**ABSTRACT**

Leishmaniasis is caused by several species of the genus *Leishmania* belonging to the protozoan parasites which are transmitted by the blood-sucking sandfly. It is a major public health parasitic disease which is distributed globally. This neglected tropical disease mostly affects people in developing or underdeveloped countries, triggering about one million cases each year. Clinically this disease is classified into cutaneous, mucocutaneous and visceral depending on the species of the parasite, host immunity, and vectors which are *Phlebotomus* in the Old World and *Lutzomyia* in the New World. The visceral type is dangerous and can lead to mortality. Even though leishmaniasis is an ancient illness, its treatment is still challenging. As of now, the available treatments for leishmaniasis suffer from drawbacks such as high toxicity, high cost, and the need for parenteral administration. Furthermore, there are reports of resistant leishmanial parasites making the current treatments ineffective. To overcome this, researchers are exploring the potential of nanotechnology to encapsulate antileishmanial drugs in nanocarriers. This approach offers several advantages over the free drug, such as minimizing the adverse effects and improving treatment effectiveness. The present chapter is an attempt to highlight the conventional drugs, and other treatments including newer options for instance nanostructure formulations like liposomal amphotericin B and other newly investigated nanoparticles as promising and prospective curing approaches for leishmaniasis.

**KEYWORDS**
Leishmaniasis, Nanocompounds, Treatment, Drug resistance, *In vivo*, *In vitro*

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## INTRODUCTION

Protozoal parasites include many pathogenic species that impact human and animal health such as *Leishmania* spp. *Entamoeba histolytica*, *Toxoplasma gondii*, *Sarcosystis spp*. and others (Shani et al., 2012; Shnawa, 2017; Swar and Shnawa, 2020). Some of them are considered dangerous and lead to death like *Plasmodium falciparum* (Ezzi et al., 2017). More than twenty diverse *Leishmania* species reason the zoonotic tropical disease leishmaniasis, which is transported by more than ninety dissimilar phlebotomine sandflies, particularly in low-income tropical countries. The World Health Organization stated that leishmaniasis recorded 700,000 to 1 million new cases each year across the world and up to 30,000 mortalities. Clinically this disease is classified into cutaneous, mucocutaneous and visceral depending on the species of the parasite, host immunity, and vectors. The visceral type is the greatest danger and can lead to mortality. The illness impacts individuals who are among the most impoverished in the world and is linked to inadequate nutrition, displacement of populations, substandard housing, a compromised immune system, and limited financial means (WHO, 2023).

### Life Cycle

Distinct forms of leishmaniasis are caused by infection with different species of *Leishmania*. For instance, infection with either *L. infantum* or *L. donovani* causes visceral leishmaniasis, whereas cutaneous leishmaniasis is caused by infection with any of several distinct *Leishmania* species. The disease-causing protozoan parasites are transmitted by sandflies, which inject infective promastigotes when they take a blood meal. The parasites are engulfed by the mammalian macrophages through receptor-mediated endocytosis, where they transform into amastigotes that multiply through binary fission. Sandflies become infected by ingesting infected cells when they take a meal of parasite-containing blood from an infected human or animal. The amastigotes transform into promastigotes and mature into metacyclic promastigotes that are infective to humans in the gut (Stuart et al., 2008). Various life cycle stages of the parasite have been shown in Fig. 1.

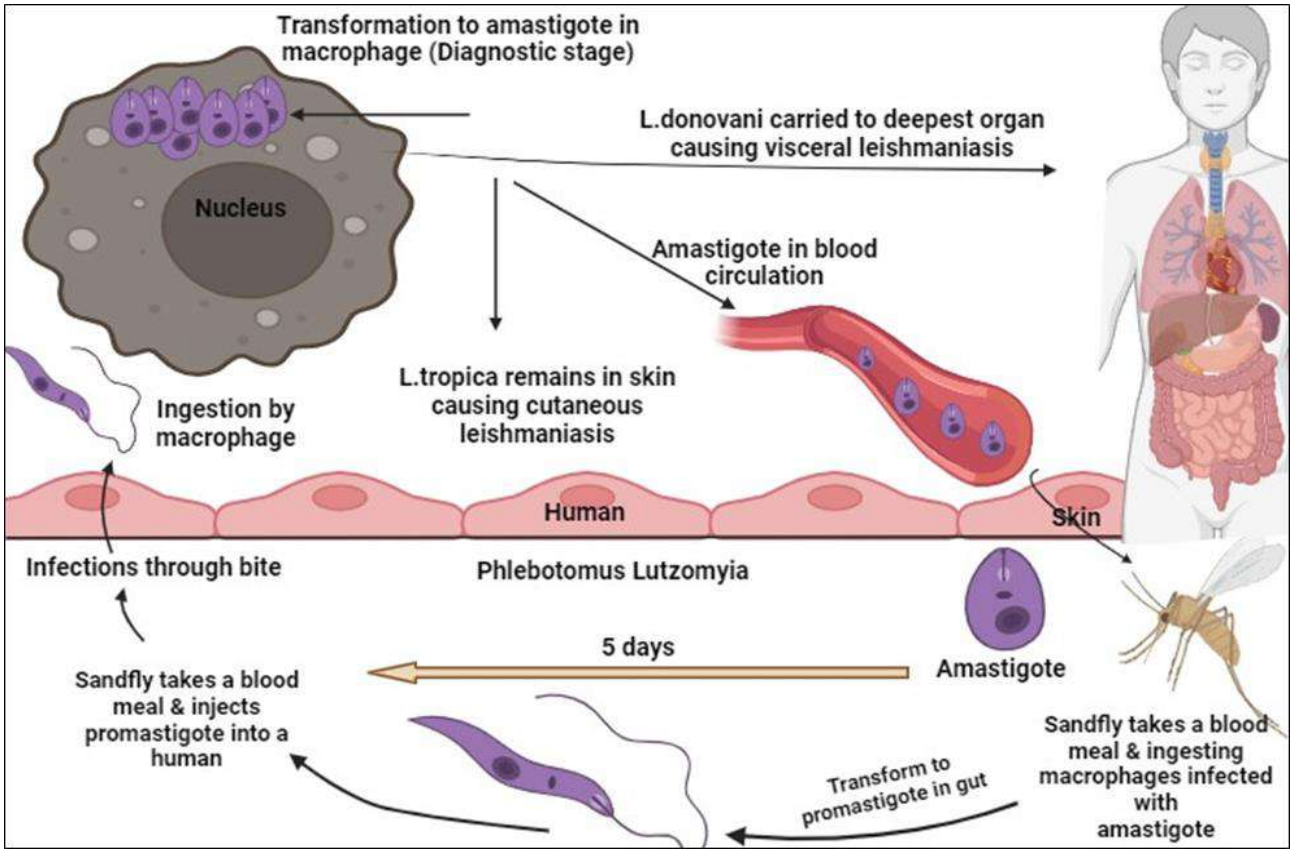


**Fig. 1:** The life cycle of *Leishmania spp*

**Treatment of Leishmaniasis**

Limitations in leishmanial infection chemotherapy are obvious. The reduced clinical curing percentage, drug resistance by the parasite, toxicity, and expense, all suggest novel active alternatives are essential in combating this disease (Uliana et al., 2018). Drug resistance of *Leishmania* spp. also arises, such as against antimonial drugs (Ashutosh et al., 2007). The following description refers to Fig. 2 which depicts an amastigote organism present inside a phagolysosome, which is located inside a macrophage host cell. It explains how antimonial enter the parasite and how the parasite develops resistance mechanisms (Ponte-Sucre et al., 2017).

Nanotechnology has emerged as a promising area of multidisciplinary research in recent times, owing to its extensive use in various fields of science. Silver, Gold, Zinc, Nickel and other nanoparticles (NPs), which have exceptional properties such as large surface-to-mass ratios, quantum structures, and the ability to adsorb and transport other compounds (drugs, probes and proteins), are recommended for various illnesses as drugs. Nanoparticles play a crucial role in medical applications due to their unique characteristics. In recent years, the greener fabrication of metal oxide nanoparticles has gained a lot of attention due to their ease of use, environmental friendliness, accessibility, and non-toxicity. Additionally, nanoparticles are being tested as anti-microbial substances to enhance the shelf life of food products (Shnawa et al., 2022; Shnawa et al., 2023).

The most effective treatment for cutaneous and visceral leishmaniasis is still represented by the liposomal amphotericin B formulation. However, its efficiency varies depending on the patient's immune system, the type of disease and the area where it is encountered. Additionally, the high cost, side effects and requirement for parenteral administration make it difficult to use in developing countries (Frézard et al., 2023).

Chemotherapy's efficiency in treating leishmaniasis is mostly dependent on two factors: the compound's microbicidal activity and the immunological response that is produced during the treatment. The anti-leishmanial properties of phytochemicals and metal atoms in biogenic nanoparticles can target potential *Leishmania* sites by a variety of approaches, such as membrane damage, ROS-mediated apoptosis, and enzyme inhibition (Mehta and Shaha 2006; Baiocco et al., 2011). *Leishmania* parasite death caused by ROS has a long history of being reported (Murray, 1981; Mallick et al., 2015) and metal nanoparticle-induced intracellular ROS generation can result in lipid and protein oxidation, genotoxicity (DNA damage), and ultimately, cell (parasite) death (Nadhman et al., 2016). Since leishmanial parasites only have one mitochondria, maintaining mitochondrial integrity is crucial to the parasite's survival and its ability to be a desirable target for chemotherapy (Kowaltowski and Vercesi 1999). AgNP is known to cause eukaryotic organisms to undergo necrotic or apoptotic death, oxidative stress, disruption of the integrity of the mitochondrial membrane, changes in the expression of energetic genes for survival, and pathways leading to genetic damage when exposed to toxic concentrations (Kruszewski et al., 2011).

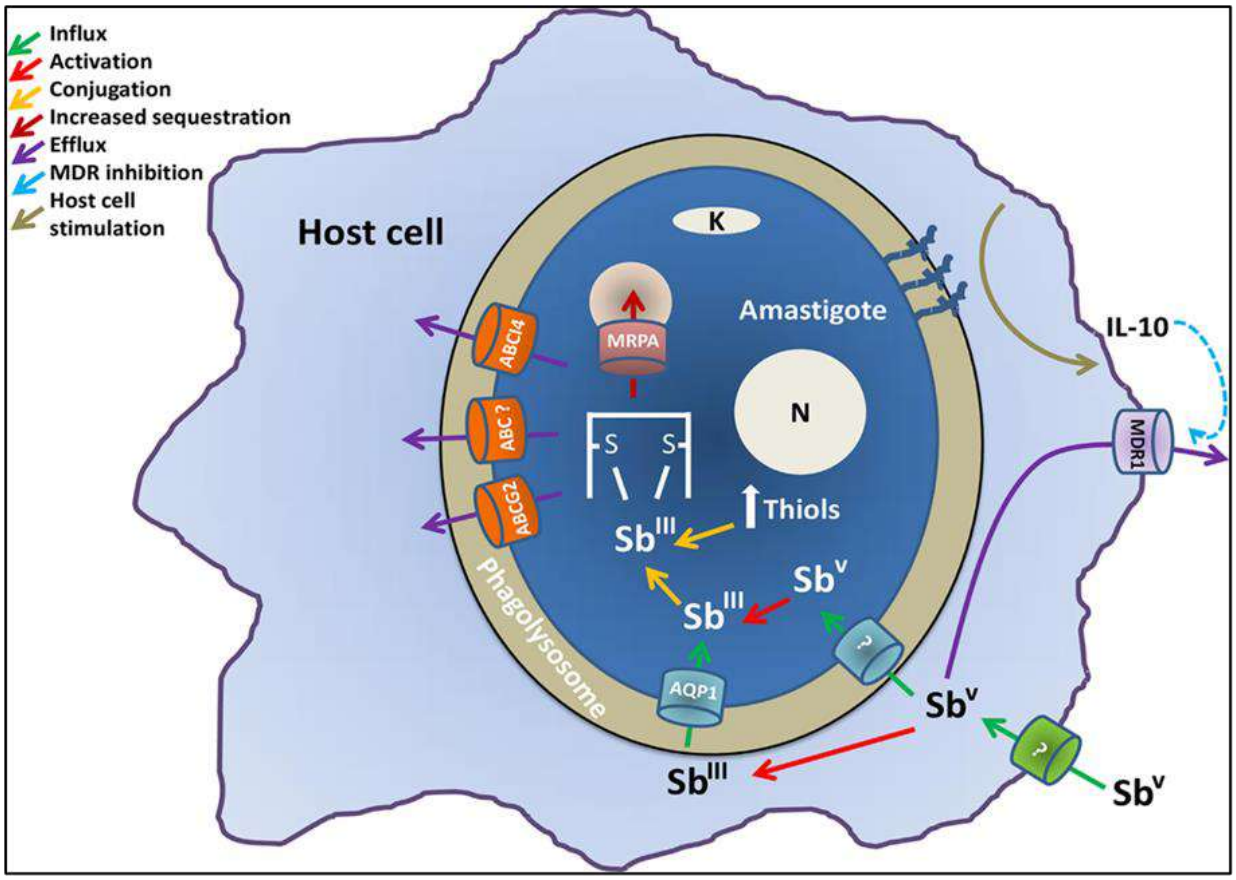


**Fig. 2:** Molecular mechanisms of antimonial drug resistance in *Leishmania*. ABC, ATP-binding cassette; AQP, aquaporin; DR, drug resistance; IL-10, interleukin 10; MDR1, multidrug resistance protein 1 (Ponte-Sucre et al., 2017).

Allahverdiyev et al. (2011) proposed that the wide surface areas, tiny diameters, and capacity of NPs to bind groups containing sulfur and phosphorus would enhance their antileishmanial actions. In support of this perspective, it was found that Ag-NPs lowered the parasites' metabolic activity and proliferation values when compared to the control groups. Another characteristic aspect of metal nanoparticles is their spherical form, which permits cell entry via phagocytosis (Kalangi et al., 2016). Silver ions (Ag+) have been shown to damage the outer membrane in bacterial infection models. Macrophages are known to undergo phagocytosis that is dependent on H+ release during the creation of phagolysosomes, which causes the medium to become acidic (pH 4-5). The lower pH increases the ability of silver nanoparticles to undergo oxidation, which releases silver ions (Ag+). This suggests that AgNPs release more Ag+ when they come into contact with acidic organelles like phagolysosomes, which benefits macrophages' mortality from *L. amazonensis* (Xiu et al., 2012; Awad et al., 2021) It has been proposed that increased cellular ROS generation causes the integrity of the mitochondrial membrane to break down, which leads to cell death (Banki et al., 1999). Fanti et al. (2018) suggest an increase in local ROS generation and a loss in mitochondrial integrity were the outcomes of the AgNp-bio promastigotes treatment, as it prevents *L. amazonensis* growth by affecting the mitochondrial function of the parasite. Similar to the reference medication, silver nanoparticles also inhibited the proliferation of *L. major* amastigote stages. Additionally, they concluded that *L. major*-caused cutaneous leishmaniasis can benefit from the use of nano-silver to prevent secondary infection (Mohebali et al., 2015).

Moreover, AgNp-bio's effect on macrophages infected with *L. amazonensis* was demonstrated by a reduction in the number of infected cells, intracellular parasites per macrophage, and promastigotes recovered from both infected and treated cells (Baiocco et al., 2011).

When Ag and Au nanoparticles are used as leishmanicidal agents, they will act as a vast reservoir of silver and gold ions, destroying the invaded parasite and providing a non-enzymatic source of ROS. It has been confirmed by electron spin resonance spectroscopy that silver ions can produce free radicals, and these radicals harm microbial cells in many ways (Kim et al., 2007). Mainly, macrophages generate elevated reactive oxygen species (ROS) to eliminate infections such as fungi, viruses, and parasites. On the other hand, the *Leishmania* parasite evades a few signalling cascade moieties to escape detection by macrophages and live inside phagolysosomes. When macrophages produce ROS intracellularly, NPs oxidize, increasing the amount of ROS produced (Alti et al., 2020). AgNPs could stimulate macrophages to produce ROS, which significantly decreased amastigote growth without causing macrophage population death (Lodge and Descoteaux, 2006).

Moreover, phytochemicals (capping agents) generated from AgNPs may also have beneficial differential effects in macrophages that are infected or uninfected. These effects can enhance antileishmanial activity by protecting the host cells through immunomodulatory mechanisms (Awad et al., 2021).

As mentioned above *Leishmania* inhibits the enzymes involved in the formation of ROS, hence passing on the oxidative damage induced by ROS (Mehta and Shaha 2006). Researchers have discovered that trypanothione reductase is a crucial enzyme that regulates *Leishmania's* polyamine-dependent redox metabolic activity. Therefore, it is a desirable target for the development of leishmanicidal treatments. According to recent research, substances containing gold have antileishmanial properties by blocking the enzyme trypanothione reductase (Ilari et al., 2012). Two crucial enzymes for Leishmanial survival are trypanothione reductase and trypanothione synthetase. These enzymes are essential for both delivering the reducing equivalents needed for DNA synthesis and protecting *Leishmania* from oxidative damage. Trypanothione synthetase and reductase are not found in the mammalian host, and *Leishmania* is known to be sensitive to ROS, which makes these enzymes a selected target for antileishmanial drugs. Furthermore, the thioredoxin glutathione reductase enzyme is effectively inhibited by gold-based drugs. This disruption of the intracellular redox balance causes oxidative stress to be induced, which in turn has lethal effects (Kuntz et al., 2007). Baiocco et al. (2011) indicate that *Leishmania's* trypanothione reductase can be considerably inhibited by ferritin-encapsulated silver nanoparticles. Leishmania parasites have a unique defence mechanism that involves a special small molecular weight thiol called trypanothione (T[SH]2) that aids the parasite in surviving within the host macrophage by providing exceptional oxidant and chemical defence. The NADPH-dependent trypanothione reductase (TryR) recycles trypanothione disulfide (TS2) to maintain the reduced state of T[SH]2. T[SH]2 plays a central role as a reductant, and it is assumed that it regulates the activation of iron-sulfur cluster proteins (Fe/S) in addition to this role (Kumar et al., 2019). Moreover, microbial Fe-S-containing proteins can be harmed by metal ions (Xu and Imlay 2012) which are crucial for the activation of many enzymes, such as fumarase and aconitase. These enzymes serve a role in *Leishmania's* energy metabolic reactions and electron transport chain. Fe-S-containing enzymes can be harmed by silver, zinc, and copper, according to studies (Xu and Imlay 2012; Ahmad et al., 2020) and a drop in these enzymes' biological activity will prevent *Leishmania* from growing. Also, *Leishmania* survival depends on reduced thiol moieties because these molecules aid in the neutralization of intracellular ROS. The cellularly reduced glutathione can be directly oxidized by metal nanoparticles, reducing these essential molecules' cellular deposits. the ability of decanethiol functionalized silver nanoparticles (AgNps-SCH) to kill *Leishmanian* promastigotes and amastigotes of various strains and species shows that AgNps-SCH has a high leishmanicidal potential (Isaac-Márquez et al.,2018).

Caspases, such as caspase-3, are involved in the activation of loss protease, which causes *Leishmania* cells to die (Zangger et al., 2002). AgNPs significantly activated the caspase-3 activation, as demonstrated by their influence on promastigotes' Caspase-3-like activity. Similarly, in THP-1 and AMJ-13 cells, AgNPs green produced by *Annona muricata* extract induced apoptosis via mitochondrial damage and the activation of the p53 protein pathway (Jabir et al., 2021). Other findings additionally demonstrate that *Leishmania* parasites become incapable of infecting macrophage cells following short exposure to nanoparticles. It has been proposed that the extract and nanoparticles disrupt the parasites' surface proteins, which cause infection. Furthermore, the parasite's amastigote form is unable to enter macrophage cells. This may be attributed that the macrophage cells release free radicals on activation with a nontoxic concentration of the extract and nanoparticles (Allahverdiyev et al., 2011). The mechanism of the effectiveness of metal nanoparticles against *Leishmania* is illustrated in Fig. 3.

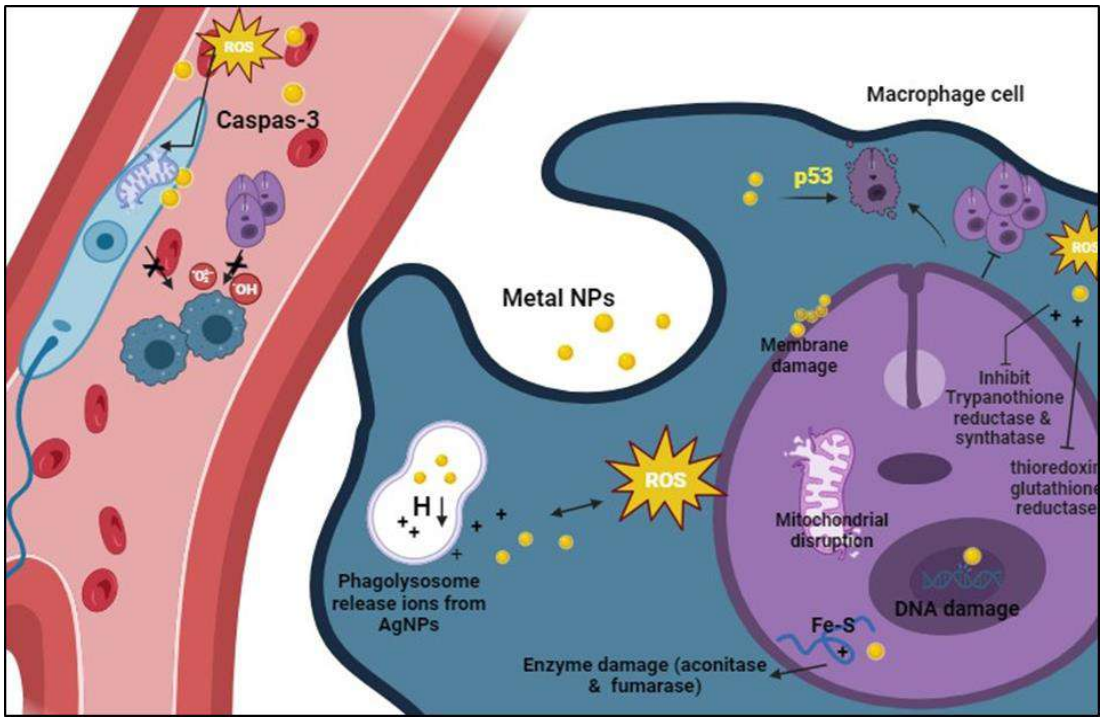


**Fig. 3:** Schematic diagram of the mechanism of anti-leishmanial activities of metal nanoparticles

The efficiency of different nanoparticles against *Leishmania* has been demonstrated in Table 1.

**Table 1:** The efficiency of different nanoparticles against the *Leishmania*

| NPs | Source | Model | *Leishmania* | Effect | Reference |
|---|---|---|---|---|---|
| Amp loaded Polycaprolactone (PCL) NPs | Chemicals | *in vitro* | *L. tropica* and *L. donovani* | Maximum parasite suppression was demonstrated by the measured IC50 of the produced nanoparticle formulation for both *L. tropica* KWH23 and *L. donovani* amastigotes, which was found to be much lower than that of control-free Amp B and AmBisome®. | (Saqib et al., 2020) |
| Ag–Au BNPs | fenugreek, coriander, and soybean (leaves) | *in vitro* | *L.donovani* | The Au-Ag BNP forms showed potent antileishmanial actions against promastigotes, with IC50 values ranging from 0.03 to 0.035 g/mL.<br>The synthesized NPs caused an apoptotic-like death in the promastigotes and enhanced the antileishmanial activity of macrophages.<br>The number of intracellular amastigotes in macrophages was reduced by 31-46%. | (Alti et al., 2020) |
| AuNPs | *Cannabis sativa* | *in vitro* | *L.tropica* | AuNPs exhibit a strong anti-*L. tropica* amastigotes effect, as evidenced by an IC50 of 171 ± 2.28 µg/ml. | (Hameed et al., 2020) |
| AgNPs | *Cuminum cyminum L* (seed) | *in vitro* | *L.tropica* | Both Bio-AgNPs and AgNPs greatly reduced the growth rates of promastigotes and the metabolic activities of amastigotes, thus inhibiting *L. tropica* promastigotes and amastigotes excessively.<br>Additionally, the infection index rates of macrophages demonstrated the exceptional anti-amastigote activities of Bio-AgNPs. Additionally, to destroy *Leishmania* parasites, Bio-AgNPs stimulated macrophages to produce NO. | (Bagirova et al., 2020) |
| $Fe_3O_4$ and Fe3O4@PO NPs | Chemicals | *in vitro and in vivo* | *L.major* | The Fe3O4 and Fe3O4@PO NPs exhibited IC50 values of 62.3 ± 2.15 µg/mL and 31.3 ± 2.2 µg/mL, respectively.<br>The mean number of parasites and the mean diameter of the lesions were found to have significantly decreased in the infected mice treated with NPs. The production of NO was significantly stimulated by both NPs in a dose-dependent manner.<br>When pre-incubated in NPs at a concentration of 5 µg/mL, the promastigotes infect 41.7% and 28.3% of the macrophage cells, respectively. | (Albalawi et al., 2021 a) |
| CuNPs | *Capparis spinosa* (fruit) | *in vivo and in vitro* | *L.major* | The findings showed that CuNPs significantly lowered the rate of proliferation of L. major amastigotes and, in a dose-dependent way, induced the production of NO, especially when combined with MA.<br>Moreover, CuNPs showed no sign of cytotoxicity. Infected mice treated with CuNPs showed a considerable decrease in the mean number of parasites, especially when combined with MA in a dose-dependent manner. The lesions treated with doses of 100 and 200 mg/mL of CuNPs, respectively, their mean diameter decreased by 43 and 58 mm. | (Albalawi et al., 2021b) |
| AgNPs | *Commiphora myrrha* (oleo-gum resins) | *in vitro and in vivo* | *L. major* | - At the higher concentrations 100, 150 l/100 l/100 ul showed a significant inhibitory effect for the MSNPS.<br>- After receiving MSNP treatment in vivo, lesions were fully cured in 21 days. | (Awad et al., 2021) |

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| AmB-loaded chitosan NPs (AmB-CNPs) | Chemical | *In vitro* | *L.tropica* | After 72 hours of incubation, AmB-CNPs showed 90% and 84% parasite suppression, but free AmB showed very little efficiency (only 65% and 67% inhibition of the promastigotes and axenic amastigotes parasite burden). When it came to reducing parasite viability, the AmB-CNPs were significantly more effective than free AmB. When compared to free AmB, the AmB-CNPs' half-maximal inhibitory concentration (IC50) values were significantly lower. | (Sohail et al., 2021) |
| AgNPs | Ginger rhizome extract | *in vitro* | *L. major* | Results for macrophages and *L. major* promastigotes treated with the greatest amount of NPs (40 mg.kg-1) were reported to be 7.3% and 32.2% viability percentage, respectively.<br>Following a 72-hour incubation period, the mean number of amastigotes within each macrophage decreased in contrast to the control groups by 1.25 and 2.5 mg.kg-1 of Ag-NPs.<br>Furthermore, this parasite strain of L. major had an IC50 value of 2.35 mg.kg-1 after 72 hours of exposure. | (Mohammadi et al., 2021) |
| AgNPs | chemical | *in vitro* | *CL* | In comparison to the other concentrations, the concentration (6 ug/ml) showed the highest level of inhibition at 96 and 120 hours. | (Hanoon et al., 2022) |
| AgNPs | *Eucalyptus camaldulensis* | *in vitro* | *L. tropica* | The highest inhibitory effect on parasites was at the highest concentration of AgNPs (3.75 g/mL), which resulted in a 90% reduction in parasite growth. | (Zein et al., 2022) |
| Curcumin-coated silver nanoparticle (Cur@AgNPs) | Turmeric (curcumin) | *in vitro* and *in vivo* | *L. major* | The nanoparticle demonstrated strong antileishmanial activity with an IC50 of 58.99 g/ml for promastigotes and an EC50 of 58.99 g/ml for amastigotes.<br>The size of the CL lesion was dramatically reduced in the BALB/c mice that were being treated for infection with Cur@AgNPs. | (Badirzadeh et al., 2022) |
| $Fe_3O_4$ | Annona squamosa (peels) | *in vitro* | *L.tropica* | Fe3O4 is effective in inhibiting the cutaneous *L. tropica* parasite's frontal flagella. When the parasite numbers were reduced by the nanoparticles within 96 hours after the start of growth, the parasite's total amount of protein was significantly reduced as well. | (Mahdi Barkash et al., 2023) |
| AgNPs | *Coffea arabica green* (seed) | *in vitro* | *L.major* | The NPs' IC50 values against promastigotes and amastigotes were 65. 4 and 47.70 µg/mL, respectively. At 250–1000 µg/mL concentrations, it demonstrated a substantial decrease in IL-10 expression levels between NPs-treated and Glucantime®-treated macrophages, but it showed a significant increase in IL-12P40, a Th1 cytokine. | (Sharifi et al., 2023) |
| AgNPs | *Thymus Vulgaris* | *In vitro* | *L.major* | AgNPs had a significant effect on L. major promastigotes and amastigotes, and their effects were inversely correlated with concentration.<br>AgNPs, with an IC50 value of 3.02 µg/mL after 72 hours, decreased the growth rate of L. major amastigotes.<br>Flow cytometry data demonstrated 69.51% apoptosis and the toxic impact of AgNPs on promastigotes after 24 hours. | (Zaki et al., 2023) |
| Ag/Cu NPs | sugarcane bagasse | *in vitro* | *L. donovani* and *L. amazonensis* | -The Ag/Cu NPS had robust leishmanicidal properties, with IC50 values of 2.909 ± 0.051 for *L. donovani*, 3.580 ± 0.016 for *L. amazonensis* | (Snoussi et al., 2023) |
| AgNPs | *Astragalus spinosus* (aerial parts) | *In vitro* | *L.major* | The diameter of CL lesions significantly decreased when various concentrations of AgNPs and AgNPs+MA were treated. AgNPs activated caspase-3 in a significant way, especially at ½ IC50 and IC50 doses. The CC50 values for AgNPs and MA were determined to be 612.5 and 789.8 µg/mL, respectively. | (Majeed et al., 2023) |

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| AgNPs | - | *In vivo* | *L. major* | Comparing treated vs untreated mice, the treatment group's infected animals showed reduced inflammatory responses and increased fibroblast activity. These findings suggest that this substance may help reduce inflammation and encourage the development of fibroblasts, which are helpful for tissue regeneration and wound healing. | (Ebrahimzadeh et al., 2023) |
| MTCAgNPs and emulgel-loaded MTC-AgNPs | chemical | *ex vivo and in vivo* | *CL* | MTC-Ag exhibited a strong inhibitory effect on intracellular amastigotes, with 90% inhibition at the maximum concentration, according to the in-vitro anti-leishmanial assay.<br>The IC50 value of MTC-Ag was found to be four times lower than that of AgNPs. At 80 µg/Ml.<br>the cytotoxicity experiment revealed 33% viability of macrophages for AgNPs and 83% for MTC-Ag. | (Shakeel et al., 2023) |
| ZnNPs and ZnNPs-MA | Lavender (leaves) | *in vitro and in vivo* | *L.major* | The mice given ZnNPs+MA showed a full improvement in their CL lesions.<br>ZnNPs and ZnNPs + MA had IC50 values of 43.2 and 12.6 µg/mL, respectively.<br>The levels of mRNA expression for iNOS, TNF-α, and IFN-γ were shown to be dose-dependently increased, while IL-10 caused a downregulation in this regard. ZnNPs significantly increased the activation of caspase-3 while giving no significant damage to normal cells. | (Ghasemian Yadegari et al., 2023) |
| $Fe_2O_3$NPs and ZnO NPs | *Tavernier glabra* | *in vitro* | *Leishmania sp.* | The ZnO NPs and $Fe_2O_3$ NPs exhibited strong anti*leishmania*l activities with IC50 = 76.3 ± 2.08 and 90.4 ± 1.031. | (Khan et al., 2023) |
| ZnONPs | *Clinopodium vulgare* (leaves) | *in vitro* | *L. tropica* | The promastigote and amastigote forms of the parasite were found to be effectively killed by the cytotoxicity at various doses, with mortality rates of 77% and 74% at 400 g/mL. | (Arif et al., 2023) |
| SS-AuNPs | *Scrophularia striata* | *in vitro* | - | The anti-leishmanial activity of SS-AuNPs was determined to be effective; the IC50 values for the anti-promastigotes and anti-amastigote activities of SS-AuNPs were calculated to be 0.12 µg/ml and 0.07 µg/ml, respectively. | (Royaa Alizadeh et al., 2023) |
| AgNPs | *Ericaria amentacea seaweed* | *in vitro* | *L.infantum, L. tropica, and L. major* | The synthesized AgNPs exhibited significant anti-leishmanial action, as evidenced by inhibitory concentration values ranging from 27.16 µg/mL to 38.18 µg/mL. These results indicate the possibility of using AgNPs produced from the seaweed *Ericaria amentacea* to treat leishmaniasis. | (Mohamed Abdoul-Latif et al., 2023) |
| CeNPs-TEP | *chemical* | *in vitro* | *L. donovani* | In *L. donovani* Ag83 promastigotes, exposure to CeNPs-TEP resulted in 62–82% cell death after 24 and 48 hours, respectively.<br>Following the administration of CeNPs-TEP, oxidative stress and depolarization of the mitochondrial membrane were detected.<br>*L. donovani* Ag83 cells' production of ROS increased by approximately 2.2 times after being exposed to CeNPs-TEP. | (Yadav et al., 2023) |
| AgNPs | *Nyctanthes arbor-tristis* (leaves) | *in vitro* | *L.donovani* | The leishmanicidal effect of AgNPNAs on *L. donovani* promastigotes is demonstrated by their efficacy (IC50 = 11.46 ± 0.120 µg/ml), and a higher safety level (IC50 = 136.33 +/- 0.881 µg/ml) in healthy macrophages, suggesting their potential as an antileishmanial drug. | (Roy Chowdhury et al., 2023) |

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| CaONPs | - | *in vitro* | *L. tropica and L. infantum* | In an in vitro study, CaO NPs shown to be more efficient against *L. infantum* than L. tropica. The IC50 values of CaO NPs within 72 hours were 19.81 µg/ml for L. tropica and 22.57 µg/ml for L. infantum, indicating the impacts of NPs on promastigotes. For L. tropica, the estimated percentages of normal, apoptotic, and necrotic cells were 82.6%, 14.81%, and 2.69%, and for *L. infantum*, they were 73.6%, 23.89%, and 2.58%. | (Dalir Ghaffari et al., 2023) |
| AgNPs | *filamentous fungus Fusarium oxysporum* | *in vivo and in vivo* | *L.amazonensis* | Promastigotes exhibited morphological and ultrastructural alterations due to the synergistic antileishmanial action of Np on promastigote forms and *L. amazonensis*-I infected macrophages. phosphatidylserine exposure, mitochondrial depolarization, lipid-storage body buildup, autophagic vacuoles, a rise in NO and ROS, and plasma membrane damage. Reduced percentages of infected cells and amastigotes per macrophage were seen. | (Alves et al., 2023) |
| Chitosan nanoparticles (CNPs) | *Fumaria parviflora* | *in vivo and in vivo* | *L.major* | *In vitro*, CNPs inhibited the growth of L. major promastigotes by 84% and 96%. CNPs had an IC50 value of 68.4 µg/ml. The best results from the amastigote assay indicated that only 2% of macrophages were infected with amastigotes in CNPs. The mean diameter of the lesions was significantly reduced in vivo testing. | (Simin et al., 2024) |
| Lin@ZNP | Chemical | *in vitro and in vivo* | *L.major* | The formed ZNPs significantly increased the percentage of early and late apoptotic cells in promastigotes and amastigotes, demonstrating their antileishmanial actions. After being exposed to LZNPs, macrophages' levels of iNOS, IFN-γ, and TNF-α gene expression were all increased in a dose-dependent manner. | (Albalawi et al., 2024) |
| AgNPs | Paullinia cupana Kunth | *in vitro* | *L. amazonensis* | The RAW 264.7 macrophages showed minimal cytotoxicity, and AgNPs' leishmanicidal activity against *L. amazonensis* was higher than that of the drug miltefosine. | (Lima et al., 2024) |

**Conclusion**

Leishmaniasis is caused by a parasite *Leishmania*, and is an infectious neglected disease. Sandflies transmit the disease by biting humans. The promastigote form of the parasites can infect humans and once they are internalized in macrophages, they transform into the amastigote form. Current treatments for Leishmaniasis are not adequate because they are expensive, have high toxicity, are administered parenterally, and resistant parasites have already been reported. Therefore, nanotechnology can be used to encapsulate antileishmanial drugs in nanocarriers, which have several advantages over other drugs. These carriers can reduce adverse effects and promote better treatment outcomes. Nanoparticles were the most frequently utilized nanocarriers. Some articles showed positive outcomes with optimistic results and demonstrated the effectiveness of the nano-encapsulated drug against parasites in both *in vitro* and *in vivo* settings.

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Chapter 25

# The Science behind Fluoride Nanoparticle Its Impact on Oral Wellness

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**ABSTRACT**

Fluoride nanoparticles have been beneficial in promoting health since the discovery of their role and the start of their usage in the field of dentistry. This chapter provides an in-depth exploration of the science behind fluoride nanoparticles and their impact on oral and overall health. In the year 2007 WHO World Health Assembly declared that one of the fundamental rights to human health is the availability of fluoride for the prevention of caries. The fluoride nanoparticles have various methods of synthesis and are found as an adjunct to various compounds. They could be obtained from natural and artificial sources in the surrounding. They have strengthening, mineralizing and antimicrobial characteristics that are beneficial for the human body and teeth. However, measures should be taken in the use of fluoride as it can be harmful in some instances despite having many benefits. Fluoride nanoparticles and nanotechnology have encouraging applications in the present and future of human health.

**KEYWORDS**
Fluoride, Nanoparticles, Health, Oral, Mineral, Nanotechnology

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## INTRODUCTION

Frederick McKay found brown stains on teeth in Colorado Springs in 1901, which sparked interest in fluoride studies. In 1909, Dr. G.V. Black became involved in McKay's research. Water was suspected because of the uneven enamel and decay resistance they discovered. Their idea was validated by McKay's visit to Oakley, Idaho, in 1923, when he saw youngsters with stains from a water pipeline. Additional research at Bauxite, Arkansas, by H. V. Churchill, the head chemist of ALCOA, showed that the water had high fluoride content. The presence of fluoride was verified by photospectrographic examination. This discovery led to the deduction that excessive fluoride levels in water caused enamel discoloration.

Dr. H. Trendley Dean was motivated to prevent tooth decay by introducing fluoride to drinking water based on previous studies on fluorosis conducted by McKay and Black. Dean proposed the theory that tooth decay may be prevented by safe fluoridation of water. The first city to fluoridate its water supply was Grand Rapids, Michigan, in 1945, marking the start of a 15-year program. The U.S. Surgeon General first funded the study, which was later taken over by the NIDR, which looked at schoolchildren's tooth decay rates. Eleven years later, Dean revealed a startling discovery: the incidence of dental decay in children born following fluoride treatment had decreased by over 60%. For the majority of individuals, tooth decay would now be avoided for the first time in history thanks to this discovery, which promised to transform dental treatment (NIDCR, 2000). Water fluoridation was recognized by the CDC as one of the top ten significant public health accomplishments of the 20th century because of the significant decrease in tooth decay that ensued.

**Nanoparticles**

The engineering and utilization of materials with sizes ranging from 1 to 100 nm is referred to as nanotechnology. Microscopic objects having at least one dimension less than 100 nm are known as nanoparticles. Because of their very large surface area, nanoparticles frequently have unique size-dependent characteristics. Furthermore, the length of a particle at the nanoscale is less than either the light wavelength or the de Broglie wavelength of the charge carrier, which consists of electrons and holes (Anwar, 2018).

**Fluoride Nanoparticles**

There are a plethora of established techniques for creating fluoride nanoparticles. Every one of them has benefits and downsides of their own. The prerequisites for nanoparticles —such as their size and phase composition, fineness, ability to disperse in water, physiologic solution, etc. determine the synthesis protocol to be used (Fedorov P, 2011). Two primary approaches to synthesizing nanoparticles, which are generally categorized into top-down and bottom-up techniques, are used.

Top-down or destructive procedures are synthetic methods with a broad spectrum of applications that reduce bulk materials to nano-scale particles. Examples of these techniques include mechanical milling, sputtering, thermal decomposition, laser ablation, and nano-lithography. On the other hand, the technique of creating substances from atoms to clusters to nanomaterials is known as a bottom-up or constructive approach, and it has several uses in the production of nanomaterials through pyrolysis etc (Foong, 2020).

One of the most popular top-down methods for producing distinct nanoparticles is mechanical milling, which is used to mill and post-anneal nanoparticles during syntheses that include milling multiple components. One factor influencing the mechanical milling process is plastic deformation. This process produces the shape of the particles and causes them to fracture, which reduces their size; then there is cold-welding, which increases their size (Rudolph, 2019). Because of its simplicity, the sol–gel method is one of the most widely used bottom-up techniques. It uses a chemical solution as a precursor for discrete particles in a wet chemical process. Metal oxide and chloride are two precursors that are frequently utilized in the sol-gel process. After that, the precursors are mixed, shaken, or sonicated in a host liquid. There are two phases in the completed system: a liquid and a solid. The nanomaterials are recovered using a separated phase and a variety of methods, such as centrifugation, filtering, and sedimentation (Catauro, 2018; Mao, 2018).

Some of the compounds that are a source for the release of fluoride nanoparticles from various fluoride sources like dentifrices and other products are as follows:

**Sodium Fluoride NaF;** is most commonly used in water fluoridation and toothpastes as the most common fluoride source in mouth rinses. Sodium fluoride is not irritating to the gingivae, tastes well, and is quite stable chemically. Furthermore, it doesn't discolor dental restorations or the tooth tissues. Sodium fluoride-containing chitosan anhydrous nanoparticles have been created. Even at 104 parts per million, pure sodium fluoride solutions showed no antibacterial action. The nature of the counter-ion (sodium or potassium) has no bearing on this. Upon dissolving in water, dissociation happens instantly (Ullah, 2015).

**Stannous fluoride SnF2;** Stannous fluoride (SnF2) is a combination of fluoride ligands and a tin(II) (Sn(II)) metal ion coordination center. Its chemical bonding is fairly strong. Through its potential to promote enamel mineralization and reduce gingival irritation and bleeding, this drug has been shown to effectively manage and prevent both dental caries and gingivitis. It may also have a broad-spectrum microbicidal action and be able to drastically alter the microbial composition of dental biofilms (Algarni, 2015) the release of fluoride ions, in this case, requires hydrolysis.

**Calcium fluoride CaF2NPs:** The use of calcium fluoridenanoparticles (CaF2NPs) to raise the fluoride content of the oral cavity has become commonplace. CaF2NPs can prevent *Streptococcus mutans* from producing exopolysaccharide (Kulshrestha, 2015). These NPs have two functions: they inhibit the growth of biofilm and encourage the remineralization of dental enamel (Moraes, 2021).

**Sodium Monofluorophosphate; Na2PO3F;** also, upon dissolving in water, it dissociates instantly. Due to the widespread recognition of MFP's cariostatic and microbicidal properties, it has been used in toothpastes and other oral health products. Its molecular structure is made up of tetrahedral [PO3F] − structural units with an unbroken P–F link (Ericsson, 1980).

**Silver(I)-Diammine Fluoride (SDF);** Mizuho worked in Yamaga's laboratory in 1969 to conduct research on silver(I)-diammine fluoride (SDF) for her PhD thesis. This resulted from an effort to create a combined product that would combine fluoride's enamel-protecting qualities with the antibacterial qualities of silver(I) cation [Ag(I)]. Manufactured by Morita in 1970, Saforide was the first commercial product (Yamaga, 1972).

**AmineHydrofluorides;** these salts consist of the fluoride ion plus a cationic surfactant, such as ammonium salt. Dissociation occurs instantly and completely upon dissolving in water (Epple, 2018).

**Physical Sciences of Fluoride**

Fluoride has a preventative impact on caries, or cavities, which makes it an essential component of health, mainly oral. It is a catalyst that helps saliva naturally remineralize; however, it works best in the presence of phosphate and calcium ions. The incidence of cavities has been dramatically decreased by using fluoride-containing home dental care products. However, rather than fluoride itself, the counterion is primarily responsible for the antibacterial activity of fluorides.

Although it never exists in a free state in nature, halogen fluoride is widely dispersed across the Earth. As fluoride compounds, which are parts of minerals in rocks and soil, fluorine can only exist in conjunction with other elements. It is well recognized that fluoride is necessary for the mineralization of teeth and bones because it builds up in the body's hard tissues (María Dolores, 2011).

**Fluoride in Dentistry Health**

**Sources**

In our environment, food, respiration, and fluoridated items could serve as the ways through which fluoride enters the body. Since fluoride is a part of the natural environment, it remains prevalent in the lives of individuals. Fluoride concentrations, however, might differ between geographical areas. Because of its tiny atomic radius, it is the element that is most electronegative and reactive from a chemical perspective. Due to its strong reactivity, it is often bonded as inorganic fluoride and not found in its unadulterated form. It makes up 0.06-0.09% of the weight of the Earth's crust and is ranked 13th in terrestrial abundance. The hydrosphere, biosphere, lithosphere, and atmosphere are all home to fluorine. A considerable amount of fluorine may be found in volcanic rocks. Some of the ways it enters the environment include through volcanic eruptions, rock breakup, and a range of human activities (coal burning, mineral processing, fertilizer manufacturing and use, and industrial facilities). Every body of natural water contains fluoride. Seawater has 1.2–1.5 parts per million of fluoride. Typically, freshwater concentrations are between 0.01 and 0.3 parts per million. Fluoride concentrations in water can be higher in the vicinity of volcanic hot springs. Normal soil fluoride buildup is minimal. A few hundred parts per million of fluoride may be accumulated by certain plants, the most well-known of which is the tea plant (Petersen, 2004; Yeung, 2008; Cagetti, 2012).

Apart from these, there are several other ways of fluoride delivery to the human body. Some of them are discussed as follows:

The most common way to provide fluoride systemically is by water fluoridation, which involves adding a certain quantity of fluoride to the public water supply. The WHO advised fluoridating public water systems as the primary means of delivering fluoride for the purpose of enhancing dental health. This practice was first implemented in the United States in 1945. Although Fluoride is naturally present in fresh water, the concentration of the element varies according to the source and region. The typical range of Fluoride content in natural water is 0.01 ppm to 100 ppm. When fluoridating water, sodium fluoride and fluorosilicic acid are frequently utilized (Harrison, 2005).

We utilize edible salt on a regular basis and it's an essential part of our diet. In areas without a centralized water supply or with intermittent water supplies, another option to water fluoridation has been salt fluoridation. It has effects that are comparable to fluoridating water. Salt receives an addition of 90–350 mg/kg fluoride. The main advantage of salt fluoridation over water fluoridation is that it allows users to choose whether or not to use it. Because salt consumption raises the risk of hypertension, certain individuals with systemic diseases cannot be provided fluoride with salt (Cameron, 2021).

A mouthwash containing 1.0 µg F/g of Salvadorapersica (miswak) has been used as an antimicrobial agent to improve oral wellness (Sofrata, 2008).

The delivery of fluoride has also been accomplished by adding fluoride to milk. Milk fluoridation is a less effective way to provide fluoride when compared to water. The addition of fluoride to milk results in insoluble complexes that hinder the absorption of fluoride (Tressaud, 2008).

The use of toothpaste is crucial for preserving dental health. Its detergent activity aids users in getting rid of dirt and plaque. Using toothpaste to polish the surface of teeth helps stop dirt and germs from building up. Since toothpaste is used by people on a regular basis in modern life, it can be a source of several medicinal compounds, including Fluoride. Fluoride-containing toothpaste was initially made commercially accessible in the 1970s. Sodium fluoride (NaF), sodium monofluorophosphate (MFP), amine fluoride, and stannous fluoride are the main forms of fluoride added to toothpastes. The availability of fluoride in the oral cavity may also be impacted by the other chemicals in toothpaste. This is particularly true for abrasives that contain calcium because of their ability to deactivate the fluoride. It has been shown that using fluoridated toothpaste reduces dental cavities by 25% more effectively than using non-fluoridated toothpastes (Kidd, 2005; Davies, 2009).

Patients who are very susceptible to dental caries are advised to use mouth washes in addition to toothpaste. Sodium fluoride (NaF) is the active ingredient in mouth rinses that delivers fluoride. Mouthwashes that are widely accessible without a prescription have 0.05% NaF, or 226 parts per million of fluoride. The benefit of fluoride-containing mouth rinses is that they are less viscous than toothpastes, which makes it possible for the fluoride to enter hard-to-reach places including cracks, narrow pits, and interproximal regions (Cameron, 2021).

For the management and prevention of dental cavities, fluoride varnishes have been used since the 1960s in addition to conventional fluoride administration methods. Fluoride varnish is not a consumer product and cannot be applied by a general practitioner or dental hygienist. Varnishes are topical therapies that provide fluoride by depositing calcium fluoride under and on top of carious lesions. Fluoride is supplied for a long time by the calcium fluoride, which acts as a fluoride pool. It is advised to employ fluoride varnishes therapeutically for the treatment of xerostomia, active caries, root surface caries, hypersensitive dentine and enamel areas, and patients with mental or physical impairments (Kidd, 2005; Davies, 2009).

There are several types of fluoride gels that are used, for example, after radiation therapy for cancer patients; stannous fluoride gels (0.4% SnF2, or 970 ppm of Fluoride) have been added to artificial saliva to lessen dental caries. These gels are efficient at stopping root surface caries. In addition to tasting terrible, stannous fluoride gel can discolor teeth. Acidulated phosphate fluoride (APF) gels with high concentrations can be utilized as an alternative. Nor does it stain dental restorations or the tissues that make up teeth. On the other hand, restorations may get etched and discolored with APF or stannous fluoride gels. For patients who have ceramic or composite resin metal-ceramic restorations, APF gels should not be utilized (Anusavice, 2013).

For clinical use, a variety of fluoride-releasing dental restorative materials are available. These materials serve as a fluoride reservoir in addition to repairing missing or injured tooth tissue. When fluoride is accessible from other sources, including toothpaste and mouthwash, these materials replenish the quantity of fluoride that they release into the oral cavity. Among the many distinctive qualities of glass ionomer cement (GIC) are its strong chemical adherence to tooth hard tissues and its capacity to release and replenish fluoride ions. Glass ionomer materials exhibit two different sorts of Fluoride release patterns: a burst of initial release and a prolonged period of continuous release. The restoration's age, the material type and permeability, the amount and degree of fluoride exposure, and other factors all affect the capacity of the repair to absorb fluoride again (Zafar, 2015).

Fluoride levels in dental plaque and saliva may be kept much higher with the use of slow fluoride release devices. Oral disorders are best managed with the use of bio-adhesive formulations. These preparations have the ability to stick to certain tissues, such as the mucosa. A co-polymer of chitin created by partial deacetylation is called chitosan. It is frequently utilized in oral applications due to its mucoadhesion, biocompatibility, low toxicity, and controlled medication release. Because these nanoparticles have better bio-adhesion to the hydrated oral mucosal surface, they can act as a reservoir for both the first surge and continued release of fluoride (Keegan, 2012).

**Metabolism**

Following ingestion, 90% of the fluoride is absorbed by the gastrointestinal system (up to 25% in the stomach and around 77% in the proximal section of the small intestine). Feces include the remaining 10%. Fluoride is absorbed, then moved into the bloodstream and dispersed throughout the body. Twenty to sixty minutes after consumption is the average time of peak concentration. Fluoride ions are attached to plasma proteins in the plasma. Seldom does the concentration rise over 0.06 ppm (parts per million). It is not homeostatically regulated in the blood and is typically around 0.01 ppm. While toddlers retain about 50% of fluoride, adults only keep about 36% of it. Of that, 1% is located in soft tissue and 99% is found in calcified tissues like teeth and bones. Saliva and perspiration only slightly excrete the leftover portion of the absorbed fluoride; the remainder is eliminated through the kidneys into the urine. Thus, the kidneys are the sole organ in humans that helps to maintain the body's fluoride concentration. Numerous factors can affect the metabolism of fluoride. The most significant ones are: food, genetic predispositions, physical activity, circadian rhythm, hormones, kidney function, altitude, hematocrit, and acid-base problems. The amount of fluoride absorbed by the placenta in pregnant women is determined by the quantity of fluoride in the mother's circulation. Fluoride is absorbed by the placenta at low concentrations. Usually, the placenta has 60% of the mother's bloodstream content. When fluoride concentrations reach beyond 0.4 parts per million, the placenta acts as a barrier to prevent fluoride from passing through and protects the fetus from excessive fluoride exposure. Mother's milk can also absorb fluoride through the plasma, but the concentration is not very high (Kanduti, 2016).

**Mechanism of Action**

One important way that fluoride helps with caries management is through the formation of fluorohydroxyapatite. In low, prolonged concentrations, fluoride can bind to the surface of apatite crystals under acidic challenges, preventing demineralization. Fluoride in solution can create extremely supersaturated fluorohydroxyapatite, which speeds up the remineralization process, when pH is restored. When partially dissolved minerals and fluoride are present, a mineral is created that tends to incorporate fluoride and exclude carbonate. Fluoride is incorporated into the mineral structure of the enamel, strengthening its resistance to future acidic challenges and preventing the formation of caries. Moreover, in carious lesions, the production of fluorohydroxyapatite at the expense of hydroxyapatite helps to preserve a protective surface layer. This layer acts as a barrier against further enamel deterioration by reducing the rate at which demineralizing chemicals diffuse into the lesion. All things considered, fluoride exposure-induced fluorohydroxyapatite production is crucial for improving enamel resistance to demineralization, encouraging remineralization, and helping to avoid dental cavities overall (Ten Cate, 2008).

Wherever there is a vulnerable location, the acids that the plaque bacteria create dissolve minerals (calcium, phosphate, and fluoride) and permeate through the plaque into the enamel. Demineralization happens when minerals percolate into the oral environment from the tooth. Remineralization results if this process is reversed, the mineral is reabsorbed into the tooth, and the broken crystals are reassembled. It was discovered that fluoride has a somewhat intricate role in the process of remineralization. Fluoride works by preventing the loss of minerals at the crystal surface and by promoting the remineralization of calcium and phosphate in a way that makes them more resilient to acid assault in the future. It was anticipated more than thirty years ago by researchers that fluoride at low doses would promote remineralization. When hydroxyapatite dissolves in a solution, fluoride traces cause the solution to become extremely

supersaturated in relation to fluorohydroxyapatite. This will expedite the remineralization process. Calcium ions are drawn to the surface of partly demineralized crystals by fluoride adsorption. The additional fluoride increases resistance to demineralization as well as remineralization (Rošin-Grget, 2013).

Sub-millimolar concentrations of fluoride block some enzymes by directly attaching fluoride ions (F-) or hydrogen fluoride (HF) to the target enzyme, hence impairing the enzyme's normal activity. Fluoride inhibits a number of enzymes, including phosphatases, urease, enolase, P-ATPase, heme catalase, and heme peroxidase. Numerous bacterial activities and virulence factors may be impacted by this inhibition. Fluoride can also weaken the proton gradient/motive force in bacterial cells at micromolar concentrations. As a result, the cytoplasm becomes more acidic, which hinders the production of intracellular polysaccharides (IPS), glycolysis, and the phosphotransferase sugar transport system (PTS). The typical proton gradient across the bacterial cell membrane can be disrupted by fluoride's ability to function as a transmembrane proton carrier. This interference further prevents the synthesis and export of macromolecules, which impacts the development and metabolism of bacteria. Fluoride's antimicrobial actions are mostly due to its capacity to block enzymes and disrupt bacterial metabolic processes. It also adds to its cariostatic qualities by targeting critical pathways involved in bacterial virulence and survival (Koo, 2008).

**Efficacy and Safety**

Following the Frederick McKay discovery, a link was found between lower caries rates and mottling incidence. Extensive investigation revealed the involvement of fluoride anion in both processes. The next step was to make a concentrated effort to figure out how to weigh the possible benefits of fluoride against any potential drawbacks. Notable is the World Health Organization's (WHO) placement of the innovative metallodrug-type agent SDF on its list of important medications for the treatment and prevention of dental decay (WHO, 2019).

As of right now, fluoride has been introduced to toothpaste, communal water sources, and other medications. The amount of fluoride present in these items has not been shown using any conventional measuring units throughout this time. A parts-per-million (ppm) measure has occasionally been used, as well as a percentage of net weight or volume. This frequently causes misunderstanding among medical professionals and the general public when they attempt to assess the safety and effectiveness of materials and products containing fluoride that they come into contact with on a daily basis. There are also instances when some groups have used fluoride supplementation as the foundation for their political beliefs. A comprehensive analysis of no fewer than 87 instances of youngsters being toxically exposed to high fluoride levels has been documented, children as young as six years old unintentionally consumed fluoride-containing mouth hygiene products. Nonetheless, a dental professional stated that two youngsters started to exhibit symptoms after receiving fluoride therapy. In the lone surviving instance, a 13-month-old kid fatally consumed an unidentified amount of a pesticide containing sodium fluoride (drop in serum calcium was noted post-mortem). Around one-third of them had gastrointestinal symptoms and sleepiness. These symptoms included nausea, vomiting, stomach discomfort, and diarrhea; only three of these showed signs more than one hour after consumption (Augenstein, 1991).

**Toxicity**

The toxicity of fluoride can be divided into acute and chronic toxicity.

Overconsumption can be hazardous and cause acute fluoride toxicity. It is advised by the ADA that a maximum of 120 mg of fluoride (264 mg of sodium fluoride) be administered at one time. 5 mg/kg body weight has been established as the minimal amount that might result in toxic signs and symptoms, including fatality, and that should prompt therapeutic action and hospitalization. Infants have died after taking as little as 250 mg. If the patient is not treated, the estimated fatal toxic dosage of sodium fluoride for an adult male is 2.5 to 5g (Clarke R, 1974; Martínez-Mier, 2011).

Acute fluoride poisoning frequently manifests as nausea, vomiting, and a decrease in blood calcium levels, which can result in muscular tetany. Additionally, discomfort and cramping in the abdomen, rising hypocalcemia and hyperkalemia may happen and can result in unconsciousness, seizures, and cardiac arrhythmias. When someone consumes too much fluoride, they usually die within 4 hours; if they live for 24 hours, their prognosis is considered favorable. Four distinct mechanisms contribute to the toxic effects: (a) burning the tissues; (b) blocking nerve function (because of calcium affinity); (c) cellular poisoning (related to enzyme inhibition); and (d) blocking heart function **(**Harris, 2004).

Chronic fluoride toxicity consequences may result from prolonged systemic fluoride consumption, which can occur from a variety of sources such as drinks, water, dental floss, and professional therapy. Dental and skeletal fluorosis, derangement of muscle fiber, headaches, skin rashes, neurological symptoms like IQ decline, anxiety, trepidation, tingling in the toes and fingers, GI issues like nausea and abdominal pain, weakened immunity, and urinary tract malfunctions are some of the manifestations of fluoride toxicity.

Both adults and children can develop skeletal fluorosis. It takes a long time to become noticeable until the illness reaches a more severe state. Walking and moving about is made more difficult by fluoride, which is mostly deposited in the joints of the neck, knee, pelvic, and shoulder bones. Skeletal fluorosis symptoms are comparable to those of arthritis or spondylitis (Arora, 2006).

Enamel loses its sheen when it is exposed to too much fluoride. Dental fluorosis can present as white, opaque patches on the tooth surface in its moderate form or as severe pitting of the teeth and yellowish brown to black stains in its severe form. This discoloration might appear as horizontal stripes or as patches. The degree of dental fluorosis is often

determined by the quantity of fluoride exposed to children up to the age of 8 or 10, since fluoride only stains teeth that are still growing in the jawbones and behind the gum line (Aoba, 2002).

**Controversies and Debates**

Concerns over a potential connection between fluoride and cancer surfaced again in 1990. A peer review was then underway for the National Toxicology Program's report on its 24-month carcinogenic research involving rodents. The expert panel came to the conclusion that the study offered "equivocal evidence" of carcinogenicity. According to this categorization, the data support the hypothesis that a little rise in neoplasms might have a chemical basis. The finding was based on the observation that osteosarcoma appeared to arise in a dose-response manner in male rats. For a substance to be listed as a carcinogen, it must clearly cause cancer in two or more species. Fluoride is not listed in the most recent NTP carcinogen database.

The National Cancer Institute then carried out a thorough investigation and concluded, **"**There were no patterns in cancer risk that they could attribute to consuming water that has been fluoridated" (Whitford, 1992).

A research on the benefits and drawbacks of fluoride was issued by the New York State Department of Health. The Review of Fluoride: Benefits and Risks was released by the U.S. Public Health Service *ad hoc* Committee on Fluoride. The two investigations did not discover any connection between fluoridating the water supply and any other adverse outcome, including skeletal fluorosis, systemic disorders (cardiovascular, renal), cancer, genotoxicity, reproduction, or Down syndrome. Rather, they came to the conclusion that dental fluorosis has become more common in the United States in the recent past (Kaminsky, 1990).

**Current Research and Future Directions**

The potential impact of fluoride nanoparticles, nanomaterials, and nanotechnology on the future of dentistry is encouraging.

The nanoscaled metal fluorides in discussion are synthesized via a non-aqueous fluorolytic sol-gel synthesis method. The metal fluorides produced by this synthesis procedure are nanoscale and dissolve in water-based solutions at low fluoride concentrations. Because of their poor solubility, these nanoscaled metal fluorides have an advantage over conventional fluoride treatments in that they can build a depot within caries regions and release fluoride ions gradually. The gradual remineralization of the enamel is made possible by this regulated release mechanism. Furthermore, these metal fluorides' nanoscale size (<15 nm) allows them to build a reservoir inside the enamel lesion and enter the enamel matrix, which may improve their remineralization capacities in comparison to conventional fluoride agents (Zirk, 2019).

Composed of parts ranging in size from 1 to 100 nanometers, nanorobots have a diameter of around 0.5 to 3 microns. In the form of diamond or fullerene, carbon will be the main component. By responding to specific algorithms, nanorobots would allow medical professionals to accurately carry out cellular and molecular processes. When it comes to pharmaceutics, diagnostics, dental therapy, boosting natural immunity, mending brain damage, altering cellular DNA sequences, and repairing cellular damage, nanorobots may also prove useful (Kanaparthy, 2011).

Orthodontic nanorobots could have the ability to manipulate all periodontal tissues, including the gingiva, periodontal ligament, cementum, and alveolar bone, enabling quick and painless correction motions (Chandki, 2012).

**Conclusion**

The impact of fluoride nanoparticles on human wellness cannot be overemphasized. From the discovery of its function to the current day, where some advanced methods are used to synthesize the most appropriate and beneficial fluoride nanoparticles, it has become a part of human health. It is imperative that research continues to make it safer and even more beneficial over time. The contentions surrounding fluoride have been well debunked, but at the same time work needs to be done to make this information comprehensible and available to the global populace. The future indicates a very promising and hopeful role of fluoride nanoparticles and nanotechnology in general in human health from diagnostics to drug delivery and the first nanorobots are going to be a remarkable milestone in the history of mankind. Summarizing the broad research and progress made discussed here in this chapter, it can therefore be stated that fluoride nanoparticles have a substantial role in human health.

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Chapter 26

# Nanoparticles-induced Toxicity and its Impact on the Nervous, Immune, and Respiratory Systems of Humans

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## ABSTRACT

Nanoparticles (NPs) have great promise in the medical field, a better understanding of their effects on human health is crucial because of their potential for negative effects. The current studies on NP-induced toxicity in the respiratory, immunological, and neurological systems are covered in this review. Cellular balance in the nervous system can be compromised by NPs, resulting in inflammation, oxidative stress, and disturbed functioning. Further, the blood-brain barrier and the creation of protein corona on NPs make things difficult. The immune system has two sides of the blade. NPs can stimulate the immune system efficiently, but they can also be suppressed and high dosages or prolonged exposure can activate it permanently. The main mechanisms involved are unbalanced immune mediators, impaired cell migration, and disturbed immunological homeostasis by modified signaling. These small-sized nanoparticles (NPs) also create a critical threat to the respiratory system as they can penetrate quite deep into the lung space at the alveolar level, thus compromising the clearance systems and potentially damaging the gas exchange barriers. NPs can worsen conditions like asthma and COPD. The extent of this toxicity depends on factors like NP characteristics, dose, and exposure duration. Understanding these interactions is crucial for creating efficient NP-based treatments and therapies. This review provides an in-depth analysis of the toxicity caused by NPs in these important systems, emphasizing the need for more studies and research to guarantee the safe use of nanotechnology in medicine.

**KEYWORDS**
Nanoparticles, Toxicity, Immune system, Nervous system, Respiratory system

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## INTRODUCTION

Over the past few years, one of the fastest-growing research interests in the biomedical field is nanomedicine (Manzanares and Ceña, 2020). NPs are a specific class of particles with sizes ranging from 1 to 100 nm. According to ISO technical specification 80004, NPs are precisely defined as "nano-objects with all three external dimensions" whose longest and shortest axes do not substantially differ at the nanoscale (Ziental et al., 2020).

Applications of nanotechnology, especially nanoparticles (NPs), are paving their way very quickly into several fields, including medicine, physics, and environmental sciences. In medicine, disease prevention, diagnosis, and therapy are facilitated by nanotechnology and NPs (Khan et al., 2019). This has been made possible by sole developments in medication delivery systems, diagnostic tools, and pharmacological therapies (Biswas et al., 2012). Specifically, nanotechnology is essential for improving sophisticated imaging agents that can see physiological changes at the cellular and tissue levels and identify specific biomarkers (Xuan et al., 2023).

For the drug delivery system or vaccination, it is now preferable to administer medication in the form of NPs (nanocapsules) instead of loose medication (soluble antigens) (Chowdhury et al., 2021). These particles can efficiently

prolong the medicine's shelf life by preventing premature deterioration. Their shape and other characteristics facilitate their uptake by immune system defenders and set off a potent immunological response. They can also be coated in specific manners to engage directly with immune cells (Taki and Smooker, 2015). Monoclonal antibodies, like Herceptin, can be employed as targeted agents for molecular identification of the desired structure with great specificity and medication administration (Dammes and Peer, 2020). By using short peptides and smaller molecules we can enhance the polyvalent binding pathway's binding tendency. The concurrent binding of many ligands to receptors, or the binding phenomenon, is amplified to a high degree of intensity and is known as the polyvalent phenomenon (Kianfar, 2021). Simple diffusion or translocation, which is an energy-independent mechanism that depends on the NP concentration gradient as well as other variables like liposolubility, might allow nanoparticles to enter the cells (Correia et al., 2022). However, NPs also most frequently enter cells by an energy-dependent process called endocytosis, which is defined as the ingestion of materials from the external environment by vesicles produced from the cell plasma membrane (Manzanares and Ceña, 2020). By functioning as three-dimensional antibody carriers to replace conventional enzymes, gold NPs increase the sensitivity, specificity, assay time, and reproducibility of ELISA, PCR, and immuno-PCR assays. This results in ultra-sensitive, highly stable, and quickly produced visually detectable points of care (POC) for clinical applications (Tabatabaei et al., 2021).

There are three main routes by which nanoparticles might enter the body; through the mouth, nose, or skin (direct contact). The most common method is the inhalation route, which exposes the victim to airborne nanoparticles from outside like industrial operations or combustion that can cause fibrosis, genotoxicity, or inflammation by penetrating deep into the lungs (Yah et al., 2012). NPs are ingested by contaminated food, water, or mouth-to-mouth contact; they may enter the bloodstream or be excreted during digestion. In contrast, dermal contact is exposure through consumer goods or production processes, and the penetration depth is determined by the properties of NPs and the state of the skin (Sajid et al., 2015). The size, shape, surface area, and composition of nanoparticles (NPs) all have an impact on their distinct toxicological properties but interactions with biological molecules remain a major factor (Park et al., 2011). Many organ systems raise health concerns; inhalation can cause respiratory problems like asthma and chronic obstructive pulmonary disease (COPD) and also can worsen the preexisting conditions, and passing through the blood-brain barrier increases the risk of neurological disorders including conditions like neurodegenerative illnesses. Reproductive effects, immune system overload, and disruption of hormone balance are also quite considerable. Concerns regarding NPs' exposure and the development of cancer also result from persistent inflammation in cells, disruption of cell signaling, and DNA damage (Xuan et al., 2023).

It is necessary to address growing worries about the safety of nanotechnology in light of its potential to bring about significant advances in the field of medicine. Although nanoparticles have great potential for medicine delivery and diagnostics, there are always concerns regarding their potential adverse impacts on human health (Buzea and Pacheco, 2019). The focus of this chapter is on three major systems; the respiratory system, in which lungs are vulnerable to damage and inflammation caused by NPs, which may result in chronic illnesses and cancer; the nervous system, where nanoparticles may be able to cross the blood-brain barrier (BBB) and cause neurodegenerative diseases; and the immune system, that can respond abnormally to nanoparticles, possibly leading to autoimmune diseases or immunosuppression. Understanding these potentially harmful effects is essential for directing serious research and guaranteeing patient security in newly developing nanoparticle-derived treatments and procedures.

**Different Types of Nanoparticles used in Medicine**

Nanoparticles are classified into four different classes; zero-dimensional (0-D) like quantum dots, one-dimensional (1-D) like nanotubes, two-dimensional (2-D) like nanosheets, and three-dimensional (3-D) or bulk nanomaterials like bulk powders (Joudeh and Linke, 2022). Various types of nanoparticles are used in medicine like polymeric nanoparticles, magnetic nanoparticles, liposomes, carbon nanotubes, quantum dots, dendrimers, metallic nanoparticles, polymeric nanoparticles, etc. (Bhatia and Bhatia, 2016).

**Polymeric Nanoparticles**

These are made from various polymers; the adjustable characteristics of these NPs make them an adaptable vehicle. By specifically engineering them to encapsulate specific medications, enables the focused therapeutic approaches (Zielińska et al., 2020).

**Magnetic Nanoparticles**

Since they have their magnetic characteristics, they can be influenced by external magnetic fields. By using this capability of these NPs, therapeutic medicines can be precisely targeted and delivered to particular anatomical sites within the body by manipulating them, increasing the treatment efficacy and reducing systemic adverse effects (Materón et al., 2021).

**Carbon Nanotubes**

Special physicochemical characteristics and the small size of carbon nanotubes make them hollow, cylindrical structures; this offers them the ability to directly carry drugs into cells. Intracellular medication administration is possible

due to its capacity to cross cellular membranes, which could enhance the preciseness of the treatment for several medical conditions (Rathinavel et al., 2021).

**Metallic Nanoparticles**

These NPs are unique due to their special optical, electrical, and catalytic capabilities. They are usually made of metals like gold (Au), silver (Ag), platinum (Pt), and many other metals along with their oxides. They have much-improved surface area-to-volume ratios, which makes them effective drug delivery and diagnostic imaging agents. To increase the metallic biocompatibility of NPs and their specificity for targeted therapy, they can also be made compatible with biomolecules or targeting ligands (Chandrakala et al., 2022).

**Physiochemical Characteristics of Nanoparticles**

The efficacy of NPs is affected by many properties like; size, shape, surface area, elasticity, magnetism, stiffness, corona, surface chemistry, surface electrical charge, hydrophobicity, hydrophilicity, ligand binding, etc. (Sabourian et al., 2020).

**Size**

When engineering nanoparticles (NPs) for effective cellular uptake, size is a crucial design factor. In general, irrespective of the composition of NPs the ideal size range is between 10 and 60 nm. However, the mechanism for penetration can be affected by size directly, with NPs of smaller sizes possibly favoring particular pathways. This highlights how important it is to take size into account in addition to other aspects when designing NPs for particular cellular delivery (Augustine et al., 2020).

**Shape**

The form and structure of nanoparticles (NPs), such as spherical, rod-like, etc., have a significant influence on how well they will interact at the cellular level along with considering their size. NPs with a higher aspect ratio (length-to-diameter) typically exhibit higher levels of cellular absorption via either passive or active mechanisms. Studies have shown that longer forms, such as rods or nanotubes, have a little lower absorption rate than spheres due to their longer wrapping times, but they are still able to pass through cell membranes more effectively (Hadji and Bouchemal, 2022).

**Surface Charge**

The surface charge itself has an important effect on the cellular uptake of NPs. Endocytosis becomes easier by the attraction of positively charged NPs to the negatively charged cell membrane. Studies have shown that negatively charged nanoparticles (NPs) via unspecific routes may also be taken up by cells. The surface charge of the NPs makes the interaction with biological fluids important as it directly affects the density of proteins (Bilardo et al., 2022).

**Elasticity and Surface Area**

Elasticity of nanoparticles is important and as well as is complicated which is revealed by experimental difficulties in quantifying it. Due to the greater surface area and improved membrane penetration of NPs, stiffer nanoparticles are ingested more effectively into the cell. Techniques like AFM (atomic force microscopy) to assess the stiffness have shown soft NPs have different absorption rates. Using computational models such as DPD (dissipative particle dynamics ) the impact of the elasticity on the uptake of nanoparticles by cells can be explained (Kiio and Park, 2021).

**Hydrophobicity and Hydrophilicity**

Nanoparticles' interaction with cells is greatly influenced by their attraction to water, it is observed by their hydrophobicity and hydrophilicity. Hydrophobic nanoparticles mostly have greater cellular absorption, which could be possibly a result of interactions with the fatty core of the cell membrane. Hydrophobic NPs may bind more proteins in biological fluids, which perhaps increases absorption even more. Furthermore, the way NPs enter cells may also be influenced by their hydrophobicity and corona formation; hydrophobic NPs may enter directly, while hydrophilic NPs may be engulfed (Anderson et al., 2018).

**Ligand Binding**

Nanoparticle (NP) ligands are essential for medication delivery and cellular absorption. Depending on the ligand's characteristics and the core material, they can affect how readily NPs enter cells. Furthermore, ligands can be specifically selected to prolong circulation duration by stalling immune system clearance. Ligands that bind to particular receptors on target cells are employed in targeted delivery to guide NPs to the desired target site (Zein et al., 2020).

**Nanoparticles' Interactions with Human Systems**

Nanoparticles for therapeutic uses have immense potential for targeted drug delivery, but their success is hinging on interactions within the human body. Very rapid clearance of these NPs is a major problem (Zelepukin et al., 2020). Proteins are attracted to the clumped NPs, making them an open target for the immune system scavengers

especially in the liver and spleen (Ren et al., 2022). Scientists to overcome this, prefer nanoparticle surfaces with materials like PEG, which acts like a cloaking agent for NPs, reducing unwanted protein binding and hence extending the circulation time (Yetisgin et al., 2020).

A delicate balance is required for this approach. Surface modification can alter the NPs' journey in our favor but can also affect is focus on the target, despite size being another factor (Hoshyar et al., 2016). Too-large nanoparticles may be ingested by the immune cells, whereas the kidneys may remove too-small ones too. The ideal zone lies between 10 and 100 nanometers, which permits prolonged circulation and possible buildup in target regions like tumors (Ahmed et al., 2021).

Two main strategies are involved in understanding nanoparticles; passive and active. Passive targeting takes advantage of the leaky blood vessels in tumors for accumulation, while active targeting, for precise cell binding, equips nanoparticles with specific surface molecules. Nanoparticles with shape-influencing uptake entry into the cell enter via endocytosis; immune cells readily absorb the rod-shaped particles (Iversen et al., 2011). Surface charge is crucial, as positively charged particles trigger the immune responses, and neutral/negatively charged ones evade detection from immune cells. Depending upon the pH of the cell, by adjusting the surface charge we can control particle release. Optimizing these factors is vital for nanoparticle therapeutics as it helps NPs reach the target site effectively (Augustine et al., 2020).

**General Mechanism of Toxicity of Nanoparticles**

Various mechanisms are involved in the toxicity of nanoparticles in humans, with surface charge playing a significant role. Nanomaterials when interacting with cellular membranes, potentially may cause distortions or disruptions (Chen and Bothun, 2014). Membrane distortions are more likely due to the positively charged NPs and are taken up more readily by cells compared to negatively charged or neutral ones (Soto et al., 2008). Surface charge greatly influences the interaction of NPs with cellular proteins, affecting their cellular uptake (Fleischer and Payne, 2014). Organelles such as mitochondria are also at great risk of the nanoparticles' interaction producing reactive oxygen species (ROS) and NPs even penetrating cell nuclei, potentially leading to genotoxicity (Li et al., 2022). Furthermore, Nanoparticle-induced toxicity can damage the cytoskeletal proteins upon exposure, which may alter cytoskeletal integrity and cellular functions (Prabhu and Poulose, 2012).

In human cell biology, reactive oxygen species (ROS) production is an important factor in the toxicity of nanoparticles (Yu et al., 2020). The operations of mitochondria are frequently disrupted due to ROS formation triggered when the cellular environment is exposed to NPs, being an important hub for ROS synthesis during cellular energy production (Sharma et al., 2012). This oxidative stress produced as a result, will harm proteins, DNA, and membranes within cells due to an imbalance between the formation of reactive oxygen species (ROS) and antioxidant defenses in the matrix (Horie and Tabei, 2021). Such oxidative stress might affect cellular processes and potentially contribute to a variety of disorders depending on the exposed organ or system. Importantly, the effect of ROS on the toxicity of nanoparticles varies depending upon the characteristics of the nanoparticles and the kind of cells that are exposed to them; certain nanoparticles also have antioxidant qualities that can prevent the production of ROS (Li et al., 2023).

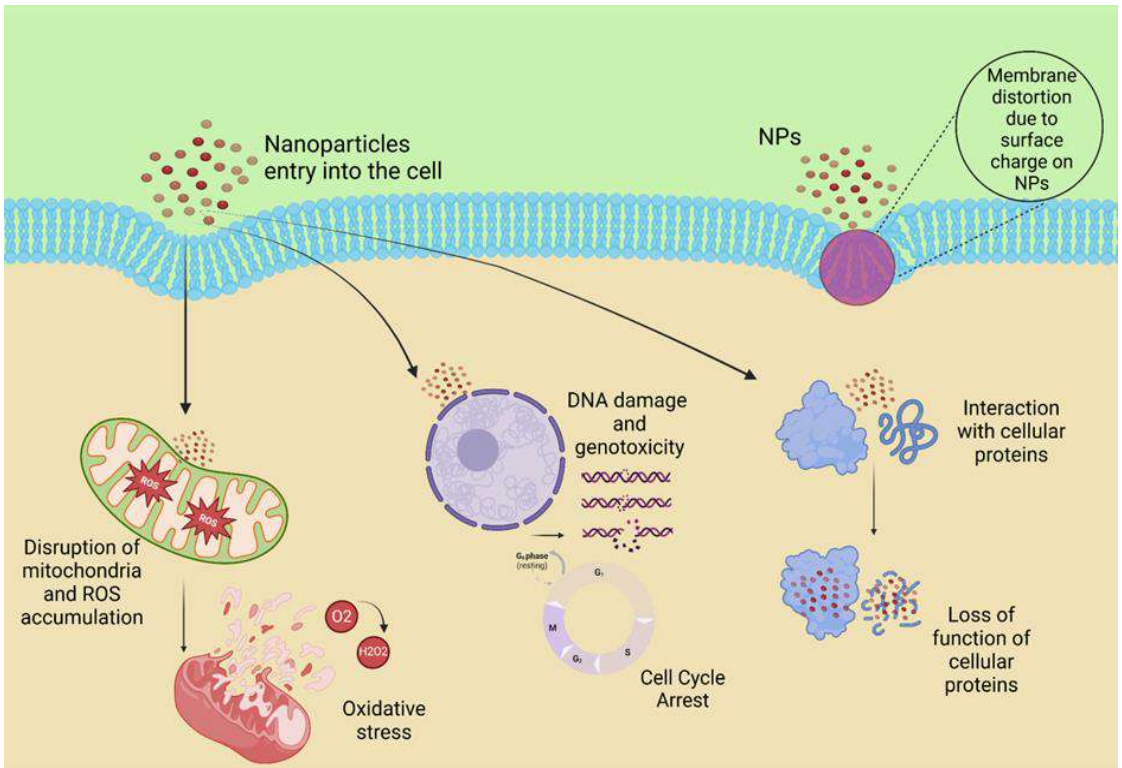


**Fig. 1:** General Mechanism of Action of NPs and their effects in the cell. Created with BioRender.com

**Nanoparticle Toxicity Induced in the Nervous System: A Guardian Confused**

In the human nervous system, the most remarkable and distinctive elements are the brain and its cognitive capacities. It is the system that is primarily responsible for controlling the body's physiological and thinking processes (Sousa et al., 2017). The central nervous system and the peripheral nervous system make up the two main branches of the nervous system, which is made up of nervous tissue. The brain and spinal cord are parts of the central nervous system, whereas the cranial and spinal nerves are parts of the peripheral nervous system (Catala and Kubis, 2013). An increasing number of systemic disorders have been linked to neurological and mental health conditions, the most notable being immunological, bioenergetic parameters, and disruption of the gut flora (Lyu et al., 2021).

Nanoparticles present interesting approaches for the administration of medications and neuroimaging in neurological applications but using them raises issues regarding possible neurotoxicity. Nanoparticles can upset the redox balance of cells, which may result in oxidative stress and decreased mitochondrial activity leading to critical situations (Feng et al., 2015). When interfering with neuronal signaling by activating glial cells they might also cause neuroinflammation (Wu and Tang, 2018). Due to their interference with the body's waste disposal mechanism. Nanoparticles can accumulate toxic chemicals within neurons. On the other hand, some nanoparticles can cause neuronal malfunction or even death by triggering signaling pathways (Sawicki et al., 2019).

There are a variety of processes NPs can disrupt cellular redox homeostasis by generating reactive oxygen species (ROS). Fenton reactions are one of these mechanisms in which transition metals are on the surface of the NP or electron transfer from biomolecules to the NP. Excessive production of ROS disrupts cellular function by damaging proteins, lipids, and DNA inducing toxic effects (Zhao et al., 2024). Mitochondria, the powerhouses of the cell, are particularly vulnerable to ROS-mediated damage. This may result in decreased energy synthesis, which would intensify the creation of ROS and set off a vicious cycle that ultimately leads to cell death and neurological dysfunction (Nalika and Parvez, 2015). Glial cells, including microglia and astrocytes in the brain, release pro-inflammatory mediators such as cytokines and chemokines when activated by certain nanoparticles (Poupot et al., 2018). Normal neuronal signaling, neuronal repair mechanisms, and the integrity of the blood-brain barrier (BBB) are disrupted and compromised by neuroinflammation. Foreign molecules may infiltrate the brain parenchyma in a compromised BBB aggravating the neuronal inflammation and other neuronal injury (Sharma et al., 2021).

Nanoparticles entering biological fluids, acquire a layer of adsorbed proteins known as the protein-corona layer. This corona can alter and manipulate a nanoparticle's surface characteristics, affecting how well it interacts with cells and even changing the functioning of proteins (Rahman et al., 2013). Disrupting essential proteins involved in synaptic transmission, neuronal signaling, or cellular homeostasis may also result in neurotoxicity (Mishra et al., 2021). Nanoparticles can physically damage the cell membrane or intracellular organelles with sharp edges or rigid structures. Furthermore, nanoparticles may disrupt autophagy, the cellular waste disposal system mechanism of the body. The accumulation of damaged proteins and organelles within neurons aggravates cellular dysfunction and potentially initiates cell death pathways (Wang et al., 2017). Recent studies indicate that the gut microbiome might be affected by NPs, which is a complex ecology of bacteria that lives in the intestines. The gut microbiota regulates behavior and brain function through the gut-brain axis. Neurodegenerative diseases like Parkinson's disease can be indirectly influenced by NPs that alter the composition of the gut microbiome (Diao et al., 2021).

**Nanoparticle Toxicity Induced in the Immune System; A Double-edged Sword**

There are different physicochemical characteristics like dose, exposure route, surface chemistry, corona formation, and several factors linked to the toxicity of nanoparticles (NPs) (Sukhanova et al., 2018). The other physicochemical characteristics, including size, shape, surface charge, and chemical composition of NPs greatly influence their interaction with immune cells. Regarding surface charge smaller NPs with positive charge usually have more immunostimulatory effects (Yung et al., 2017). The other important factors are the dosage and the exposure method with nanoparticles (NPs). Inflammation is readily caused by higher dosage while long-term exposure through ingestion, inhalation, or other route might lead to immunosuppression or permanent immunological activation (Ali et al., 2019). Nanoparticles (NPs) have a unique surface chemistry, when they enter biological fluids, they can adsorb biomolecules and create a protein corona layer. The corona layer changes the way NPs interact with the immune cells, and disturbs the intensity of immunological response by modifying their bioactivity (Rahman et al., 2013).

Nanoparticles can stimulate the immune system and cause inflammation, which makes them harmful to the immune system as they can alter the way the immune system works. Pattern recognition receptors (PRRs) are used by immune cells like macrophages and dendritic cells to identify nanoparticles (Silva et al., 2017). Pro-inflammatory cytokines and chemokines are released as a response to these recognitions triggering an inflammatory response (Nishanth et al., 2011). Although inflammation helps us fight infections normally, prolonged or high levels of inflammation on the other hand can cause tissue damage and play a role in the onset of severe autoimmune conditions. Irregularities may arise in immune responses from the interaction of immune cells with NPs, raising the possibility of dangers to both immune system function and the general health of the being (De Matteis, 2017).

Through immunosuppression, nanoparticles cause harm to the immune system; this is generally the case with high dosages or extended exposure. Elaborating on the mechanisms that are involved in this phenomenon, due to the accumulation or survival of NPs in the circulation immune cell count may decline (Shen et al., 2021). Nanoparticles

hinder the ability of macrophages and neutrophils to take in and destroy foreign particles, which makes it harder for them to efficiently remove infections from the system (Kodali et al., 2013). Furthermore, antigen presentation which is essential for immune cells to deliver antigens to T-lymphocytes and start a specific immune response may be hindered by nanoparticles. When these factors come together, the body experiences immunosuppression and weakened immune functioning, making it more vulnerable to immunological-related conditions that worsen the scenario (Kang et al., 2017). Through several different processes, it has been learned that nanoparticles (NPs) upset the immune system's delicate balance. A potential mechanism of action for NPs is through their ability to modify the production of immune mediators, such as cytokines, chemokines, and other signaling molecules that are essential for immune control (Ernst et al., 2021). An unbalanced immunological response, marked by either excessive inflammation or immune suppression, may result from this change. NPs can directly hinder the ability of immune cells to counter threats adequately by restricting their capacity to migrate to the infected or inflammatory regions (Chen et al., 2018). NPs can disrupt the cellular signaling pathways that regulate immunological responses in general, which leads to abnormal immune suppression or activation of the system. The complex nature of NPs and their influence on the immune system is highlighted by many processes (Liu and Tang, 2020).

| NP | Type | Damage caused | System Affected | References |
|---|---|---|---|---|
| SiO2-Silica Oxide | Inorganic NP | ROS, oxidative stress, and apoptosis | Brain (Nervous System) | Shim et al., 2014; Mitchell et al., 2020 |
| CNT-Carbon nanotube | Inorganic NP | Neurotoxicity, Accumulation in subcellular compartments | Brain and Spinal Cord | Facciolà et al., 2019; Mitchell et al., 2020 |
| Dendrimers | Polymeric NP | Cell lysis | Respiratory and Immune System | Thakare et al., 2022; Madaan et al., 2014; Mitchell et al., 2020 |
| Poly(propylene imine) glycodendrimers | Polymeric NP | Damage membrane integrity | Nervous system | A Dobrovolskaia, 2017; Franiak-Pietryga et al.,2022; Mitchell et al., 2020 |
| Ag | Metal NP | Silver NPs can penetrate the neutrophils and induce ROS overproduction leading to atypical neutrophil cell death [ | Immune system | Shim et al., 2014; Mitchell et al., 2020 |
| Liposomes | Lipid-based NP | Macrophage Apoptosis by stiffening of ER | Immune system | Inglut et al., 2020; Mitchell et al., 2020 |
| Positively charged Lipid Np | Lipid-based NP | Proinflammatory response releases cytokines and interferons, hepatotoxicity | Immune system | Kedmi et al., 2010; Mitchell et al., 2020 |
| Quantum Dots | Inorganic NP | Reduction of cell viability, genetic material damage, and accumulation results in inflammation and lung injury | Respiratory system | Wu and Tang, 2014; Mitchell et al., 2020 |

**Nanoparticle Toxicity Induced in the Respiratory System**

The nose, pharynx, larynx, trachea, bronchi, and lungs which are made up of many alveoli, blood vessels, lymphatic vessels, nerves, pleura, and other tissues are among the organs collectively referred to as the respiratory system. These organs exchange gases between the human body and the outside air (Marcus, 2010). The size, shape, and surface chemistry of NPs mostly determine how much of them deposit in the respiratory system. Smaller NPs tend to accumulate in the lower respiratory tract, where they can interact with lung cells by penetrating the alveolar region (Zhang et al., 2022).

To eliminate inhaled foreign materials the respiratory system uses a special mucociliary clearance system, a defensive mechanism that involves the ciliary movement and mucus secretion in the respiratory tract. Nanoparticles (NPs) could inhibit this essential process by changing the characteristics of mucus that is being produced or interfering with ciliary activity directly. NPs have the potential to alter the mucus layer's composition or viscosity, which would hinder the effective release of trapped particles (Iyawe and Omorogiuwa, 2018). Furthermore, NPs can interfere with the cilia's ability to beat in harmony, which makes it harder for them to move mucus and trapped particles out of the airways. As a result, the respiratory system becomes prone to the build-up of inhaled particles in the respiratory tract raising the possibility of inflammation and infections (Katare et al., 2024).

A protein corona made up of adsorbed proteins is acquired by nanoparticles (NPs) upon entry into biological fluids. This corona alters the NPs' surface characteristics and then influences how they interact with lung cells in the tract (Konduru et al., 2017). When lung epithelial cells or immune cells get NPs, the protein corona may sometimes make them potentially harmful to the cells. As a result, one factor that contributes to NPs' harmful effects on the respiratory system is their changed bioavailability as a result of protein corona development (Liu et al., 2020).

The fragile epithelial layer that divides the bloodstream from the air space is known as the alveolar barrier, and it is essential for effective gas exchange in the lungs. Through a variety of methods, including direct physical harm or the inducement of enzymatic release, nanoparticles (NPs) can breach this barrier and cause the integrity of the barrier to deteriorate. As a result, this disruption makes lung dysfunction more severe, which causes fluids and inflammatory cells to flow into the airspaces (Farcal et al., 2013).

The lungs' first line of defense, alveolar macrophages, attack and eliminate foreign particles in the system. But when these macrophages are overpowered and outnumbered in a sense by nanoparticles (NPs), "frustrated phagocytosis" incomplete engulfment occurs (Brown et al., 2007). The NLRP3 inflammasome, a complex that starts a strong inflammatory response, can be activated by this incomplete process. In lung disorders such as COPD, there is a strong connection of Chronic activation of the NLRP3 inflammasome (Sharma et al., 2018). Exposure to nanoparticles (NPs) might aggravate respiratory conditions that are already there, such as COPD, asthma, or chronic lung disease CLD. NPs can increase airway sensitivity, making individuals more sensitive to allergens or irritants, and can stimulate a strong hypersensitivity response. In the respiratory system, they can also trigger inflammatory reactions that worsen these types of conditions (Inoue and Takano, 2011).

The degree of lung damage caused by NP is dependent on several factors, such as individual NP type which is Based on their surface characteristics, composition, and ability to produce reactive oxygen species (ROS), different NPs (such as metal oxides, plastic nanoparticles, and carbon nanotubes) have varying degrees of toxicity. Dose and length of exposure, longer exposure times, and higher doses greatly increase the probability and intensity of side effects in the system at cellular levels (Gill et al., 2007).

**Conclusion**

In conclusion, even though nanoparticles present fascinating prospects for improving medicine, it is critical to fully understand any potential toxicity. The negative effects of NPs on the neurological, immunological, and pulmonary systems have been discussed in this review chapter. There is a significant risk of potential injury, ranging from brain cellular balance disruption to immune system impairment and compromised respiratory system clearance processes. The effects of NP characteristics, dose, and exposure duration need to be carefully considered throughout the process of research and development. By acknowledging these challenges and concerns, and focusing on more studies and research, we can make sure that the potential benefits of nanotechnology in healthcare exceed the risks.

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Chapter 27

# Nanoparticles-based Therapeutic Approaches towards Resistant Tuberculosis with Advances in Drug Delivery Systems

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**ABSTRACT**

Despite the significant progress made in the field of medical science and therapies, tuberculosis (TB) continues to be a leading cause of mortality and socioeconomic devastation for millions of individuals globally. This issue has been a persistent concern for humanity throughout recorded history and prehistoric times. The efficacy of tuberculosis treatment depends on several factors, including patient adherence to prescribed treatment regimens, malnutrition, smoking habits, comorbid conditions such as HIV, and insufficient supervision by healthcare personnel. These factors lead to the development of resistance against antituberculosis drugs. To overcome resistance, an investigation of carrier-based drug delivery systems is underway, exploring these advanced technologies' potential. Nanoparticles and nanoparticle-conjugated drugs reduce the drug dosage and duration of therapy significantly. These formulations exhibit enhanced drug bioavailability and therapeutic efficacy, even when administered at low therapeutic doses, because they evade phagocytic clearance, mucociliary mechanisms, and hepatic metabolism, and have efficient alveolar absorption. Additionally, it has the potential to minimize the duration of chemotherapy. Further studies may be conducted to investigate their adverse effects in animal models as well as in humans.

**KEYWORDS**
Tuberculosis, Resistance, Toxicity, Nanoparticles, Bioavailability

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## INTRODUCTION

### Tuberculosis and its Significance

Tuberculosis is a highly lethal communicable illness that is attributed to the pathogenic microorganism known as *Mycobacterium tuberculosis (Mtb)* (Mitchell et al., 2007). The Nobel Prize was awarded to Robert Koch in 1882 for his significant discovery. *Mtb*, an intracellular acid-fast positive bacillus bacterium, has developed several methods to evade macrophage-induced phagocytosis (Cooper, 2009; Rohde et al., 2007). The pathogen is widely recognized as a highly thriving organism that can establish long-term persistence within its host for several decades, all while remaining asymptomatic (Stewart et al., 2003).

According to the World Health Organization (WHO) estimation, tuberculosis leads to morbidity and mortality up to a significant level on a global scale. One-third of the global population (9M new cases and 2M deaths) is infected by Mtb yearly. The remaining individuals infected with Mtb do not exhibit any symptoms (Organization, 2010). Tuberculosis is responsible for a higher number of annual fatalities compared to all other infectious diseases, making it the second leading cause of mortality among infectious diseases, following AIDS (de Lima et al., 2017). Consequently, the WHO declared tuberculosis a global health emergency in 1993 (Organization, 1993). *Mycobacterium* predominantly targets the pulmonary system; however, it can potentially affect various anatomical regions, including the circulatory, lymphatic, renal, and central nervous system, bones, and joints (Bhowmik et al., 2009). The efficacy of tuberculosis treatment is contingent upon several factors, including patient adherence to prescribed regimens, malnutrition, smoking habits, comorbid conditions such as

HIV, and insufficient supervision by healthcare personnel. One of the primary challenges associated with the present tuberculosis chemotherapy lies in the distribution of the drug within the body. When administered intravenously or orally, the drug is disseminated throughout the blood circulation, resulting in a significant proportion of substances failing to attain their intended targets. These molecules remain in the body, leading to undesirable side effects. Pharmaceutical substances exhibit a brief duration of presence in the bloodstream and are swiftly eliminated, hence imposing constraints on their efficacy (Greenblatt, 1985). To address the obstacles presented by antituberculosis medications and enhance tuberculosis therapy's success rate, developing novel tuberculosis treatments is vital to surmount these problems.

The effective management of tuberculosis in clinical settings continues to pose significant challenges. Under the strategy of directly observed therapy, a considerable proportion of regions with a high prevalence of tuberculosis are targeted with tuberculosis therapy. The WHO DOTSC program (Directly Observed Treatment, Short Course) has not achieved full success in effectively eliminating TB in the developing countries of Asia and Africa, which continue to bear a significant burden of the disease (Dye et al., 2005).

The treatment of tuberculosis (TB) is accompanied by significant challenges, mostly stemming from the extended length of treatment and the need for continuous and frequent administration of several medications. These factors contribute to a high likelihood of low adherence or noncompliance among patients receiving current therapy. The primary factor contributing to the disease reemergence and multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis development is the low compliance of patients (Chan and Iseman, 2008). Therefore, the occurrence of MDR-TB and XDR-TB in underdeveloped nations is expanding at a higher rate. The human immunodeficiency virus (HIV) infection, commonly known as AIDS, continues to play a significant role in the rising prevalence of TB. Such interaction creates favorable conditions for the growth of Mtb within a host with weakened immune function (Gladwin et al., 1998). The urgency surrounding the proliferation of MDR and XDR strains, coupled with the dearth of viable treatment options, underscores the imperative to cultivate novel and efficacious antituberculosis (TB) medications. This imperative is driven by the necessity to combat drug resistance, expedite treatment duration, and enhance patient devotion (Griffiths et al., 2010). The current study elaborates on different types of tuberculosis along with various alternative approaches toward tuberculosis treatment along with their merits and demerits.

**Type of Tuberculosis**

Tuberculosis exists in two distinct manifestations, namely latent tuberculosis (LTB) and active tuberculosis (ATB) as shown in Figure 1. In the context of LTB, the bacteria responsible for the infection persist in a dormant state within the human body. This phase has the potential to endure for an extended duration. Typically, the condition is managed by administering a single medication over nine months. In the context of active tuberculosis, the bacteria undergo multiplication and dissemination within the human body, resulting in tissue destruction (Bhowmik et al., 2009) .

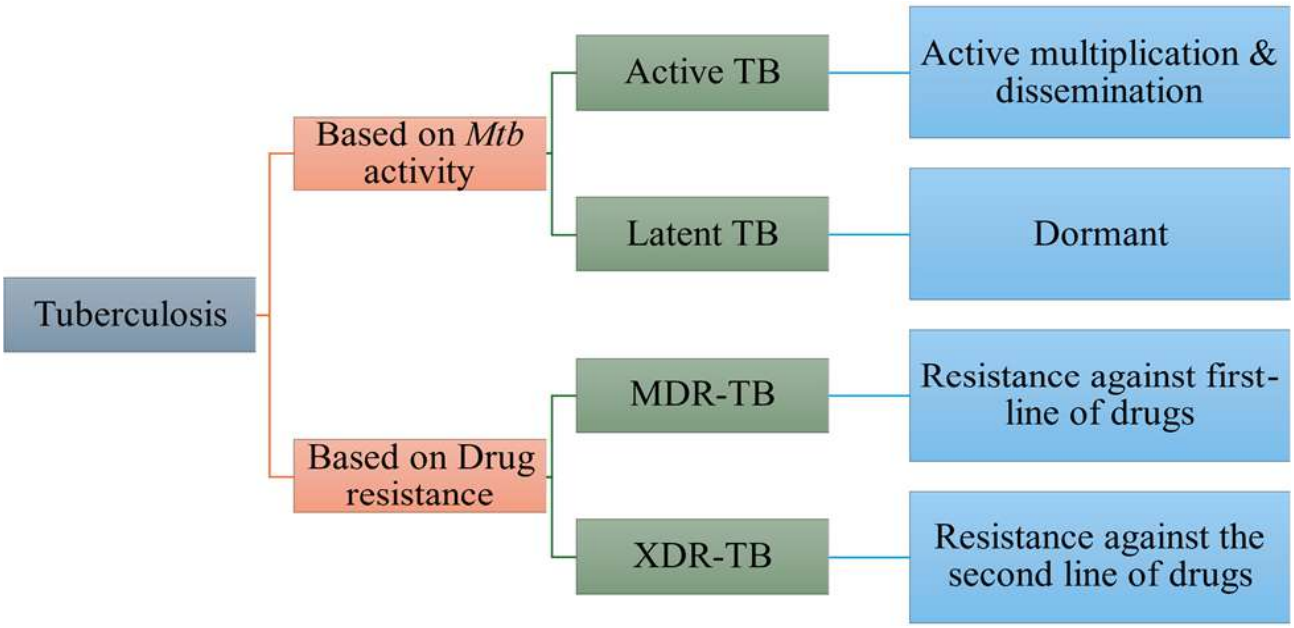


**Fig. 1:** Elaborating on different types of tuberculosis with reasoning

**MDR-TB**

MDR-TB refers to a very arduous variant of TB, characterized by its resistance to a minimum of two of the conventional anti-TB medications out of four first-line therapeutic drugs (Hammer et al., 2013). The emergence and rapid spread of MDR-TB might be attributed to insufficient or inconsistent treatment practices. Currently, the therapeutic regimen for drug-resistant tuberculosis spans nearly two years. Moreover, this treatment approach is characterized by its

intricate nature, high cost, and considerable toxicity, hence posing significant challenges to the survival and well-being of patients suffering from multidrug-resistant TB (Girma, 2015). The management of MDR-TB involves the utilization of second-line medications. Numerous secondary pharmaceutical agents possess deadly properties and induce severe adverse reactions. The treatment regimen for MDR-TB typically lasts for at least two years or maybe longer. This therapeutic approach necessitates the administration of injectables daily. These various elements present a substantial challenge to governmental bodies and healthcare centers. The lack of distinctive, uncomplicated, and cost-effective interventions for multidrug-resistant tuberculosis renders this task exceedingly challenging (Van den Boom et al., 2024).

**XDR-TB**

Comprehensively, the emergence of XDR-TB represents a significant threat to the well-being of the general population. It is a notably severe form, exhibiting resistance to fluoroquinolone antibiotics and injectable medicines of the second line (Wright et al., 2006). The issues mentioned above render the treatment of XDR-TB more challenging. In 2006, an outbreak of XDR-TB occurred in the province of KwaZulu-Natal, located in South Africa. It was observed that out of the 53 individuals who became infected with the disease, 52 of them succumbed to the illness within a short span of a few months (Van den Boom et al., 2024). According to estimates, a significant proportion of patients diagnosed with XDR-TB, namely 70%, were projected to experience mortality within one month following diagnosis. According to estimations provided by the World Health Organization (WHO), around 5% of cases of MDR-TB are classified as XDR-TB. The topic of drug regimens is significant in the field of medicine. Drug regimens refer to the prescribed schedules and dosage.

**The first line of Medications**

Typically, tuberculosis is managed with the administration of a combination therapy consisting of first-line medications, including isoniazid, rifampin, pyrazinamide, and ethambutol, over an extended duration of several months. The use of these medicines orally has demonstrated remarkable efficacy against Mycobacterium tuberculosis (Grange and Zumla, 2002; Prasad et al., 2021).

**The second line of Medications**

When *Mycobacterium tuberculosis* (Mtb) strain exhibits resistance to isoniazid and rifampin, which are considered to be highly potent first-line treatments, it transforms into a more intricate manifestation of tuberculosis referred to as MDR-TB. Aminoglycosides (kanamycin and amikacin), polypeptides (capreomycin, enviomycin, and viomycin), thioamides (prothionamide, ethionamide, and cycloserine), and fluoroquinolones (levofloxacin, ciprofloxacin, and moxifloxacin) are commonly combined as second-line drugs for the treatment of MDR-TB (Grange and Zumla, 2002; Prasad et al., 2021). According to the cited source, second-line medications exhibit higher lethality rates and are associated with increased costs compared to first-line drugs. Additionally, the duration of therapy with second-line drugs may be significantly prolonged.

**The third line of Medications**

The third-line medications utilized in the treatment of tuberculosis encompass linezolid, rifabutin, thioridazine, vitamin D, arginine, and macrolides, including thioacetazone and clarithromycin (Grange and Zumla, 2002; Prasad et al., 2021). Like other medications used to manage tuberculosis, third-line medicines exhibit limited effectiveness or lack established efficacy (Gill et al., 2022; Lalloo and Ambaram, 2010).

**Alternative Approaches**

The investigation of carrier-based drug delivery systems for treating tuberculosis is underway, exploring these advanced technologies' potential. Biodegradable polymers, liposomes, and microspheres were produced to mitigate the required dosage and treatment time (Hari et al., 2010; Minhas et al., 2020). The medications are released progressively, exhibiting a high concentration and minimal toxicity in comparison to commonly utilized drugs.

While the existing antituberculosis medications have demonstrated efficacy, it is imperative to promptly devise techniques to ensure the effective administration of these therapies. In this context, nanotechnology emerges as a promising avenue for advancing drug delivery systems that are more impactful and efficient in treating tuberculosis. Additionally, it represents a potent approach for creating and administering the next-generation tuberculosis vaccines.

In drug delivery systems, nanotechnology has the potential to enhance the admissibility of chemotherapeutic drugs, facilitate the controlled release of drugs, and ultimately improve the bioavailability of these medications. The optimal nano-particle size ranges from 50 to 200 nanometers for administration and drug localization through inhalation.

The potential advantages of nanosized particles include their ability to evade phagocytic clearance by gradual release from the lungs and their ability to be cleared by mucociliary mechanisms without eliciting immune responses. The efficient absorption of drugs via alveolar epithelium and their elevated lung bioavailability reduces drug dosage and sustains therapeutic concentrations. The concurrent administration of a lower dosage, coupled with the elimination of hepatic metabolism and prevention of GIT absorption, is anticipated to decrease systemic adverse effects and improve tolerance. The present approach to addressing resistant strains of *Mycobacterium* involves the development of novel anti-TB medicines. These antibiotics are specifically developed to achieve two objectives: to reduce the duration of the therapeutic

course and to minimize potential drug interactions with existing anti-TB medications (Dahanayake and Jayasundera, 2021; Moretton et al., 2010). In the present scenario, nanotechnology emerged to overcome the constraints posed by several aspects, namely: (a) enhanced patient compliance and adherence to treatment regimens, (b) significant technological limits, and (c) the targeting of bacterial reservoirs such as alveolar macrophages (Mazlan et al., 2021; Sosnik et al., 2010).

Exploration of nanotechnology-based therapy is increasing for its applications as a potential alternative to the conventional administration of antibiotics or other pharmaceuticals. This approach involves using drugs encapsulated within nano-particles, thereby providing controlled access to the therapeutic agents (Dahanayake and Jayasundera, 2021).

**The Role of Nanoparticles in Tuberculosis Treatment**

The delivery of nanobeads exhibits a notable characteristic of gradual, prolonged, and regulated release from a particle that undergoes biodegradation. Various animal models have been utilized in attempts to create a polymer-based antibiotic therapy against *M. tuberculosis*. Regrettably, none of these models have met the anticipated standards or successfully replicated all the characteristics of human tuberculosis (Mazlan et al., 2021).

Nanoparticles, colloidal particles with diameters below one micrometer (submicron), are employed as carriers for drug delivery purposes. In the context of therapeutic applications, medications have the potential to be covalently immobilized onto the surface of particles or they can be integrated within the particle's matrix (Kreuter, 2004; Minhas et al., 2020). Nanoparticles consist of biocompatible and biodegradable substances, including polymers of different origins *Viz;* natural origin (albumin and gelatin), synthetic nature (polylactides and poly alkyl cyanoacrylates), or solid lipids (NLCR and SLNR) (Chutoprapat et al., 2022; RH, 1995).

Nanoparticles exhibit higher cellular uptake efficiency than bigger molecules, rendering them a suitable platform for transportation and delivery. The carriers have been modified to facilitate controlled, gradual, and sustained release of drugs (Rudhrabatla et al., 2020; Wissing et al., 2004). Numerous techniques have been extensively examined in various literature reviews on synthesizing and analyzing nanoparticles.

Three key advantages are associated with the applications of drug delivery systems. Firstly, it offers high constancy and a longer period for drug release. Secondly, it has a high carrier ability, meaning several pharmaceuticals can be contained within the matrix. Lastly, this system is associated with fewer adverse effects when compared to conventional drugs. The enhancement of bioavailability through the implementation of delayed, prolonged, and controlled drug release mechanisms. The assessment of the feasibility of other modes of administration, such as oral delivery and inhalation. The reduction of adverse effects and the promotion of enhanced adherence to treatment protocols.

**The Administration of ATD Nanomedicine by Oral Route**

The uptake of nanoparticles commonly transpires through several mechanisms, including M-cell-based transcytosis, intracellular absorption and transportation facilitated by epithelial cells within the intestinal mucosa, and uptake by Peyer's patches. The stability and sustained release properties of nanoparticles enable the possibility of oral delivery of pharmaceuticals. The efficacy of orally administered anti-TB medicines has been validated by Pandey et al. (2004).

The treatment of tuberculosis involved the utilization of three prominent antitubercular medications, specifically rifampin, isoniazid, and pyrazinamide. The medicines were synthesized using solvent evaporation and double emulsion procedures and subsequently encapsulated by PLG NPs (Buya et al., 2021; Pandey et al., 2004b). Following a solitary oral injection of drug-loaded PLG NPs, drug concentrations in the plasma of mice remained over the minimum inhibitory concentration (MIC90) for 6 to 9 days. Although eliminating free medicines from plasma occurred within 12-24 hours after oral treatment (Pandey et al., 2004b), the administration of nanoparticle-bound medications (5 oral doses with an interval of 10 days) resulted in total bacterial clearance from the organs in mice infected with Mtb. The same effect was only generated after administering 46 doses of free medicines. Notably, comparable results regarding the pharmacokinetics, bio-distribution, and chemotherapeutic efficacy of the formulation were seen when the study was conducted on larger animals such as Guinea pigs (Mendoza-Muñoz et al., 2021; Pandey et al., 2005).

A Significant factor contributing to the suboptimal adherence of patients to antituberculosis drug (ATD) treatment is the dose frequency and length of chemotherapy. To improve patient adherence to ATD treatment, WHO has recommended the inclusion of ethambutol in the intense phase of chemotherapy. This medicine has been shown to increase the sputum conversion rate (Chae et al., 2021; Pandey and Khuller, 2005b). Therefore, the potential chemotherapeutic efficacy of PLG NPS-encapsulated EMB, in combination with three other encapsulated front-line ATDs, was assessed. Following administration of a singular oral therapeutic dosage of drug-loaded NPs to mice, administered drug concentrations persisted in the plasma for durations of 3 days, 6 days, and 8 days for EMB, RIF, and INH-PZA, respectively (Ahmad and Khuller, 2008; Veronica et al., 2023).

**Lipid ovate-conjugated Oral Antibody Delivery**

Polymeric nanoparticles function in the gastrointestinal tract as bio-adhesives. Including ligands, often called bio-adhesive ligands, substantially enhanced PLG nanomedicine. Mucosal ligands called lectins have been demonstrated to enhance nano-particle adherence to mucosal surfaces, boosting medication absorption and bioavailability (Gabor et al., 2004; Golshani et al., 2022). As wheat germ agglutinin receptors (WGARs) are found on epithelium of alveoli and intestine, they can transport pharmaceuticals orally and aerosolically (Abu-Dahab et al., 2001; Alavi et al., 2020). Additionally, it has

been demonstrated that wheat germ agglutinin's covalent attachment to PLG increases the effectiveness of anti-TB medications (Sharma et al., 2004; Usharani et al., 2023). Compared to uncoated PLG-NPs, animals administered oral/aerosol wheat germ agglutinin-coated PLG nanoparticles had extended plasma levels of 6–7 days for RIF and 13–14 days for INH and PYZ. Complete bacterial clearance was achieved with three oral dosages of these lectin-coated nanoparticles (LC-NPs) after every fourteen days. For fifteen days, the liver, spleen, and lungs contained all three medications (Sharma et al., 2004; Usharani et al., 2023). Because of its low immunogenicity, it is also widely used in medication delivery(Clark et al., 2000; Surwase et al., 2022).

Lectins may enhance the particles' prolonged adhesion to the intestine, allowing for augmentation in the absorption time and concentration gradient between the serosal and luminal sides of the membrane, which accounts for the drugs' long-lasting circulation when encapsulated (Horvath et al., 2012).

**The Administration of ATD Nanomedicine by Intraperitoneal Route**

The most common type of tuberculosis is pulmonary TB, and the respiratory route offers a special way to deliver ATDs straight to the lungs. Direct drug administration to the lungs holds promise for lowering systemic toxicity and achieving greater drug concentration at the primary site of infection. Improved mucosal adhesion, particle delivery, and net drug delivery to the lungs are features of inhalable NPs (Pandey and Khuller, 2005b).

**The Administration of ATD Nanomedicine by Intravenous Route**

There are three injectable medication delivery methods. Among these, administering medication intravenously boosts bioavailability and makes all drug molecules instantly available (Pandey and Ahmad, 2011). A novel and potentially effective treatment for mycobacterial infections is clofazimine. However, due to its decreased solubility, this medication was restricted. A new strategy was used to solve this issue. A nanosuspension of clofazimine with a particle size of 385 nm was created. After receiving this formulation intravenously, mice infected with *M. avium* showed a marked decrease in the number of colony-forming units in their lungs, spleen, and liver (Peters et al., 2000; Stadler et al., 2023). It was discovered that the effects of clofazimine nanocrystalline were comparable to those of the liposomal formulation that served as the study's control (Rabinow, 2004).

**Liposomes-based Drug Delivery Systems**

Liposomes are tiny, closed vesicles with an aqueous portion encased in a phospholipid bilayer (Gregoriadis et al., 1974). Due to their unique capacity to encapsulate hydrophilic and hydrophobic medicines, they have been extensively researched as a viable drug delivery platform for bioactive molecules. In animal models, liposomes have been tested to continuously deliver anti-TB medications to analyze therapeutic efficacy (Mazlan et al., 2021; Sosnik et al., 2010). A select few medications, such as doxorubicin for breast cancer and amphotericin B for fungal infections, have been licensed for use in humans (Zhang et al., 2008).

After administration, phagocytic cells quickly identify these carriers and remove them from the bloodstream. Liposomes are typically PEGylated to prolong circulation durations and prevent removal or clearance. A well-researched study found that gentamicin could be incorporated into liposomes and assessed the antibacterial activity in a mouse model compared to other drugs (Klemens et al., 1990). The encapsulated medication significantly decreased the bacterial burden in the liver and spleen; nevertheless, sterilization was not observed. Different liposome-entrapped second-line antibiotics produced similar results (de Paula et al., 2020; Leitzke et al., 1998).

There are two key benefits of employing liposomes when alveolar macrophages vigorously consume them: they effectively combat intracellular infections (Gonzalez Gomez and Hosseinidoust, 2020; Salem et al., 2005). Liposomes must be given intravenously or by respiratory means since they are vulnerable to intestinal lipases. PEG can be added to liposomal formulations to decrease nonspecific absorption by the liver and spleen's mononuclear phagocyte system (MPS) (Deol and Khuller, 1997; Shrivastava et al., 2023).

After giving liposome-encapsulated medications (isoniazid or rifampicin alone) twice a week for six weeks to Mtb-infected mice, the drugs were shown to be more effective in curing mycobacterial infection than the free drugs. When frontline drugs were co-administered with liposomes, the dose was successfully lowered to one weekly dosage for six weeks. Histopathological analysis revealed no evidence of hepatotoxicity, corroborated by serum albumin, alanine aminotransferase, and alkaline phosphatase levels (Pandey et al., 2004a; Shrivastava et al., 2023).

**Microemulsions as Possible Vehicles for Anti-TB Drug Delivery**

Hoar and Schulman first proposed the idea of microemulsions in 1943 (Hoar and Schulman, 1943). "Micro-emulsion is a system of water, oil, and an amphiphile (surfactant and co-surfactant), which is a single optically isotropic and thermodynamically stable liquid solution," as defined accurately by Danielsson and Lindman (Danielson and Lindmann, 1981).

Because of their high solubility, ease of production, high absorption rates, and thermal stability, microemulsions have recently garnered a lot of attention for the creation and design of novel drug delivery systems (Sarciaux et al., 1995). They contribute to decreased toxicity (Bhargava et al., 1987), resistance against enzymatic hydrolysis (Pouton, 1997), and improved drug bioavailability. Microemulsions consist of minuscule droplets that exhibit thermodynamic stability. Since the

droplet diameter in stable microemulsions typically falls between 10 and 100 nm, these systems are also called nano-emulsions (Eccleston, 1992). Microemulsions are widely used in colloidal drug delivery systems to achieve controlled release and medication targeting. Based on composition, there are three types of microemulsions: (a) water-in-oil (w/o), (b) bi-continuous, and (c) oil-in-water (o/w) (Lawrence and Rees, 2000). Talegaonkar et al. investigated a method of microemulsion concentration for oral drug administration (Talegaonkar et al., 2008). This study proved that RIF worked well and that it might be able to solve the issue because reducing the dosage reduces toxicity.

**Solid Nanoparticles-based Anti-TB Medication Delivery System**

Oral administration is prohibited due to liposomal breakdown by intestinal lipases. In addition to having many advantages over liposomes and polymeric nanoparticles, the SLNs are nanocrystalline nanosuspensions in water that may be taken orally. The production method also uses small amounts of organic solvents. The medication in SLN is primarily encapsulated in a solid lipid matrix to generate lipid nanoparticles (Tanwar et al., 2014). Because they are derived from physiological lipids, SLNs have higher tolerability, scaling-up feasibility, the capacity to integrate hydrophobic or hydrophilic medicines, and increased stability of medications when incorporated (Kaur and Singh, 2014).

SLNs have improved encapsulation efficiency and longer/higher stability, and they need less organic solvent than liposomes and polymeric nanoparticles. Mice receiving a single oral dose of ATD-loaded SLNs showed drug recognition in their plasma starting three hours later and continuing until day eight (Pandey and Khuller, 2005b). The least inhibitory concentration (MIC90) was reached or exceeded by plasma drug concentrations at each time peak, while free pharmaceuticals were eliminated from the bloodstream 12 hours after oral treatment (Kaur and Singh, 2014). In Guinea pigs, the formulation's potential for chemotherapy was predicted through the respiratory route. It was shown that an extended medication release remained stable in organs for 7 days and in plasma for 5 days. In place of 46 traditional dosages, seven formulation doses completely cleared the bacilli (Pandey and Khuller, 2005b). ATD-loaded SLN was assessed orally, yielding better outcomes because the drug's level could be maintained in organs for nine to ten days and in plasma for eight days. In mice infected with M. tuberculosis H37Rv, five oral doses of drug-loaded SLNs administered every tenth day were sufficient to achieve complete sterilization in the lungs/spleen; in contrast, the administration of 46 oral doses of the free drug was required to accomplish the same effect. An antitubercular medication based on SLN significantly decreased the recurrence rate at dose and increased bioavailability (Pandey et al., 2005).

**The Niosomes-based Drug Delivery System for Tuberculosis Treatment**

Niosomes are liposomes that exhibit thermodynamic stability and share similarities with colloidal particles. Self-assembling nonionic surfactants and a hydrating combination of cholesterol in an aqueous medium generate them. The niosomal bilayer system comprises nonionic surface-active molecules with a single chain without charge. In contrast, liposomal structures contain phospholipids with double chains, which can be either neutral or charged. Niosomes are characterized by their nanometric dimensions, rendering them tiny in size. The particle size spans from 10 nanometers to 100 nanometers.

Niosomes have emerged as highly promising carriers for targeted drug delivery systems, employing several strategies for drug targeting. Using niosomes as a drug delivery system with regulated and sustained release properties has demonstrated significant potential in various applications and therapeutic approaches. This has resulted in the enhancement of bioavailability and the ability to maintain a therapeutic impact for an extended duration (Kazi et al., 2010).

Karki et al. (year) developed a niosomal drug delivery system for antitubercular drugs, specifically isoniazid. This method shows promising promise for creating a low-dose, efficient, and successful treatment in combating tuberculosis (Karki et al., page number). Niosomes have the potential to serve as oral controlled release devices as well. As an illustration, hydrophilic antitubercular medicines like isoniazid and pyrazinamide, which can be taken orally, exhibited prolonged drug release from the tyloxapol niosome membrane (Mehta and Jindal, 2013).

**The Alginate-Based Drug Delivery System for Tuberculosis Treatment**

Guluronic acid and mannuronic acid are the constituent monomers of alginic acid, which is a naturally occurring copolymer. The US Food and Drug Administration has authorized the oral administration of alginate as a supportive treatment for reflux esophagitis. Alginate is a biopolymer that occurs naturally and has been increasingly utilized in several fields. In their study, Gonza'lez-Rodr'ıguez and con-workers successfully synthesized alginate nanoparticles loaded with ATD using ionotropic gelation. The encapsulation effectiveness of the nano-particles was found to be relatively high, with rifampicin ranging between 80% and 90%, isoniazid and pyrazinamide between 70% and 90%, and ethambutol between 88% and 95% (González-Rodrıguez et al., 2002). Hence, these findings support the superiority of alginate nanoparticles compared to other poly (lactic-co-glycolic acid) nanoparticles (Ahmad and Khuller, 2008).

In a separate investigation, significant findings were documented by introducing chitosan into the alginate solution. Adding chitosan to alginate enhanced pharmacokinetics and therapeutic efficacy, resulting in a 50% reduction in medication dosage.

According to the source cited as (Pandey and Khuller, 2005a), An additional benefit of alginate nanoparticles is their ability to accommodate higher medication loadings while minimizing polymer consumption.

Nevertheless, there is a growing concern regarding the potential adverse consequences of the organic solvents

employed for polymer suspension. Alginate, a water-soluble polymer that does not include organic solvents, has been successfully utilized to treat *M. tuberculosis* in mice and Guinea pigs through oral and aerosol delivery (Ahmad et al., 2005). Due to its hydrophilic nature, alginate exhibits resistance to rapid elimination by the mononuclear phagocyte system upon intravenous injection. This study presents findings indicating a prolonged half-life of circulation. The method mentioned above has clear advantages compared to conventional neutral polymers or liposomes.

The researchers have successfully created alginate nanoparticles (NPs) that have been stabilized using poly(L-lysine)-chitosan. These nanoparticles can encapsulate antituberculosis drugs (RIF, INH, PZA, and EMB). The drug-to-polymer ratio was maintained at a value of 7.5: 1, deemed superior to the ratio of 1: 1 observed in PLG NPs. Therefore, the alginate formulation demonstrates a higher drug loading capacity while utilizing a smaller amount of polymer. According to the literature, the formulation exhibits a sustained release profile in plasma for 7 to 11 days and in organs for 15 days after administering a solitary oral dose (Satyanarayana and Srivastava, 2007).

**Inferences and Prospects for the Future**

In both emerging and underdeveloped nations, infectious diseases are prominent health concerns. In developing nations, a significant proportion of the impoverished population lacks adequate access to essential medications, with nearly one-third affected. The prevalence of tuberculosis significantly affects developing nations. In this circumstance, the management of tuberculosis (TB) is hindered by the presence of drug-resistant strains of TB, which poses a significant obstacle. This study aims to address this issue by developing and synthesizing improved pharmaceutical agents that can effectively decrease the duration of therapy, mitigate drug toxicity, and enhance the bioavailability of medications. To date, except for a limited number of medications, such as quinolones and rifampicin, there have been no advancements in ATD therapy.

Furthermore, developing an efficacious tuberculosis vaccine has proven to be as challenging. The objective is to identify a resolution for eliminating the transmission of the causal organism. However, this task is challenging, complex, and intricate due to the diagnostic challenges, the presence of multidrug resistance, and the low adherence of patients to treatment. Several novel prospective antituberculosis medications are now under development. However, these drugs are accompanied by significant limitations, including insufficient research, high financial burden, challenges in targeting multidrug-resistant and latent bacilli, as well as potential drug toxicity. Several factors motivate the pursuit of novel and distinctive alternative therapeutic medications. Additionally, it has the potential to minimize the duration of chemotherapy. All these factors are crucial in significantly reducing the cost of treatment, minimizing interactions with anti-HIV medications, and enhancing the management of multidrug-resistant tuberculosis (MDR-TB) and latent tuberculosis (TB). Based on the evidence, it may be inferred that nanoparticles possess significant promise in tuberculosis treatment. The primary benefits of DOTS, including extended shelf life, reduced dose frequency, and improved drug absorption, contribute to its increased convenience and cost-effectiveness. Using oral and inhalation routes for medication administration further enhances nanoparticle feasibility.

In conclusion, the efficacy of this technology will be contingent upon the toxicological characteristics associated with comprehending the destiny of polymeric constituents within nanocarriers within the human body. According to this viewpoint, drug delivery systems composed of natural polymers such as alginate and chitosan present a promising perspective. However, further investigations are necessary to explore their potential fully. It is imperative to conduct suitable clinical trials to determine the potential efficacy of a nanoparticle-based drug delivery system as a promising alternative for enhancing patient adherence to tuberculosis treatment.

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Chapter 28

# Role of Cerium Oxide Nanoparticles in Medical Applications

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**ABSTRACT**

The biomedical applications of cerium oxide nanoparticles are highly appreciable due to their biocompatibility, stability, antioxidant properties, antibacterial properties, and redox potential. Their redox potential makes them capable of removing oxidative stress from the cell. The SOD and catalytic mimetic proprieties make them excellent scavengers of ROS and RNS due to the cerium oxide ion $Ce^{3+}/Ce^{4+}$. The potential to scavenge ROS inflammation modulation witnesses their role as a potential therapeutic agent against various biological disorders of neurological, cardiovascular, and cancer types. In addition, Cerium oxide nanoparticles can be employed to update various diagnostic approaches like MRI, CT, and PET scans and be used in advancing diagnostic tools. This article discusses the advancement in the field of biomedicines with the exploration of nanoceria will have significant impacts on the diagnosis, management, and treatment of diseases providing better health facilities and improved disease monitoring tools and techniques to the health sector, thereby improving the quality of life and health style in favor of patients. The dopping of nanoceria with different substrates and chemicals and the formation of nanocomposite will help improve the detection, diagnosis, and treatment of disorders of potential concern.

**KEYWORDS**
Cerium Oxide, Nanoparticles, Alternatives, Medical applications

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## INTRODUCTION

Nanotechnology has positively impacted the lives of living beings as nanomedicines by having extensive scope in the various fields of medicine from the atomic level to the supramolecular level (Bobokulova, 2024; Haleem et al., 2023). Nanoparticles are nano-sized particles having a diameter of one-thousandth of the thickness of a hair; comprised of atoms and molecules. Nanoparticles have a vast role in the diagnostic field, targeted drug delivery to the site and treatment as in the case of various chronic and infectious diseases causing high morbidity and mortality like HIV, cancer, cardiovascular diseases, and many others. The lack of safe and ineffective drug delivery to the target site makes it ineffective to treat infectious diseases (IDs), so the safe and effective transport of drugs in their nanocarriers to the target site is ensured to aid available treatment modalities and overcome drug toxicities (Kirtane et al., 2021; Malik et al., 2023).

This field emphasizes works related to the construction and use of materials and structures that demonstrate specific behavior at small dimensions, such as less than 100nm in one or more dimensions. The potential of nanotechnology is evident in the field of medicine for drug delivery and diagnostics, as an imaging tool, bio-sensing, medical implant, anti-inflammatory, antioxidant, anticancer, and antimicrobial agent, and for the treatment of various ailments in current nanomedicine (Riehemann et al., 2009).

Cerium oxide Nanoparticles (CeNPs) have a promising role as inorganic metal oxide nanomaterial in the biomedical field because of their unique physical and chemical properties, biological properties, antioxidant, catalytic properties, durable chemical stability, large surface area, size and biocompatibility. Cerium oxide nanoparticles exhibit two states; low oxidation state of $Ce^{3+}$ and a high oxidation state $Ce^{4+}$ and thus forming $CeO_2$, redox catalysts, thereby possessing antioxidant properties. This characteristic helps cerium oxide nano-particles eliminate ROS by reducing oxidative stress on

cells and tissues for potential medical uses. Furthermore, the fluorite crystal property of $CeO_2$ brings oxygen vacancies in their crystal lattice structure due to the transition between two trivalent ($Ce^{3+}$) and tetravalent ($Ce^{4+}$) states, thereby increasing its redox properties of MONPs. The Fluorite crystalline structure of the cerium oxide provides the high reactive surface area for the neutralization of free oxygen radicals. (Das et al., 2013; Singh et al., 2020).

The surface area, particle size, and shape of $CeO_2$ can be precisely managed through several processes that impact their reactive properties and interaction among biological system (Heckert et al., 2008).

The redox properties of $CeO_2$ are the contributors to their therapeutic applications in the biomedical field. The cycling between two oxidation states trivalent/tetravalent makes them antioxidant by scavenging ROS while the redox cycling activity is supported by the presence of oxygen vacancies that are created by the absence of one or more oxygen atoms in their octane. Oxygen vacancies are the core site of high catalytic activity because of its fluorite crystal lattice. These properties make cells adaptable to oxidative damage and remove stress (Singh et al., 2011; Wu and Ta, 2021).

$CeO_2$ possesses a high level of biocompatibility and stability in its biological environment. Studies have proved that $CeO_2$ are non-invasive and non-toxic metallic oxide nanoparticles. These are helpful even at their lowest therapeutic concentrations and remain stable in physiological conditions, thereby maintaining redox activity over extended periods. Their stability is attributed to the strong Ce-O bonds and the ability to form stable colloidal suspensions in aqueous solutions (Karakoti et al., 2008).

Cerium oxide nanoparticles have been reported to adopt a great therapeutic potential of biomedical application because of its unique switching between two transition states, $Ce^{3+}/Ce^{4+}$ which is the strong base of its remarkable anti-oxidant, anti-inflammatory, anti-bacterial activity and biological mimetic activity properties (Kalaycıoğlu et al., 2020; Wu and Ta, 2021).

**Mechanisms of Action**

**Antioxidant Properties, ROS Scavenging Properties, SOD Mimetic Activity and Catalytic Activity**

There are enormous number of antioxidants available for use in medicine that inhibit the noxious effect of free radicals. Among those, inorganic antioxidants such as cerium oxide nanoparticles are of great interest because of their promising SOD mimetic activity, catalytic activity, free radical scavenging properties and anti-inflammatory properties (Naganuma, 2017).

The outstanding antioxidant properties of cerium oxide nanoparticles make them excellent candidate for the scavenging of super oxide anion ($O^{-}$**)** for SOD mimetic activity and reactive nitrogen specie (RNS) present in biological milieus. Free radicals are harmful by products of cellular metabolism as it causes significant oxidative damage to the biomolecules like lipids, proteins and DNA by causing mutation in them ending up enormous diseases, so the scavenging of free radicals is important (Gallucci et al., 2021). The interchangeable surface oxidation state of the cerium oxide nanoparticles is correlated with its redox properties that depends on the surface ratio of $Ce^{3+}/Ce^{4+}$ atoms and the efficiency of their transition between two states (Turin-Moleavin et al., 2019). This redox switch between two oxidation states is due to the deficiency of oxygen atom in its latic structure, $Ce^{3+}$ and $Ce^{4+}$ (Baldim et al., 2018). Estevez et al. (2011) studied brain ischemic slice model for the study of SOD mimetic activity that reported the reduction of SOD when introduced with nanoceria. Another study carried out by Ganesana et al. (2012) in ex-vivo system indicated the reduction of SOD mimetic activity with the use of 5 molar mole of cerium oxide nanoparticles that is equal to 580 units of SOD activity, hence showing an outstanding level of catalytic activity in this model. So, this indicates that nanoceria exhibit SOD mimetic activity through different assay and also shows as this in vivo system.

$CeO_2$ nano particles causes the neutralization of ROS through redox reaction converting harmful radicals to nontoxic materials thereby savaging from oxidative cellular damage. Chen et al. (2020); Singh et al. (2020) have reported that nanoparticles in epithelial cells hinder $H_2O_2$ and the overproduction of ROS decreases the cell death thereby protecting from cardiovascular diseases. Gallucci et al. (2021) has stated that functionalized nanoparticles play their part in biomedicine by balancing ROS level in cells where oxidative stress was induced within the cell. In this way, they play a crucial part in the field of biomedicines.

Korsvik et al. (2007) conducted first study on SOD mimetic activity in 2007. As per reports, the SOD mimetic activity and the catalytic activity of the cerium oxide nanoparticles is correlated with the concentration of surface cerium atom on cerium molecules. Higher the surface concentration of $Ce^{3+}$ molecule, the higher is the SOD mimetic activity while higher the surface concentration of $Ce^{4+}$, higher the catalytic mimetic activity of the cerium oxide nanoparticles (Walkey et al., 2015). Corsi et al. (2023) reported that SOD possess high mimetic activity when $Ce^{3+}$ is oxidized to $Ce^{4+}$ while high catalytic activity is observed with reduction of $Ce^{4+}$ to $Ce^{3+}$. A study carried out by Heckman et al. (2013) reported the in vivo SOD mimetic and catalytic activity by the cerium oxide nanoparticles.

CeNPs also exhibit anti-inflammatory properties by modulating the immune response and inhibiting the production of pro-inflammatory cytokines. This is achieved through their redox activity, which reduces oxidative stress and, consequently, inflammation. The anti-inflammatory effects of CeNPs have been demonstrated in various in vitro and in vivo models, showing high potential for treating inflammatory diseases such as arthritis and inflammatory bowel disease (Naganuma, 2017). Walkey et al. (2015) studied the effect of cerium oxide nanoparticles on the proliferation of cytokines in the retina of v*ldlr* $^{-/-}$ mice through PCR assay that inhibit the growth of pro-inflammatory cytokines and other growth factors like Tslp, Il3, Il7, Fgf1, Fgf2, Egf and Lep on single intravietral nanoceria injection at p28. This study proves that inflammatory properties of nanoceria particles are the best to treat regenerative diseases through different assay.

**Reactive Oxygen Species (ROS) Scavenging**

These are the core target of the anti-inflammatory therapies. The scavenging of the nanoceria involves a series of oxidation-reduction steps that convert super oxide free radicals, $H_2O_2$ and hydroxyl radicals to oxygen and water (Caputo et al., 2015). ROS is produced in response to the cellular metabolism in the cells as a byproduct of respiration in mitochondria. At low physiological concentrations,, it participates in cells signaling pathways in a very precise manner but at higher concentrations, it exert toxic effect on cells, tissues and DNA etc thereby leading to apoptosis and various pathological disorders. Aerobic organism being equipped with few endogenous antioxidant and various other enzymes including SOD dismutases enzymes mutates the oxygen free radical $O^-$ to $H_2O_2$ and then reduces $H_2O_2$ back to water and oxygen, thereby causing the scavenging of ROS from the cell as given in figure 1. The redox switching property is the excellent factor for scavenging because of the presence of oxygen vacancies that facilitates ROS interaction. This continuous act of redox switching allows the maintenance of antioxidant activity over a prolonged period of time (Celardo et al., 2011).

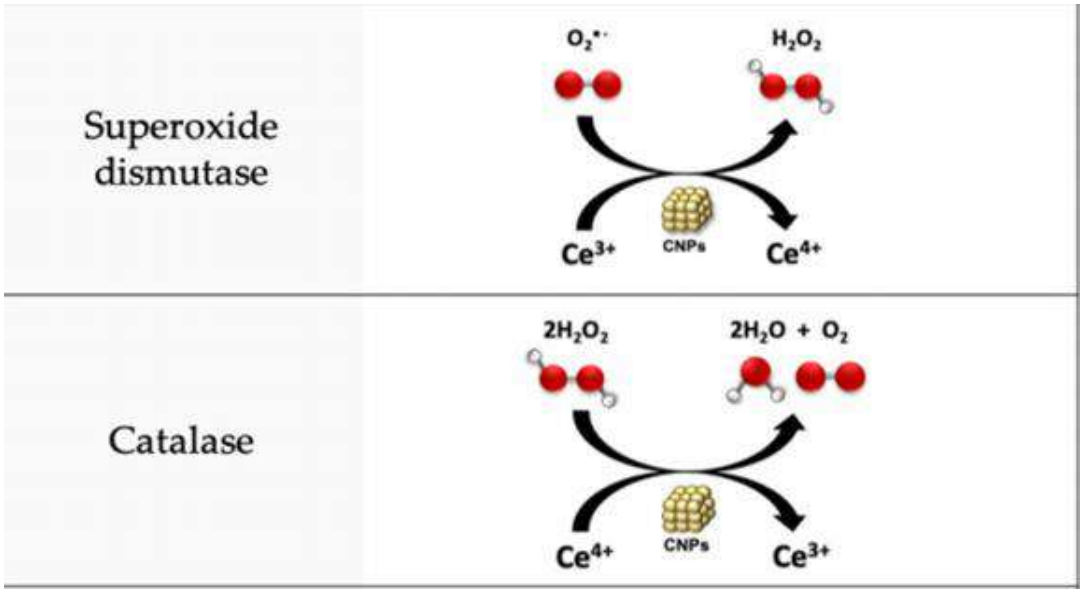


**Fig. 1:** I- The SOD activity: The reduction of SOD to $H_2O_2$ and the reduction of $Ce^{3+}$ to $Ce^{4+}$ by Cerium oxide nanoparticles II- Catalytic activity: The oxidation of Ce4+ to Ce3+ and catalysis of hydrogen peroxide back to oxygen and water.

**Therapeutic Applications**

**Neurological Disorders**

**Neuroprotection in Stroke**

CeNPs have shown promising role in protecting neurons from ischemic damage caused by stroke. Stroke results in the overproduction of ROS, leading to neuronal death and functional impairments. Studies have demonstrated that CeNPs can reduce infarct size, improve neurological outcomes, and enhance recovery by scavenging ROS and reducing oxidative stress in the brain (Kim et al., 2012).

**Treatment of Neurodegenerative Diseases**

It is well established that neurodegenerative diseases are the product of prolonged oxidative stress, thus paving the pathway towards the pathogenicity of severe neurological degeneration of CNS leading to severe mental deterioration and progressive dementia such as Alzheimer's disease (Lefevre-Arbogast et al., 2024). The core pathogenic factor of these disorders is the accumulation of amyloid-beta plaques leading to the mitochondrial dysfunction which cause oxidative stress by overproduction of ROS free radicals in the nerve cells that ultimately ends up damaging the neurons (Bartley et al., 2012). Cerium oxide nanoparticles counter the ROS overproduction by modulating anti-inflammatory response and reducing ROS level. A number of preclinical studies carried on Alzheimer's diseases and Parkinson's diseases using animal models are the witness of improving cognitive function and reduced neuroinflammation, therefore cerium oxide nanoparticles can be used in order to fix neurodegenerative disorders (Heckman et al., 2013). Nelson et al. (2016) reported that in vivo studies conducted by Kwon et al. (2016) explored the neurodegenerative therapeutic effect of cerium oxide nanoparticles in 5XFAD transgenic Alzheimer's disease mouse model by utilizing triphenylphosphonium (TTP) conjugated cerium oxide nanoparticles to treat mitochondria that not only reduced oxidative stress but also inhibited neuronal cell death. In addition to this, CNPs were also involved to prevent the activation of pro-inflammatory phenotype, the microglial cells responsible for the progression of AD. This ability of cerium oxide nanoparticles to mitigate inflammatory response by microglial cells was due to redox switch of CNPs between trivalent and tetravalent states as reported by Kwon et al. (2016). In another study conducted by Kim et al. (2024); Ranjbar et al. (2018) exposed cerium oxide nanoparticles with the brain

tissues of rats in order to explore its anti oxidative potential. CNPs were reported to lower its thiol content and institute caspase-3- activity that prevented DNA damage and lipid per oxidation by expressing its antioxidant activity, hence proved its role as a neuroprotective agent.

**Cardiovascular Diseases**

**Mitigation of Myocardial Ischemia**

The reduced blood flow to the heart is called myocardial ischemia which leads to oxidative stress to the heart. The oxidative stress thus induced can be mitigated with use of cerium oxide nanoparticles owing to their ROS scavenging properties, thereby mitigating the effects of ischemia re-perfusion.

Animal studies carried out by Niu et al. (2007) have shown that nanoceria have been known to improve cardiac function and help reduce the size of infarct following ischemic events, highlighting their potential as cardio-protective agents.

An investigated by Chen et al. (2013), it was reported that the effect of nanoparticles on epithelial cells which cause hindrance in the production of $H_2O_2$ can lead to the reduction of reactive oxygen species (ROS), ultimately decreasing cell death, thereby providing protection from cardiovascular diseases

**Prevention of Atherosclerosis**

Atherosclerosis is a cardiovascular associated endothelial tissue dysfunction characterized by the build up of plaque in the walls of the arteries induced by ROS induced severe oxidative stress. Preclinical studies have demonstrated that CeNPs can reduce the formation of atherosclerotic plaques and improve vascular function by alleviating ROS driven oxidative stress as that reported by Turin-Moleavin et al. (2019).

In one of the study conducted by Gao et al. (2023) reported that researchers observed the strong anti-atherosclerotic effect of gadolinium doping of cerium oxide nanoparticles as reactive oxygen scavenger which increased the $Ce^{3+}$ concentration on the cell surface. In addition to this, in vitro and in vivo studies of $Gd/CeO_2$ also witnessed the efficient scavenges of harmful ROS at cellular and histological level and lipid accumulation in macrophages thereby reducing pro-inflammatory factors leading to the exacerbation of atherosclerosis, thus serve as the potential diagnostic and therapeutic biomedicine for ROS induced atherosclerosis.

**Cancer Therapy**

**Radio-protection and Radio-sensitization**

Cancer patients are usually treated with minimal effective techniques such as surgery, chemotherapy and radiotherapy techniques. Such techniques provides minimal efficiency with the destruction of normal tissues along with cancerous cells (Yang et al., 2019). In contrast, cerium oxide nanoparticles are the excellent proviosioner of radio protective and radio sensitizing effect in cancer therapy that is apart from protecting normal tissues from tissue damage, seem to enhance the sensitivity of tumor cells towards radiations (McDonagh et al., 2023; Zal et al., 2018). $CeO_2$ nanoparticles provides cytoprotective effect to the healthy cells while cytopathic effect to the cancerous cells by inducing ROS species which in turn will activate RNS which interrupts the intracellular potential of cells by providing anti cancerous role (Girigoswami et al., 2023). This goal is achieved by the mechanism of radiation induced ROS scavenging while the radio-sensitization is achieved by the accumulation of cerium oxide nanoparticles in cancerous cells. This critical role elevates the efficiency of radiotherapeutic effect of CeNps with minimal side effects (Babu, 2023; Girigoswami et al., 2023; Mozafari et al., 2022). The first in vivo anti tumor exploration of cerium oxide nanoparticles as drug delivery and therapeutic purpose was first done by Alili et al. (2013) who injected A375 melanoma cell induced tumor mice with 5nm of dextran coated cerium oxide nanoparticles intraperitoneally on alternate days for about a month that showed a significant reduction in weight and volume of the tumor and CD31 as well, an endothelial cell marker that shows administered CNPs are involved to hinder the vascular endothelial cell migration.

Another study conducted on ovarian cancer in A2780 ovarian carcinoma mouse model by Giri et al. (2013) reported the significant reduction in the tumor mass and weight when injected with 3-5nm at the dosage of 0.1mg/kg BW cerium oxide nanoparticles. Furthermore, the increases accumulation of cerium oxide nanoparticles in the tumors also lead to the attenuation of angiogenesis.

**Drug Delivery Systems**

CeNPs can be functionalized with therapeutic agents for targeted drug delivery, improving the efficacy and reducing the side effects of chemotherapy. Their high surface area and ability to bind various molecules make CeNPs excellent carriers for anticancer drugs. Studies have shown that CeNPs can deliver drugs directly to tumor sites, enhancing drug accumulation in cancer cells and improving therapeutic outcomes (Xu et al., 2013).

According to the reports punished by Hu et al. (2023), researchers explored the anticancer therapeutic effect of the mitochondria targeted combined therapy system formulated with CPT and $MnO_2/CeO_2$ mediated fenton like reaction in order to elevate the mitochondrial ROS properties against cancer. CPT drug is responsible for the efficient endogenous acceleration ROS in mitochondria was used to modify TPP molecules for its excellent accumulation in $CPT@MNO_2CeO_2$-CD-TPP in mitochondria. The presence of over expressed GSH degraded the MnO2 while the CPT drug was successfully

loaded into mitochondria, thereby increasing its the ROS level along with CDT therapy, In this way it presented its excellent anti-tumor activity as drug delivery system by ROS regulation system.

**Ophthalmology**

**Treatment of Age-related Macular Degeneration**

The development of inflammation associated ocular pathologies are the result of oxidative stress such as age related macular degeneration and neovascular disease. Age related macular degeneration is associated with the loss of vision as the retina is the main site of oxidative stress due to direct light exposure that causes the degradation of photo-receptors (Ung et al., 2017). Cerium oxide nanoparticles are the most fitting candidates for removing oxidative stress and reducing inflammation from the retina of the eye. Preclinical studies conducted on different animal models are the evidence of improved retinal function by the reduction of retinal degeneration by removing oxidative stress from the retinal cells (Patel et al., 2023).

In a recent in vitro and in vivo investigation precursed by Badia et al. (2023) tested CNPs formulation prepared for topical administration against age related macular degeneration disease of retina of the eye. In vitro investigations confirmed its biocompatibility and antioxidant effect in retinal ARPE19 cells while in vivo studies conducted on DKOrd8 mouse model confirmed the effectiveness of topical formulation of cerium oxide nanoparticles by lowering the retinal inflammation and normalizing the altered retinal transcriptome of the mouse model under consideration to, thereby successfully eliminating the retinal defect.

**Protection against Neovascular Disease**

The avascularity of the cornea of the eye is associated with the pathology of the corneal vascular disease. This abnormality rises from the disruption of delicate physiological balance among the angiogenic and anti-angiogenic factors lead to corneal neovacularization. This balance is maintained by corneal limbal stem cells (Liu et al., 2018; Wen et al., 2024).

Zheng et al. (2019) explored the in vitro and in vivo cytoprotective potential of cerium oxide nanoparticles in case of neovascular defect of the cornea of the eye. The in vitro investigations of antioxidant and anti-inflammatory properties of CeNPs in human corneal epithelial cells and murine RAW264 and the in vivo investigations in a rat model associated with corneal inflammation were found to be associated with the reduction in the level of TNF-α, a pro-inflammatory cytokine, hence deceasing vascularization and opacification through down regulation of CNPS mediated ROS supression of NF- κB.

**Wound Healing and Tissue Regeneration**

**Antimicrobial Effects and Anti-inflammatory Effects**

Skin wound healing process is a complex and dynamic process and having a great socioeconomic burden to patients because of the re-occurrence of acute and chronic wounds. Cerium oxide nanoparticles possess the excellent activity in wound healing by wound closure, reducing scarring, mitigating the inflammation and enhancing antibacterial activity thereby preventing from further infections because of their unique properties (Chen et al., 2024; Thakur et al., 2019). Their anti-inflammatory properties reduce local inflammation, while their antimicrobial activity prevents bacterial colonization of wounds. Studies have demonstrated that CeNPs can accelerate the healing process and improve the quality of regenerated tissue in various wound models (Hirst et al., 2009).

$CeO_2$ express antibacterial activity against wound healing by inhibiting the growth of microbes. The positively charged cerium oxide nanoparticles when adsorb to the negatively charged cell membrane of the microbes which help them held on their surface that leads to stops the permeability of the cell membrane their by disturbing the ion pump of the microbial cell, thereby hindering its growth (Cheng et al., 2021). $CeO_2$ induces ROS in the cell causing the damage to cells DNA, proteins and other metabolites, thereby causing cell death (Chen et al., 2018).

In s study conducted by Kamalipooya et al. (2024) on antibacterial activity of wound healing against *S. aureus* found that the use of 0.1% cerium oxide nanoparticle showed high antibacterial performance. It is because the PCL/CA nanofiber mats functionalized with $CeO_2$ NPs were found to exhibit high potential of treating diabetes associated wounds..

Hirst et al. (2009) reported the enormous generation and prolonged discharge of ROS causes oxidative stress to the wound tissue which contribute towards the progressive inflammation and tissue damage, therefore the ROS scavenging ability of the $CeO_2$ NPs can be employed in order to reduce inflammation. Studies have proven $CeO_2$ can mitigate reactive oxygen specie in J774A cells and hinder the iNOS production level which admits its potentiality as therapeutic agent in treatment of inflammatory diseases.

**Enhancement of Tissue Repair**

CeNPs promote tissue repair by stimulating cellular proliferation and migration. Their ability to modulate oxidative stress and inflammation creates a favorable environment for tissue regeneration. Preclinical studies have shown that CeNPs can enhance the healing of skin, bone, and other tissues, suggesting their potential in regenerative medicine. Researchers have reported angiogenesis property of wound healing possessed by $CeO_2$. This property of $CeO_2$ NPs make them of utilization in wound healing process. Furthermore, cerium oxide nanoparticles help in wound healing by tissue regeneration and repairment by mitigating oxidative stress level that is mainly due to their pH dependent property in micro environment (Chen et al., 2024; Descamps and Emanueli, 2012) .

**Diagnostic Applications**

**Imaging Techniques**

**Magnetic Resonance Imaging (MRI)**

The paramagnetic properties of nanoceria make the eligible for use as contrast agent in MRI for the detection and characterization of lesions. The high biocompatibility and stability act as stimuli to explore their potential as theranostic agent by modulating clinical imaging application to molecular imaging techniques (Kagan et al., 2010).

Researchers conducted an analysis by using $Gd^{3+}$ doped nanoceria increases its antioxidant potential and T1 relaxitivity by providing an excellent platform for developing new theranostic agents because of its high redox and high MRI contrast potential (Del Mercato et al., 2010; Wason et al., 2013). Kolmanovich et al. (2023) have reported that in order to enhance targeted drug delivery which otherwise was impossible to achieve with the mere use of nanoceria only because of its ultra-small size and high reactivity, the redox active gadolinium doped nanoceria ($CeGdO_2$-x NPs) was delivered in LbL polyelectrolyte encapsulated polymer to the targeted tumor cells and normal cells which proved high biocompatibility, high cellular uptake and as high MRI contrast agent.

Another report mentioned by Kolmanovich et al. (2023) suggested the successful launching of Gd-doped nanoceria as $T_1$-MRI positive contrast agent by assessing the Gd-CeO2-ZrO2-PEG nano-platform in MRI scanner owing to its $T_1$ weighted MRI Images and relaxitivity ($_{r1}$). The $T_1$ weight magnetic resonance images became more brighter upon increasing the molar concentration of Gd-CeO2-ZrO2/DOX-PEG from 0-0.9mM hence proved its efficiency as theranostic agent and T1-MRI contrasting agent.

**Biosensors**

**Detection of Biomolecules**

CeNPs can be modulated as biosensors in order to detect different biomolecules such nucleic acids, glucose, and cholesterol etc. Their catalytic properties improve biosensors' sensitivity and specificity, allowing for the quick and precise identification of target molecules. The exceptional biosensing properties of cerium oxide nanoparticles stem from their redox flip between two transition states. Personalized medicine and point-of-care diagnostics benefit greatly from this use (Kumara et al., 2015).

The biosensing performance of the cerium oxide nanoparticles can be enhanced by nano fabrication of other metals as reported by Kumara et al. (2015) the nano-fabrication as Au-NP$CeO_2$ with the deposition of Au onto the surface of cerium oxide was achieved to improve their catalytic properties to be used as biosensing tool due to the synergism between Au and $CeO_2$ which showed an improved oxidation properties, hence played role as biosensing agent.

Uncovering the functions of ROS and RNS in both healthy and pathological states would require developing techniques to measure their levels in intact living tissues, as these molecules are extremely reactive and have a limited lifespan. Detection of the level of hydrogen peroxide ($H_2O_2$), the highly persistent molecules in biological system could be highly beneficial in the diagnosis and treatment of various pathologies including cancer, diabetes, rheumatoid arthritis, inflammation, and atherosclerosis in humans (Charbgoo et al., 2017).

Ujjain et al. (2014) have created a CNPs-based electrochemical biosensor that measures hydrogen peroxide by oxidizing $H_2O_2$ on an electrode surface and generating a current that is commensurate to the $H_2O_2$ concentration. They have demonstrated how to create CNPs with spherical morphologies with the help of fructose and hexamethylene-tetra-amine (HMTA), two distinct capping agents. Thus, $CeO_2$-HMTA proved to me excellent peroxidase sensing agent due to the redox switch properties of cerium ion.

Another studied carried out by Crespo et al. (2016) demonstrated the use of histamine as a biosensors in the detection of allergic response to the immune system.

**Monitoring of Disease Biomarkers**

Real-time monitoring of disease biomarkers using cerium oxide based nano-biosensors provides essential clue for the diagnosis and treatment of diseases. The exceptional sensitivity of cerium oxide nanoparticles makes it to identify low-abundance biomarkers, which promotes early disease detection and better patient outcomes. Preclinical research has shown the huge contribution of cerium oxide nanoparticles in a wide range of biosensing applications (Saifi et al., 2021).

Investigations by Kumara et al. (2015) on $CeO_2$ based graphene composite was used on to GCE (graphite carbon electrodes) in order to prepare cholesterol biosensor based on elector-generated chemiluminesence (ECL) due to its enhanced sensitivity and catalytic properties towards cholesterol that amplified the luminous ECL signals during cholesterol sensing. On the contrary, the sensing being taken place on luminol-$H_2O_2$ relies on the chemical reaction among radial anions of lumina and ROS produced less efficient signals as compared to the ECL signals produced as a result of CeO2-graphene/GCE biosensor due to low ECL luminal intensity. Hence, proving its great sensing performance of cerium oxide biosensing agents.

Reports by Dave et al. (2018) used electrochemical method for the *in situ* preparation of cerium oxide nanocubes reduced graphene oxide (nc$CeO_2$-RGO) based nano-composite through hydrazine hydrate in order to detect biomarkers for oral cancer, Cyfra-21-1, a cytokines fragment-21-1. The immunosuppressor thus generated, BSA/Anti-Cyfra-21-1/ncCeO2-RGO/ITO exhibited high level of selectivity with glucose, mucin 16, interleukin 8 and sodium chloride.

**Safety and Toxicology**

**Biocompatibility Studies**

Cytotoxicity is a major concern towards the utilization of nanoceria. A number of in vitro and in vivo studies have proven their non-toxic nature at their therapeutic concentrations due to their non-toxic nature, stability antioxidant properties and high biocompatibility. The surface modification of nanoceria improves its biocompatibility (Yadav et al., 2023). The bio-availability in lysosymes, endoplasm reticulum, nucleus and cytoplasm makes them best scavengers of free radicals, antioxidant properties protecting the cell from oxidative stress (Nyoka et al., 2020; Yadav et al., 2023). McCormack et al. (2014) studied water based and PEG coated nanoceria (PEG-$CeO_2$-NPs) for high $Ce^{3+}$ concentration and dextran associated nanoceria (Dextran-$CeO_2$-NPs) for high $Ce^{4+}$ concentration in the lattice. The later one showed high catalytic activity due to the presence of high $Ce^{4+}$ concentration in the lattice.

Sarkar et al. (2023) reported the biocompatibility of FA-coated nanoceria. The FA coated nanoceria provides excellent biocompatibility and bacteriostatic effect when observed in wiser mice model to various cell lines at 0.1% w/v concentration and proved to be effective in wounds healing process. This was achieved due to the utilization of scavenging of free radicals and antioxidant properties in wound healing process by the FA-nanoceria.

Pushpalatha et al. (2022) reported that the bio-compatible studies of nanoceria particles showed that nanoceria are non-toxic when exposed for short term at the therapeutic dose level of 0.5ug/ml to 500ug/ml but are toxic when exposed for long term. The biocompatibility of nanoceria is dose dependent.

**Cytotoxicity and Genotoxicity**

Though CeNPs are considered relatively inert, their potential for cytotoxic and genotoxic effects at high concentrations should be investigated. Research has shown that high concentrations of CeNPs can lead to oxidative stress and DNA damage in cells and therefore the need to optimize doses in potential applications. Toxicological studies of CeNPs are required for the proper use in medicine (Park et al., 2008).

De Marzi et al. (2013) investigated the in vitro antioxidant properties by studying the cytotoxic and genotoxic effect of cerium oxide nanoparticles by exposing them to three different cell lines; A549, $CaCO_2$ and HepG2 for short term and long term exposure at different therapeutic concentrations of 0.5µg/ml to 5000µg/ml. The short term exposure for about 24h to cell lines did not show any sign of Cytotoxicity but the long term exposure for about 10 days observed using MTT Assay to A549 and HepG2 cell lines affected the viability of the cell lines even at the concentration of 50µg/ml while $CaCO_2$ exhibited a bit lower level of cytotoxicity to the cell line which will be productive for the treatment of tumors in near future.

Another contribution in the form of Comet assay was conducted by De Marzi et al. (2013) in order to study the genotoxic effect induced by cerium oxide nanoparticles ($CeO_2$-NPs) that showed HepG cells line to be the most sensitive one whole A549 cell line found to induce genotoxicity even at its lowest concentrations. The final therapeutic concentrations of $CeO_2$-NPs was 500µg/ml. The comet assay results showed that the genotoxic damage was induced by the hydrogen peroxide ($H_2O_2$) can be reduced by increasing the concentration of $CeO_2$-NPs while keeping the concentration of hydrogen peroxide constant, hence protecting the cell from oxidative stress.

**Long-term Exposure and Biodistribution**

The toxicity of CeNPs in the body and its long-term fate and distribution are important for understanding the safety of these cerium oxide nanoparticles. Some research works have demonstrated that CeNPs have the ability to accumulate in different organs such as liver, spleen and lung after systemic administration. The methods of clearance and the effects of such accumulation over time should be further examined to safeguard the patients (Rzigalinski and Strobl, 2009).

**Current Challenges and Future Perspectives**

**Limitations in Clinical Translation**

Apart from having great therapeutic, theranostic and diagnostic potential, cerium oxide nanoparticles possess some drawbacks of great concern which lower their importance as practical implementation in biomedicines. These are of great challenge for their potential role in clinical settings. These included challenges related to its quality of production , good batch to batch consistency and meeting regulatory requirements. All these issues need to be addressed but that require more advancement in research, development and regulatory measurements to adopted in a coordinated manner (Sancey et al., 2015).

**Regulatory and Ethical Considerations**

There is a dire need to develop a regulatory framework for the use of nanomedicines. the development of comprehensive risk assessment fir the use of nano-biomedicine in terms of safety, efficacy and ethical considerations is the dire need of time. The regulatory firms need to develop some special guidelines for the regulation of nanomaterials to be used for the development of nano-biomedicines. The deployment of nanomedicine as therapeutic purpose also raises few ethical concerns on patient side in the sense of issuance of the consent and their long term exposure to nanomedicines (Fadeel and Garcia-Bennett, 2010).

**Future Directions in Research and Development**

Researchers and investigators need to explore more about its potentials in biomedical filed as therapeutic, theranostic and biosensing agent by making more advancement in the exploration of its specific properties like biocompatibility, toxicity, targeted drug delivery, and further antioxidant and antibacterial properties. More efforts should be done in order to formulate new techniques and delivery methods for further assistance in biomedical field. Government and private funding institutes for research purpose should play their part in keeping forward the development of nanoceria based therapeutics and diagnostics tools and techniques (Heckert et al., 2008).

**Conclusion**

Cerium oxide nanoparticles' have great biocompatibility, stability, antioxidant, antibacterial, and redox potential that make their biomedical applications are particularly noteworthy. They have efficient redox potential making them able to eliminate oxidative stress from the cell. Because of the cerium oxide ion Ce3+/Ce4+, they have high scavenging properties for ROS and RNS due to their SOD and catalytic mimic properties. Their importance as a possible therapeutic agent against numerous biological illnesses of neurological, cardiovascular, and cancer types is demonstrated by their ability to scavenge ROS and modulate inflammation. Furthermore, Cerium oxide nanoparticles can be utilized to improve diagnostic techniques such as MRI, CT, and PET scans, as well as to develop new diagnostic instruments.Research is being done to evaluate improved disease monitoring tools and techniques to the health sector, thereby improving the quality of life and health style in favour of patients.As discussed above the potential exploration of cerium oxide nanoparticles in tissue engineering field and biomedical field is likely to enhance as the filed continues to explore more about about different techniques and its application in real life.

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Chapter 29

# Revolutionizing Healthcare: The Role of Nanoparticles

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## ABSTRACT

Drug delivery methods utilizing nanomaterials show potential for personalized medicine strategies, enabling customized treatments that take into account certain patient attributes, like genetic profiles or illness stages. Researchers are currently working on creating nanoparticles that can effectively transport genetic material, such as siRNA or CRISPR-Cas9, for gene therapy. This phenomenon presents novel opportunities for the treatment of genetic problems and the mitigation of diseases at the molecular scale. To enhance the immune system's defense against cancer and infectious diseases, nanomaterials have the potential to enhance the transport of immunomodulatory drugs, including immune checkpoint inhibitors and immunological adjuvants. This has the potential to augment the efficacy of immunotherapies. It is anticipated that forthcoming drug delivery systems utilizing nanomaterials will include stimulus-responsive characteristics, enabling the release of medicines in reaction to certain stimuli, such as pH, temperature, or enzyme activity. This allows for precise control over the timing and location of drug release, thereby maximizing the effectiveness of treatment. Nanoparticles can function as contrast agents in several imaging techniques, providing earlier identification of diseases and continuous monitoring of treatment administration. The incorporation of imaging techniques and drug delivery systems enables the utilization of theragnostic applications. Efforts are currently underway to enhance nanoparticle removal from the body and mitigate the risk of prolonged buildup through the development of biodegradable nanomaterials. The ongoing progress in nanotechnology is anticipated to result in the growing significance of nanomaterial-based drug delivery systems in the realms of disease treatment and personalized medicine. Nevertheless, it is imperative to confront obstacles to biocompatibility, manufacturing processes, regulatory authorization, and the enduring safety of these groundbreaking technologies to guarantee their effective implementation in clinical settings.

**KEYWORDS**
Nanotechnology; Disease treatment; Nanobiotechnology; Nanomedicine; Diagnosis; Drug-delivery; Healthcare; Medical applications

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## INTRODUCTION

According to theory, the world unintentionally came into being when an unstable, microscopic-sized, energized particle (an atom) bursts. An entire cosmos was built from a single bit, and scientists are currently working on similar tiny particles to produce scientific wonders once more. From this point on, the field of nanoscience has emerged and established itself in all facets of science and technology (Cheng and Dou, 2022). Nobel Prize-winning physicist Richard P. Feynman suggested the concept of nanotechnology, which involves manipulating larger items and using smaller tools and particles (Tockary et al., 2019). Since they believe that nanotechnology will shape science in the future, scientists are eager to use it to their advantage in as many contexts as possible. Biological researchers, doctors, and medics have also been interested in nanoscale goods because of their distinctive qualities and behavioral traits (Hajiali et al., 2021). Nanoscale quantum phenomena are currently applied in several disciplines such as biomedical sciences, bioengineering, food technology, biophysics, biochemistry, and other domains related to biology and medicine (Husain et al., 2023). This review chapter thoroughly examines the application of nanotechnology in the field of medicine. The text briefly mentions the early applications of nanotechnology in the closing years of the 21st century, with a focus on the most advanced forms of nanotechnological uses. To take into consideration the most recent nanomedical applications, several contemporary

medical applications have also been added, such as personalized targeted therapeutics, nanomedicine, regenerative medicine, and diagnostics (Fox et al., 2019).

**Various Implications of Nanotechnology in the Health Field**

**Nanotechnology in Diagnostics**

Nanodevices are used in diagnostic sciences to quickly identify illnesses and recommend medical treatments. Nanotechnology is also used to examine illness predisposition at the molecular and cell level to find remedies (Uchida and Kataoka, 2019). Nanotechnology may improve the diagnostics sector by improving medical examination accuracy, sensitivity, and speed. One important application is using nanoparticles to target biomarkers to improve MRI, CT, and PET scans' sensitivity, accuracy, and specificity (Avula and Grodzinski, 2022). Nanotechnology-enabled point-of-care diagnostic tests may quickly and accurately detect infections, cancers, and other diseases, enabling immediate prevention and treatment (Vaishampayan et al., 2023).

**The Field of Nanotechnology and Lab-on-chip Technologies**

Nanotechnology and lab-on-a-chip technologies have revolutionized pharmaceutical delivery, personalized treatment programs, and illness diagnoses (Erkoc and Karnak, 2021). Combining these strategies has developed faster, more accurate, and cheaper diagnostic instruments (Gu et al., 2013). Science is moving forward with Lab-on-a-Chip technology; for instance, it is being explored for application against malignant and viral disorders (Enrico, 2019). The analysis of genetic data at the cellular level is the central focus of the entire procedure. The use of body fluid sampling and sophisticated gene sequencing techniques has further contributed to the revolution of nanotechnology in the search for hitherto unthinkable disease solutions. The combination of these two technologies has resulted in the creation of Lab-on-Nanoparticles, which are tiny devices with a variety of uses, such as medication administration, diagnosis, and condition monitoring (Dong et al., 2019).

Real-time monitoring and individualized treatment are made possible by the nanoscale materials used in the construction of these devices (Fox et al., 2019). Cancer diagnosis is one of the major uses of lab-on-a-chip technology and nanotechnology in healthcare. It is possible to create nanoparticles that specifically target cancer cells, enabling early diagnosis and treatment. Additionally, a variety of medical illnesses, such as infectious diseases, metabolic disorders and genetic disorders can be diagnosed using lab-on-a-chip devices (Anselmo and Mitragotri, 2019).

**Pharmaceutical Nanotechnology Applications**

The subsequent part provides a concise synopsis of nanotechnological uses in pharmaceutical sciences, illustrated diagrammatically in Fig. 1.

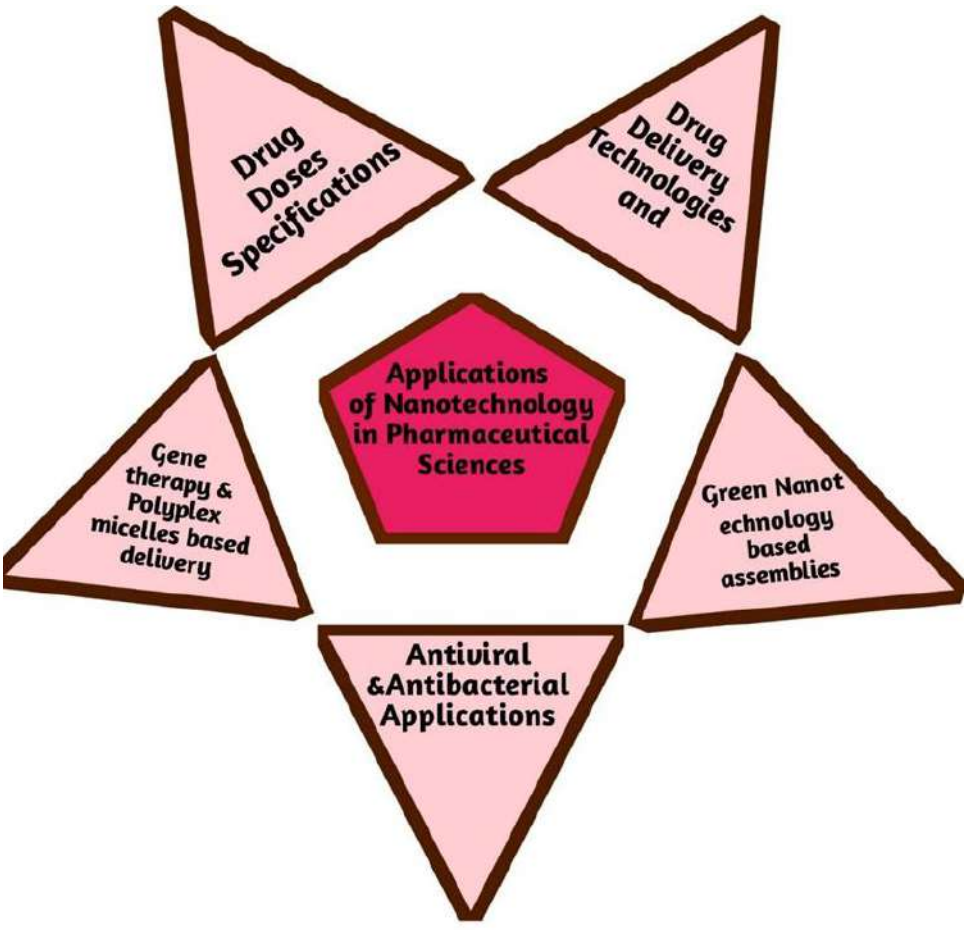


**Fig. 1:** Various nanotechnology applications

**Nanotechnology and Drug Dose Specifications**

Nanoscience allows for more effective and safer therapeutic drugs, revolutionizing the pharmaceutical industry. Nanoparticles increase medicine solubility, stability, and bioavailability, improving pharmacokinetics. Nanoparticles' dynamic behavior and complicated pharmacokinetics in vivo need careful dosage regimen design (Dirisala et al., 2020). Researchers must find the optimal nanoparticle dosages, frequency, and duration to maximize therapeutic benefits and minimize negative effects (Iravani and Varma, 2022). Medical research has produced some extremely sophisticated treatment alternatives in the past, but there is always a need for more effective ways to counter drug overdoses. In the medical sciences, a feature being considered to produce a rich technique of medication absorption is the utilization of nanoparticles as toxic drug absorbents (Kanwar et al., 2019).

**Nanoscience and Gene Therapy**

Gene therapy and nanobiotechnology often combine to produce new disease treatments. Gene therapy includes inserting DNA molecules into cells to replace damaged or missing genes to address genetic defects and other disorders (Kang et al., 2021). Nanobiotechnology is utilized in gene therapy to deliver therapeutic genes to target cells using nanoparticles (Javaid et al., 2021). The effectiveness and safety of gene therapy are increased by these nanocarriers, which shield DNA molecules from deterioration and improve their capacity to pass across cell membranes (Dirisala et al., 2022). Nanoscale technology is essential to gene therapy because it plays a fundamental role in the cellular level of genetic modifications and disease prevention (Kim and Franco, 2020). Different types of organic and inorganic particles made with nano-assemblies, both biodegradable and non-biodegradable, are being attached to gene therapy procedures through modifications. DNA can be accessed and bound by these structural combinations throughout cellular surfaces. Additionally, combinations of polymer-based nanoparticles are made for intravenous medication injections. These updated technologies provide access to new developments in nanogenetic medicine (Dirisala et al., 2020). Overall, gene therapy and nanobiotechnology are expected to enhance cancer, infectious, and genetic disease treatments.

**Antiviral and Antibacterial Applications of Nanotechnology**

The greatest strategy to combat bacteria, viruses, and other types of microscopic diseases is at the nanoscale since these diseases' causal agents operate at the minuscule level. Thus, nanotechnology offers the key to the identification and treatment of numerous bacterial, fungal, and viral illnesses (Hulla et al., 2015). Although ancient Greek medicine used metals like silver to treat illnesses, nanoscale-based material conversion has been demonstrated to enhance both traditional and contemporary therapies (Jiang et al., 2022). Research has revealed that nanosized silver particles exhibit greater reactivity in the treatment of burns and wounds due to their tiny size and ease of skin penetration (Iravani and Varma, 2022). According to research, there will soon be targeted and personalized therapy options for preventative and regenerative medicine against pathogenic and pathophysiological disorders that are based on nanotechnology. These advantages are combined with the new technology's ability to save money and time (Kang et al., 2021).

**Nanoparticle-Based Delivery Efficiency and Clinical Integration Barriers**

Delivery and clinical translation of nanoparticles are difficult. Nanocarrier buildup in reticuloendothelial system organs, notably the liver, hinders clinical translation. This buildup prevents the administered dosage from reaching the illness site and raises toxicity concerns. (Gu et al., 2013).

**Nanotechnology Applications in Regenerative Medicine**

**Use of Nanotechnology and Bone Regeneration Technology**

Nanotechnology encompasses the scientific understanding and methodologies employed to manipulate and regulate matter at the atomic and molecular levels. Bone regeneration technology utilizes ingredients that stimulate bone growth to generate new bone tissue or facilitate the healing of existing bone tissue. Scientists are currently engaged in the development of nanostructured materials as alternatives for bone grafts, aiming to achieve comparable qualities that bodily tissues and organs can readily absorb. If these investigations are successful, they will introduce a novel era of regenerative technology aimed at treating fractured bones and broken muscle fragments (Buya et al., 2020). The current research is primarily focused on conducting a comprehensive analysis of the biomineralization process. The goal is to reduce the size of bone particles, allowing them to be coupled with their crystalline properties and integrated into collagen fibers. This work aims to change osteology and bone tissue engineering by developing a composition that can enter wounded bone areas and has unique mechanical characteristics (Crommelin et al., 2020; Sahu et al., 2021). Research is underway to create knee and hip prosthetic joints and nanoscale collagen coverings. The coatings attempt to stabilize osteoblast bone development. This includes speeding healing, strengthening bones, and reducing issues.

**Regenerative Medicine and Nanotechnology**

Regenerative medicine uses cell therapy and tissue engineering to treat, preserve, enhance, and restore damaged cells, tissues, and organs (DeLuca et al., 2020). Cellular manipulation of the human body was formerly difficult. Nanoscale technology has opened up new possibilities in regenerative medicine by allowing cell and component manipulation. Nanomedicine focuses on the investigation and advancement of nanoscale substances, including silver and gold

nanoparticles, nanoshells, nanorods, dendrimers, nanocubes, carbon buckyballs and several other materials. These specific features may be applied to certain tissues and organs. Numerous research organizations worldwide are studying these nano-agents' medicinal, diagnostic, anti-viral, antifungal, and anticancer properties. Progress shows that nanotechnology will soon change cancer therapy options. Early nanotechnology diagnosis has been successfully explored and used (Tang et al., 2019).

**Table 1:** Obstacles to medicine distribution using nanotechnology

| Sr.No | Barriers Associated with Nano-Based Drug Delivery | Brief Explanation | Examples |
|---|---|---|---|
| 1 | Size and stability | The size of nanoparticles plays a crucial role in their delivery efficiency. Very small nanoparticles may be cleared quickly from the bloodstream, while larger nanoparticles may have limited tissue penetration. Additionally, maintaining the stability of nanoparticles during storage and delivery can be challenging. | Liposomal nanoparticles although initially promising for drug delivery, have faced challenges due to their size variability and instability, leading to a limited clinical translation. |
| 2 | Controlled release | Efficient release of the encapsulated drug or payload at target site is critical. It requires precise control over the release mechanism, kinetics, and release triggers (pH, temperature, and enzymes) to ensure optimal therapeutic effects. | Researchers working on tumor-targeted drug delivery have faced challenges in achieving the controlled release of drugs from nanoparticles thereby reducing their therapeutic efficacy. |
| 3 | Targeting specificity | Nanoparticles often require functionalization with targeting ligands to enhance their specificity toward diseased cells or tissues. Achieving selective targeting while minimizing off-target effects remains a significant challenge | Gold nanoparticles functionalized with antibodies for cancer-targeted photothermal therapy have faced issues related to non-specific accumulation and targeting of healthy tissues, leading to toxicity concerns |
| 4 | Biocompatibility and toxicity | Nanoparticles should be biocompatible to avoid adverse reactions and toxicity. This includes minimizing immune responses, toxicity to healthy tissues, and ensuring nanoparticles do not accumulate excessively in the body. | Carbon nanotubes showed significant toxicity concerns due to inflammation and tissue damage, limiting their clinical translation despite their potential applications. |
| 5 | Regulatory approval | Nanoparticles should be biocompatible to avoid adverse reactions and toxicity. This includes minimizing immune responses, toxicity to healthy tissues, and ensuring nanoparticles do not accumulate excessively in the body. | The regulatory approval process for nanoparticles such as liposomes or polymeric nanoparticles often involves long and complex pathways, delaying their clinical translation. |

**Implications of Nanotechnology in Surgical operations**

The subsequent part provides a concise summary of the utilization of nanotechnology in the field of surgery, accompanied in Fig. 2.

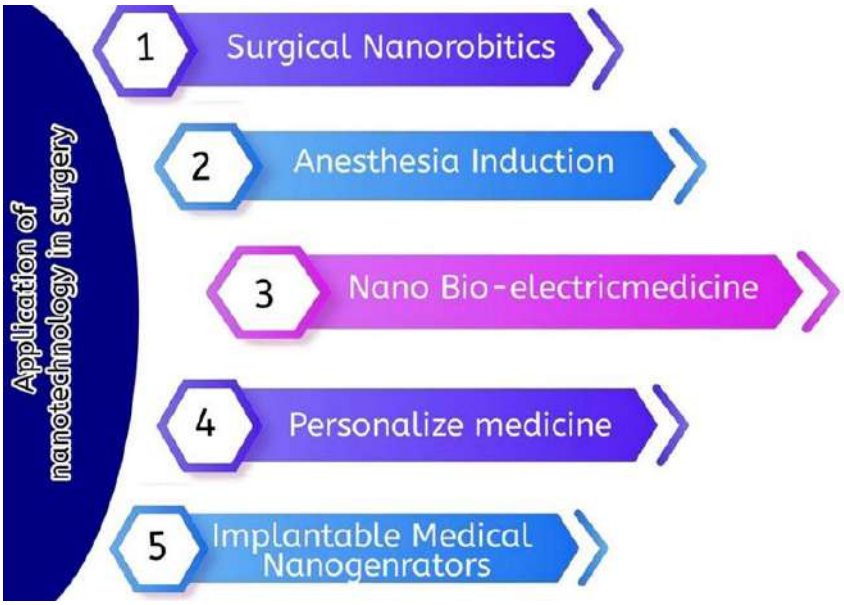


**Fig. 2:** Various implications of nanotechnology in surgery

**Nano-Bioelectric Medicine and Nanorobotics in Surgery**

Surgical nanorobotics is the development and use of tiny robots, or nanorobots, that can perform surgical procedures with remarkable precision and effectiveness (Chen et al., 2020). Nanorobots possess the capability to be directed towards precise anatomical sites within the human body by the utilization of sophisticated imaging methodologies. Subsequently, they are capable of executing many functions, including drug administration, tumor excision, and tissue restoration. In contrast, nano-bioelectric medicine entails the utilization of electrical impulses to induce the body's natural healing mechanisms. Nanoscale technologies are being used to manipulate and regulate cell and tissue electrical activity. This approach focuses on cardiac issues, chronic pain, and wound healing. Nanobioelectric medicine and surgical nanorobotics have the potential to transform medicine and enhance patient outcomes. Nonetheless, additional investigation is required to confirm the technology's efficacy and safety (Suhail et al., 2022). A surgical nanorobot for the vascular system is being developed using programming, engineering, and biology. These portable devices may test and treat lesions and germs (Talukdar et al., 2022).

**Surgical Nanorobotics/Bioelectric Medicine Surgical Nanorobotics Development**

Nanorobotics in surgery and nanobioelectric medicine. Surgical nanorobotics is the art of designing and utilizing small robots, or nanorobots, to perform surgery with accuracy and effectiveness (Chakravarthi and Balaji, 2010). The efficacy of these investigations has been validated in animal models, whereby the nanorobotics have been observed to regulate the nano scissor action. The findings have prompted researchers to conduct more trials before optimizing surgical circumstances on individuals with diseases. There is currently a burgeoning field of bioelectric medicine that incorporates biological elements to enhance the efficacy of diagnostic and therapeutic interventions. The application of this nanobioelectronic technology has been observed in the treatment of malignant diseases, cardiovascular problems, and many physiological dysfunctions inside the human body (Hu et al., 2018). Nevertheless, numerous enhancements are required to effectively implement this technology in a clinical environment for multifaceted and intricate disorders.

**Medical Nanogenerators Implants**

Self-powered, implanted medical nanosensors are nanogenerators (Modi et al., 2021). These devices convert mechanical energy from motion into an electrical spark. Chemical energy from glucose is converted into mechanical energy in the body. Mechanical energy is transformed into electrical energy by these nanogenerators. The implantable nanodevices that are currently being created in large quantities for medicinal applications can then be charged and powered by this electrical energy. The utilization of mechanical energy derived from bodily movements to produce electrical energy is a characteristic feature of implantable medical nanogenerators (IMNGs). Implantable devices can be utilized to provide power to a range of medical equipment, such as neurostimulators, pacemakers and drug delivery systems (Wen et al., 2023).

**Nanotechnology and Anesthesia Induction**

In dentistry and other sensitive treatments like brain surgery, anesthesia induction is vital. Nanorobotic suspension mixes with millions of nanoscale active analgesic nanoparticles are being developed for anaesthesia induction (Wong et al., 2013). Patients' gingival and other sensitive regions are targeted by these nanoparticles, which penetrate loose tissue deeply. On-site nanocomputers monitor and regulate chemical, temperature, and positional gradients used to manipulate nanomaterials (Mbunge et al., 2021). Nanoscale anaesthetics give the desired effect on the tooth surface quickly and uniformly. A tooth that needs surgery may be adjusted for sensitivity. Following the conclusion of surgical procedures, nanorobots are manipulated by nanocomputers in order to reinstate tooth sensitivity to its original state (Dang and Guan, 2020).

**Implications of Nanotechnology in Dental Health**

Nanodentistry has several nanotechnology applications, including dental function detection, diagnosis, treatment options, and prognosis (Mazayen et al., 2022). Nanomaterials might treat several oral health issues (Salamehet al., 2020). These nanoparticles originated from tissue engineering and biotechnology dental nanorobotics (Thakur et al., 2022). Dental nanotechnology has enhanced dental health care alternatives for enameling, orthodontic realignment, dentition renaturalization, anesthetic, and hypersensitivity therapies (Souri et al., 2022).

The mechanical dentifrobots are the nanoscale technology employed for these functions. Their job is to enhance the sensitivity of nerve impulse flow in the central part of the tooth through real-time calculation. This allows them to control the penetration and maintenance of dental tissue for normal functioning (Li et al., 2021; Yu et al., 2021). Programmable nanocomputers are used to carry out activities based on external stimuli by connecting with localised internal nerve stimuli. The aforementioned mechanistic insights have the potential to assist dental surgeons in proposing a strategic treatment approach that can be implemented by the direct utilisation of in vivo nanorobot action, employing acoustic signals, as previously explained (Wang et al., 2021).

The mechanical dentifrobots are the nanoscale technology employed for these functions. Their job is to enhance the sensitivity of nerve impulse flow in the central part of the tooth through real-time calculation. This allows them to control the penetration and maintenance of dental tissue for normal functioning (Malik et al., 2023)

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**Fig. 3:** Major applications of nano-dentistry

**Implications of Nanotechnology in Oncology Field**

**Nanotechnology and Cancer Therapy Approaches**

In order to provide efficient therapy and early diagnosis, medicine focuses on treating difficult, incurable disorders like cancer (Lal et al., 2021). Researchers are using nanotechnology to build nano_agents, molecular diagnostics kits, fluorescent materials, and personalized medications that might enhance disease detection and therapy (Dirisala et al., 2014). Researchers are experimenting with several methods to combine existing medications with nanoparticles in order to improve the specificity and targeting of pharmaceuticals in organs (Saunderset al., 2020). Nanomedicine serves as a vehicle for the delivery of numerous targeted anticancer agents, which can be targeted towards tumour locations. Furthermore, it is important to consider the tumour imaging and immunotherapy methods associated with nanomedicine when delving into the connections between nanomedicine and cancer (Wang et al., 2021). The efficacy of nanomaterials in the field of cancer therapies has prompted researchers to substitute conventional cancer therapy methods with specific therapies that can be employed either independently or in combination with existing anti-cancer medications (Wang et al., 2021). Furthermore, there is a growing emphasis on mitigating the adverse effects of chemotherapeutic medications by the enhancement of their tumor-targeting efficacy and the optimization of their pharmacokinetic and pharmacodynamic characteristics (Misra et al., 2010). In addition, nanorobotics is being used in conjunction with gene therapy procedures to combine heat-induced ablation therapy against cancer cells (Xu et al., 2022; Thwala et al., 2023).

**Nanotechnology in Cancer Diagnostic approaches**

The most obvious issue is the diagnosis of cancer. Due to delayed identification in the third or fourth stages, cancer persists. Nanotechnology is being used to identify organ cancers faster (Salleh et al., 2020). Nanotechnology provides sensitive and specific multiplexed cancer biomarker detection in extracellular and in vivo bioimaging situations. Cancer diagnosis is a promising nanotechnology use. Because of their small size, nanoparticles can pass through blood-brain barriers and cellular membranes. They are therefore perfect for delivering medications to cancer cells. In addition, they are able to recognize cancer cells and locate the illness's site and symptoms (Lal et al., 2021).

**Toxicology and Safety Analyses of Nanotechnologies**

Humans, animals, and the ecosystem are concerned about nanotechnology's potential harm (Amiri et al., 2022; Patel and Patel, 2023). Despite the lack of clarity surrounding the toxicity of these assemblies, scientists remain uncertain about the extent to which they can advance nanotechnology, particularly in the delicate field of medicine (Baroud et al., 2021). Nanotechnology can provide sensors and markers for chemical, biological, and environmental cleanup (Adir et al., 2020). These evaluations should focus on these nanomaterials' dangers before they are employed in medicine (Park, 2013). Furthermore, nanomaterial fabrication, manipulation, and use must be regulated to ensure safety (Nikalje, 2015; Pardi et al., 2015).


**Fig. 4:** Applications of Nanotechnology in Oncology field.

**Future Prospects Regarding Nano-Medical Applications**

Nanomaterials have significant potential for several biomedical advancements and industrial applications. Nevertheless, the distinctive physicochemical characteristics of these substances give rise to apprehensions over their possible ramifications on both human health and the environment. To facilitate the entry of medicinal nanomaterials into the market, numerous challenges must be addressed, including obtaining FDA approvals and permits, as well as addressing safety and ethical considerations. In recent times, regulatory entities on a global scale have directed their attention towards the establishment of suitable frameworks aimed at guaranteeing the secure and conscientious use of nanomaterials. The matter at hand necessitates a more comprehensive approach in the forthcoming years of nanotechnology research. In this context, it is imperative for review papers to strive towards furnishing researchers, policymakers, and industry experts with a complete comprehension of the contemporary regulatory developments pertaining to nanomaterials. This study emphasizes the necessity of achieving harmonization and collaboration across regulatory bodies globally by doing a thorough analysis of the existing state of nanomaterial regulation. The process of regulating industrialization affairs pertaining to nanoparticles in the field of medical sciences encompasses multiple sequential stages. It is imperative to acknowledge that the aforementioned phases offer a broad structure, nevertheless the precise particulars and procedures may differ based on the legislation and special prerequisites of individual countries or regions (Zhou et al., 2017).

**Conclusions**

Nanotechnology has the potential to transform disease diagnosis, treatment, and prevention. Nanotechnology involves manipulating materials at a nanoscale, where their properties differ from those of bigger materials. This lets them manipulate their biological, chemical, and physical aspects precisely. This opens up new treatment techniques, precise drug delivery systems, and sensitive diagnostic tools. Nanoparticles may improve medication stability, solubility, bioavailability, distribution, targeted administration, better medicines, restricted doses, and systemic side effects. Furthermore, sensors and gadgets based on nanotechnology can continuously monitor.

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Chapter 30

# Nanoscale Marvels: Understanding Nanoparticles and their Applications

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**ABSTRACT**

Nanotechnology has significantly transformed the domain of medicine, namely in the advancement of innovative methods for delivering drugs. Nanomaterial-based drug delivery systems provide numerous benefits compared to conventional techniques, such as increased therapeutic effectiveness, higher bioavailability, precise targeting, and minimized adverse reactions. This chapter offers a comprehensive examination of the uses and recent progress in medicine delivery methods that utilize nanomaterials. The initial focus of the review is on liposomes, carbon-based nanomaterials, polymeric nanoparticles, dendrimers and metallic nanoparticles used in drug delivery. Nanomaterial-based drug delivery systems are used in the third category to address cancer, cardiovascular, infectious, and neurological diseases. These systems can be designed to specifically gather at the desired location, thus increasing the effectiveness of the medication and reducing any unintended side effects. The fourth segment examines current progress in drug delivery systems utilizing nanomaterials, specifically focusing on nanocarriers that are responsive to stimuli and possess several functions. Stimuli-responsive systems can release medications when specified triggers, such as alterations in pH, temperature, or enzymatic activity, occur, resulting in drug release at precise locations. Multifunctional nanocarriers transport medications and conduct diagnostic imaging, enabling the simultaneous monitoring of distribution of drugs and treatment response in real-time. The hurdles involve regulatory factors, concerns about toxicity, the capacity to scale up, and the process of translating findings into clinical applications. Possible future breakthroughs encompass the progress of tailored nanomedicine, the use of combination therapy tactics, and the integration of emerging technology like as gene editing and artificial intelligence. Nanotechnology can provide sophisticated medication delivery systems for personalized treatment and precision cures. However, additional research and collaborations are necessary to overcome the challenges of translating these innovations into clinical settings and using them safely.

**KEYWORDS**
Artificial intelligence, Drug delivery system, Nanotechnology, Nanomaterials, Targeted delivery

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## INTRODUCTION

Nanotechnology has caused significant progress in several areas, including medicine. The incorporation of nanotechnology into drug delivery systems has demonstrated significant potential, providing a new method to improve therapeutic results while reducing adverse effects. Nanomaterial-based medication delivery systems utilize the distinctive physicochemical features and configurable surface changes of nanoparticles to accurately transport therapeutic medicines to specific locations. Nanomaterials consist of several structures, including dendrimers, liposomes, carbon nanotubes, polymeric nanoparticles, and metallic nanoparticles. Each of these shapes has its unique advantages and limits (Fu et al., 2014). Liposomes mimic cellular membranes and have the ability to encapsulate hydrophobic as well as hydrophilic drugs. On the other hand, polymeric nanoparticles offer a customizable release pattern for long-lasting drug administration (Davis et al., 2008). Researchers can customize drug carriers for various purposes due to their adaptability. Nanomaterial-based medication delivery systems improve medicine targeting and bioavailability. These systems may specifically accumulate in ill tissues or cells, decreasing side effects and boosting medication effectiveness (Fayaz et al., 2010).

In addition, nanoparticles provide protection for pharmaceuticals, preventing them from breaking down or being eliminated by the body too quickly. This helps to increase the amount of time the drugs remain in circulation and enhances their capacity to be absorbed by the body. In addition, drug delivery systems based on nanomaterials provide a distinct benefit by effectively bypassing biological obstacles, such is the blood-brain barrier, which restricts brain drug delivery. Nanocarrier surface modifications and receptor-mediated interactions may bypass biological barriers and carry drugs to previously inaccessible areas. In 2014, Chen et al., 2014 showed that this has major implications for neurological disorders and brain cancer therapy. An additional crucial factor in nanomaterial-based medication delivery is the capacity to achieve precise control over the release of drugs. These systems may manage and extend therapeutic substance delivery, minimizing the need for frequent administration and maintaining a stable medicine level. The implementation of this controlled release mechanism can improve patient adherence and mitigate the adverse effects linked to elevated medication concentrations (Duan et al., 2019). Ensuring the safety and biocompatibility of nanomaterial-based drug delivery systems is of utmost importance (Sahoo and Labhasetwar, 2003).

Scientists perform thorough safety assessments to evaluate the possible toxicity and compatibility with living organisms of these nanocarriers, resolving any issues prior to their use in clinical applications. To summarize, drug delivery systems based on nanomaterials are an advanced method that has the potential to significantly transform modern medicine (Seabra and Durán, 2015). Through the utilization of distinct characteristics of different nanomaterials, these systems provide improved medication targeting, enhanced bioavailability, and regulated drug release (Raj et al., 2012). Surmounting biological obstacles enhances the range of potential uses for nanocarriers in the treatment of difficult diseases. Nevertheless, conducting thorough safety assessments is crucial in order to fully use the capabilities of these systems for secure and efficient clinical application (Couvreur 2013).

**Current Integration of Nanotechnology**

Nanotechnology, the precise control and modification of devices and materials at the scale of nanometers, has emerged as a powerful and versatile field with numerous applications in electronic devices, energy, medicine, and environmental sciences. Nanotechnology has produced significant advancements in medication delivery systems within the medical field (Dhuria et al., 2010). Nanoparticles specifically engineered for precise drug delivery have demonstrated significant potential in the field of cancer therapy. These nanoparticles improve the efficacy of treatment and minimize harm to healthy tissues by directly delivering drugs to tumor cells. Nano-sensors, a significant advancement in the field of nanotechnology, have demonstrated immense value across multiple industries (Farokhzad and Langer, 2009). These advanced sensors are capable of detecting even the smallest quantities of certain molecules, so opening up possibilities for medical diagnostics, environmental monitoring and food security. Researchers have extensively studied nano-sensors for their ability to identify diseases such as Alzheimer's and diabetes at an early stage, which could lead to advancements in healthcare (Prausnitz et al., 2004). Nanotechnology has significantly revolutionized the electronics sector. More powerful and energy-efficient mobile phones and computers have been developed as a result of the continuous shrinking of electronic devices made possible by the usage of nanoscale transistor and components. Future electrical applications are now possible because to graphene and carbon nanotube research. Renewable energy technologies are affected by nanotechnology. Researchers created nanomaterials that boost solar cell, energy storage, and hydrogen catalytic performance (Siafaka et al., 2023).

These technological breakthroughs hold the potential to improve sustainable energy solutions, which are crucial for tackling worldwide energy concerns (Singh et al., 2023). In the midst of the advancement, nanotechnology encounters crucial obstacles. Thorough attention must be given to environmental effects, health and safety concerns, and ethical considerations. Collaboration among researchers, policymakers, and enterprises is essential in tackling these difficulties to guarantee accountable and sustainable advancement of nanotechnology (Prasad et al., 2018).

**Nanotechnology's Industrial Impacts**

**Food Industry**

Nanotechnology is revolutionizing numerous sectors, including food. This technology might improve food sustainability, safety, and quality and provide innovation avenues (Fig. 2). Nanotechnology has the potential to enhance packaging by improving its barrier properties, hence increasing the shelf life of food and minimizing rotting. Nanocomposite films and coatings possess the ability to withstand bacteria, oxygen, and moisture, hence extending the shelf life of perishable objects (Prasad et al., 2018). Nanoencapsulation transports bioactive substances including antioxidants, vitamins, and omega-3 fatty acids to particular body regions, improving nutrition absorption. This may boost consumer health and reduce vitamin shortages.

Packing materials can incorporate microscopic sensors to detect and monitor the deterioration, contamination, and pathogenic agents in food. These intelligent sensors can deliver up-to-the-minute data on the quality of food, thereby guaranteeing the safety of consumers and diminishing the occurrence of foodborne diseases. Nano-emulsions and nanoliposomes facilitate the regulated discharge of flavor components, hence augmenting the gustatory and olfactory qualities of food items (Stephan and Irvine, 2011).

In addition, the incorporation of nanostructured components can enhance the mouthfeel and texture of processed foods. Nanoparticles, namely silver nanoparticles, possess antibacterial characteristics as well as can be integrated into

packaging for food or coatings to hinder the proliferation of bacteria and fungi. This results in enhanced food safety and less dependence on chemical preservation agents (Trucillo, 2021).

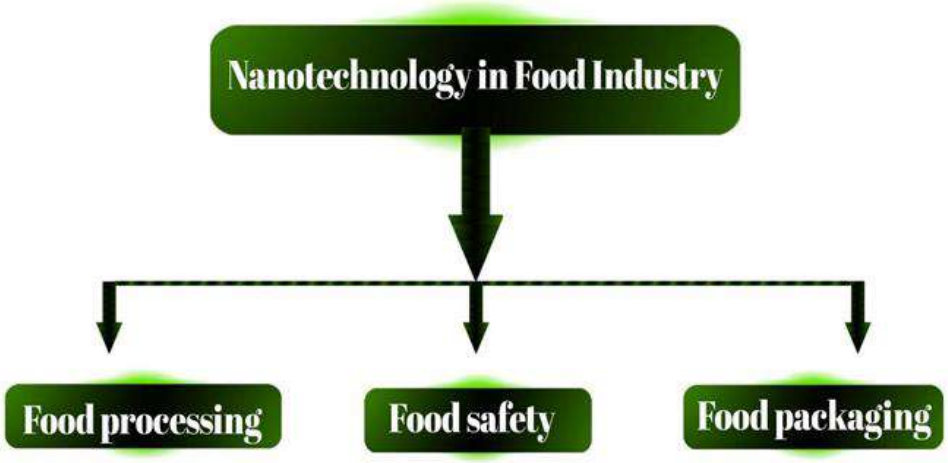



**Fig. 1:** Applications of nanotechnology in the food industry

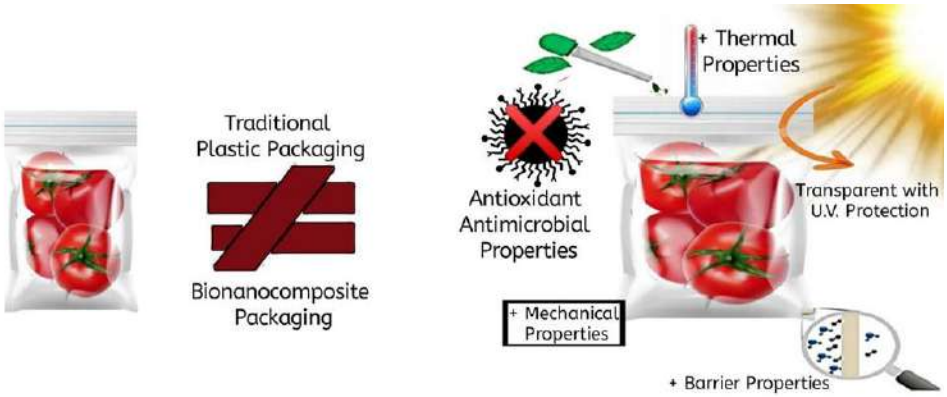



**Fig. 2:** Applications of nanotechnology in food packaging

Through improving emulsification, stabilizing formulae, and permitting precise component release, nanotechnology can improve food processing methods. Better product consistency and homogeneity result from this (Petros and DeSimone, 2010). Nanotechnology has the potential to enhance crop protection, nutrient absorption, and soil fertility in the field of agriculture. Nano-pesticides have the potential to decrease the usage of chemical pesticides, resulting in a diminished environmental effect and possible health advantages. Although nanotechnology offers significant possibilities for the food business, there are also apprehensions regarding potential hazards associated with the safety of nanomaterials. In order to assure customer safety and regulatory compliance, it is imperative for the industry to address concerns and carry out comprehensive risk assessments when exploring nanotechnology applications (Jain et al., 2011).

**Nanotechnology in the Cosmetic Industry**

Currently, nanotechnology has achieved significant advancements in the cosmetic industry, providing a diverse array of applications that enhance the stability, efficacy, and overall performance of products (Fig. 3).

Vitamins, antioxidants, and skin-beneficial substances are better delivered via nano-emulsions and nanoliposomes. They help these compounds penetrate the dermal layers, improving their efficacy and longevity (Jain, 2007). Sunscreen formulations: Nanoparticles like titanium dioxide and zinc oxide provide sunscreens with clear, broad-spectrum UV protection. The nanoparticles show better UV absorption and scattering, which reduces the visibility of white residue and increases sun protection (Jokerst et al., 2011). Nanostructured materials that mimic the composition and properties of human skin can be produced thanks to nanotechnology. These substances have the ability to moisturize the skin, enhance its ability to retain moisture, and shield it from harmful environmental factors, resulting in skin that is healthier and appears more youthful. Nano-delivery systems provide the precise and regulated administration of anti-aging substances, like retinoids and peptides, to selectively target distinct layers of the skin. This aids in diminishing wrinkles, fine lines, and skin damage caused by aging. Nanocarriers such as nanogels and nanoparticles have the ability to enclose and transport cosmetic substances with great accuracy to specific parts of the skin, enabling focused treatment for different skin diseases.

The utilization of nano-sized components in hair care items enables more efficient penetration into individual hair strands, resulting in enhanced conditioning, healing, and safeguarding against heat-induced damage and styling-related

harm. Nanotechnology facilitates the containment of fragrances within carriers that are extremely small in size, resulting in the gradual and prolonged release of odors, hence enhancing the longevity of perfumes and cosmetic items. According to Khodakovskaya et al. (2013), nanoparticles can change both consistency and texture of cosmetic formulations, leading to smoother textures, enhanced sensory experiences, and better communication. It is crucial to stress that safety issues remain the top priority, even if nanotechnology offers significant benefits in cosmetic applications. To fully investigate the possibility of nanoparticle diffusion into the skin or any other unfavorable outcomes, a thorough investigation and regulatory evaluation are important (Fig. 4).

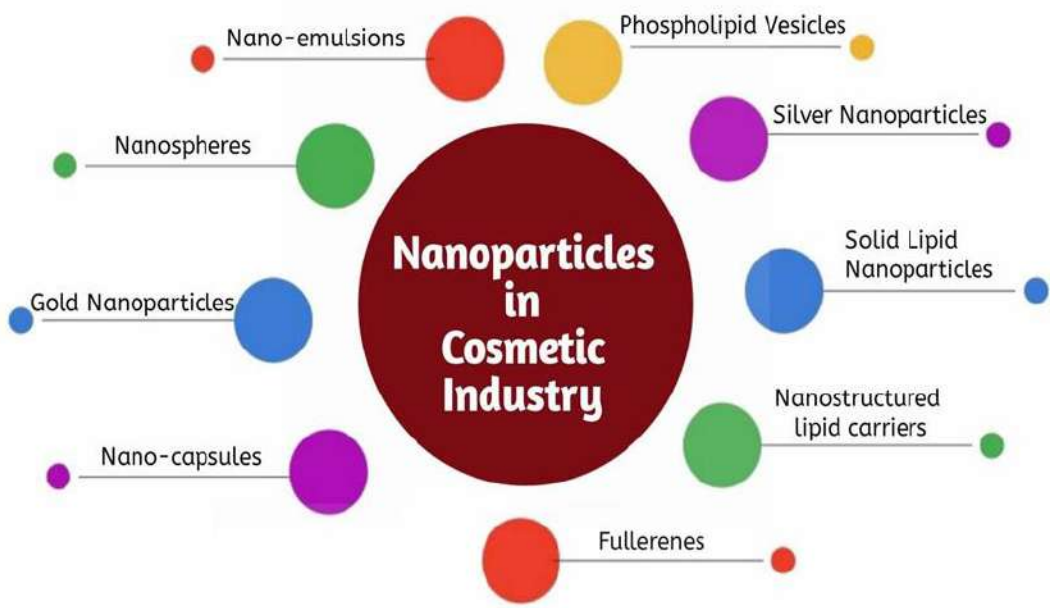
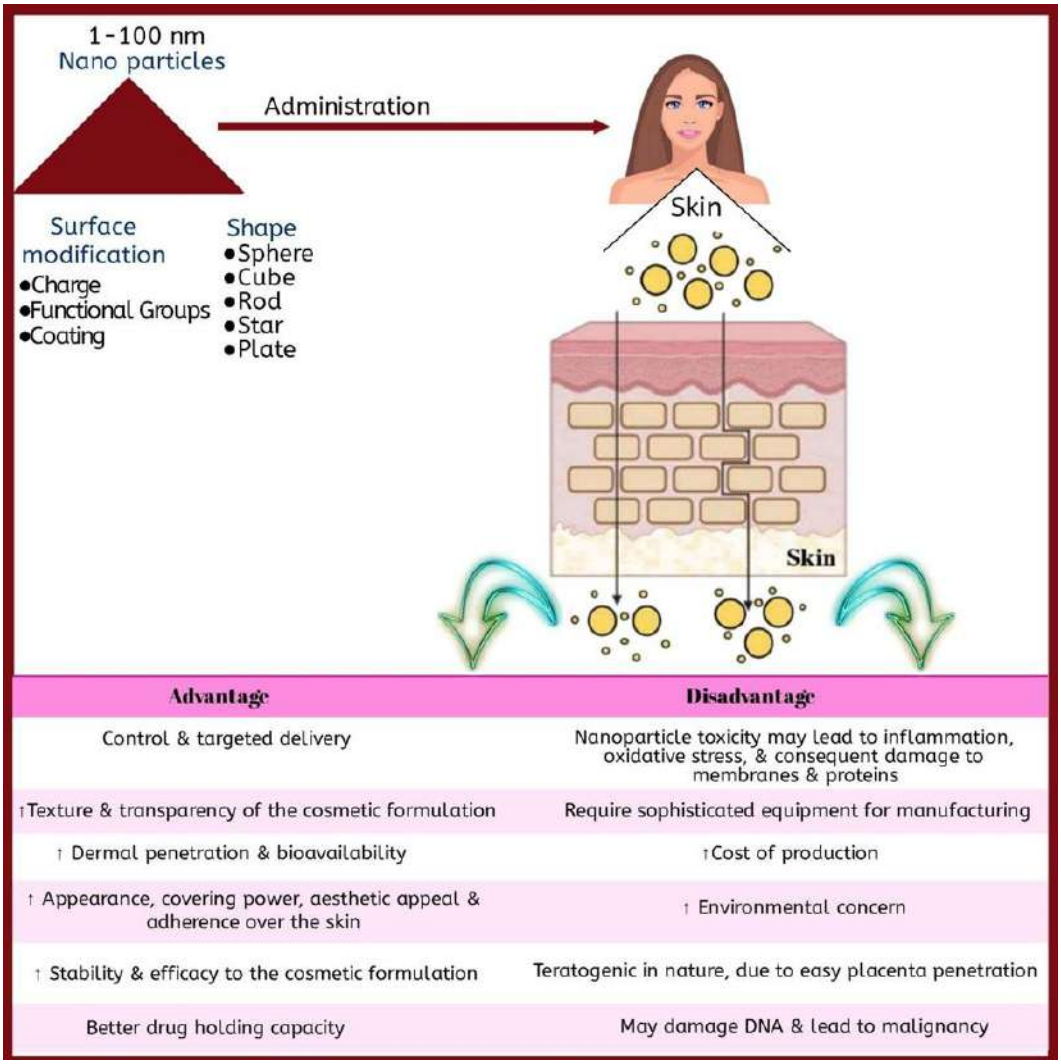



**Fig. 3:** Application of nanoparticles in the cosmetic industry




| Advantage | Disadvantage |
|---|---|
| Control & targeted delivery | Nanoparticle toxicity may lead to inflammation, oxidative stress, & consequent damage to membranes & proteins |
| ↑Texture & transparency of the cosmetic formulation | Require sophisticated equipment for manufacturing |
| ↑ Dermal penetration & bioavailability | ↑Cost of production |
| ↑ Appearance, covering power, aesthetic appeal & adherence over the skin | ↑ Environmental concern |
| ↑ Stability & efficacy to the cosmetic formulation | Teratogenic in nature, due to easy placenta penetration |
| Better drug holding capacity | May damage DNA & lead to malignancy |

**Fig. 4:** Advantages and disadvantages of nanocosmetics

**Nanomedicine**

It is possible to create nanoparticles and nanocarriers that will improve the targeted distribution of therapeutic agents and drugs to particular parts of the body (Labiris and Dolovich, 2003; Larrañeta et al., 2016). Therapeutic results are improved by targeted medication administration, which also improves drug absorption and allows for longer release with fewer adverse effects.

Nanoparticles may be adjusted with iron oxide nanoparticles or quantum dots to increase MRI, CT, and optical imaging contrast. Early disease diagnosis is simpler and more accurate using nanoprobes. Nanotechnology has improved gene therapy, photodynamic treatment (PDT), and hyperthermia for cancer. Nanoparticles can transport therapeutic genes, heat-inducing chemicals, and photosensitizers directly to cancer cells, allowing for tailored and focused treatment (Santos et al., 2022). Research has investigated the use of nanoparticles, namely silver nanoparticles, for their ability to fight against bacteria and viruses that are resistant to drugs. This has created new possibilities for controlling infectious diseases (McClements, 2012). The utilization of nanotechnology in the field of nanomedicine is constantly advancing, and ongoing scientific investigation is anticipated to provide far more groundbreaking remedies to address a wide range of medical obstacles. Nevertheless, it is imperative to do comprehensive safety evaluations and tackle any toxicity issues linked to nanomaterials in order to guarantee their secure and efficient application in clinical environments (McClements, 2020).

**Various Methods of Drug Delivery System**

The development of drug delivery technologies has significantly increased both the efficacy of pharmaceuticals and patient adherence to therapy. Various drug delivery systems have been created, each designed to meet certain medical requirements and target processes (Weng et al., 2017). There are several significant modes that should be mentioned:

1) Drug delivery via the oral route: Oral administration is still the most prevalent and convenient method for delivering drugs, despite obstacles such as low solubility and enzymatic breakdown. These issues have prompted the creation of more sophisticated drug delivery systems (Nel et al., 2006).

2) Inhalation as a method of medication delivery: By administering pharmaceuticals in the form of inhaled aerosols, they directly targeted to the respiratory system, rendering them highly efficient in the treatment of respiratory ailments such as asthma or chronic obstructive pulmonary disease (COPD).

3) Targeted medication delivery: Ligands or antibodies may target pharmaceuticals to specific cells or tissues, decreasing adverse effects and improving therapeutic results (Mihranyan et al., 2012).

**Nanoparticle-based Drug Delivery**

Medication administration with precision, extended release, and improved bioavailability are all made possible by the use of nanoparticles as carriers. Solid lipid nanoparticles (SLNs), liposomes, and polymeric nanoparticles are a few examples of these. By using small needles to deliver medications through the skin's outer layer, microneedle drug delivery offers a painless and efficient substitute for traditional injections.

1) Implantable drug delivery systems: medication-eluting stents and implanted pumps provide extended and tailored medication release, making them appropriate for chronic pain treatment and cardiovascular issues. Transdermal medicine administration uses patches to release pharmaceuticals continuously via the skin. This procedure is convenient and improves patient compliance.

2) Intranasal medication delivery involves administering pharmaceuticals through the nasal route, allowing for quick absorption into the bloodstream. Targeted drug delivery involves the use of ligands or antibodies to specifically deliver medications to particular cells or tissues. This approach reduces the occurrence of off-target effects and improves the effectiveness of the treatment (Nohynek et al., 2008).

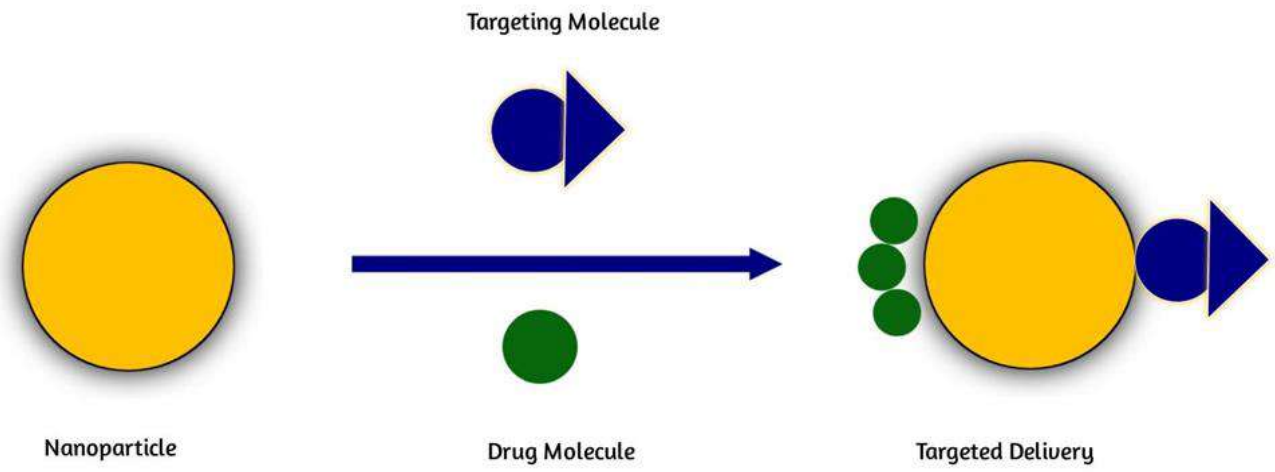



**Fig. 5:** Nanoparticle-based targeted drug delivery

3) Inhalation medicine administration involves the direct delivery of medications to the respiratory tract by aerosols. This method is highly effective for the treatment of asthma and COPD.

**Medical Nanoparticle Physiochemistry**

Medical nanoparticles possess distinct physicochemical characteristics that render them versatile and advantageous. These molecules' properties determine their drug carrier, imaging, diagnostic, and therapeutic effectiveness (Hartshorn et al., 2018). These nanoparticle physicochemical properties are relevant in medicine:

1) Size and scope: Nanoparticles usually measure 1–100 nanometers. Drug integration and ligand or targeting agent attachment are possible because to their small size and high surface area-to-volume ratio (Gupta et al., 2022).

2) Surface charge and functional groups: Nanoparticles may be surface modified to gain specific charges and functional groups for targeted connections to biological molecules, cells, and tissues. This approach of material surface modification delivers medications to particular sites and improves cell uptake (Ho et al., 2019).

3) Drug encapsulation and release: Nanoparticles may encapsulate hydrophobic and hydrophilic medicines. This encapsulation improves therapeutic effectiveness and reduces side effects by preventing degradation and allowing regulated release.

4). Biocompatibility and biodistribution: To minimize bodily reactions, nanoparticle biocompatibility is essential. Surface and material changes impact nanoparticle dispersion throughout the body and how they are digested and removed (Anjum et al., 2021).

5) Targetability: Nanoparticles can selectively bind to cells or tissues through ligand-receptor interactions, antibody targeting, or stimuli-responsive processes. Nanoparticles with specific targeting properties minimize unintended side effects and enhance the delivery of pharmaceuticals to affected regions (Afzal et al., 2022; Abdel-Mageed et al., 2021).

**Nanoparticle Drug Delivery Systems for Disease Treatment**

Nanoparticle Drug Delivery Systems (DDSs) have the potential to efficiently treat illnesses due to their numerous advantages over conventional drug delivery systemsTherapeutic systems that use nanoscale carriers to transport genetic material or drugs to specific body regions improve patient outcomes, decrease side effects, and boost treatment effectiveness (Torchilin, 2014).

One potential application of nanoparticle DDSs is targeted drug delivery. This can be accomplished by modifying the surfaces of the DDSs with ligands or antibodies that specifically attach to receptors found on the desired cells, tissues, or organs. Targeted delivery of drugs reduces exposure to healthy tissues, resulting in higher drug concentration at the illness site and improving therapeutic effectiveness (Zhang et al., 2010). Improved drug solubility: The ineffectiveness of numerous drugs, particularly those used to treat cancer, is due to their inadequate solubility. The utilization of nanoparticles to encapsulate hydrophobic medications has been found to enhance their solubility and bioavailability, thereby facilitating improved drug delivery and uptake efficiency (Worrall et al., 2018).

Anoparticle DDSs have a wide range of applications, encompassing diseases such as cancer, infections, inflammatory disorders, and neurological conditions, among others. The ongoing progress in nanotechnology and drug delivery is anticipated to sustain the significant impact of nanoparticle DDSs on disease treatment and the medical field (Xie et al., 2019).

**Nano-targeted Delivery System Drawbacks**

Nanotechnology-based delivery systems hold immense potential for enhancing drug delivery and therapeutic outcomes. These advances have several challenges that must be overcome to be applied in therapeutic settings. To ensure biocompatibility and reduce harmful effects, nanoparticles must be tested in biological systems (Yagublu et al., 2022). Safety issues arise because certain nanoparticles might cause immunological reactions or accumulate in organs. Nanoparticle delivery systems are complex and costly to produce and expand. Manufacturing procedures must be standardized and reproducible to ensure product consistency. Optimizing medication delivery and treatment efficacy requires excellent nanoparticle deposition of therapeutic ingredients. With poor encapsulation efficiency, less material can be transferred to its destination. Gradual nanoparticle instability may diminish therapeutic activity or drug release (Thanki et al., 2013).

Sufficient storage conditions and enhanced stability of nanoparticles are indispensable factors in preserving the operational longevity of nano-targeted delivery systems. It is ideal to comprehend the post-administration trajectory of nanoparticles to accurately forecast their biodistribution, duration of circulation, and elimination from the body. The utilization and fabrication of nanoparticle-based systems might entail supplementary expenditures in contrast to traditional approaches to drug delivery. It is critical to prioritize the affordability and accessibility of these technologies to facilitate their broader integration into clinical settings. Regulatory agencies mandate the submission of comprehensive safety and efficacy data before the clinical approval of medications or delivery systems containing nanoparticles. Adherence to regulatory obligations further complicates the process of commercializing nano-targeted delivery systems (Zhang et al., 2013).

Nevertheless, nanotechnology research and development are overcoming several difficulties. Novel nanoparticle formulations, surface modifications, and targeting mechanisms are being developed to increase nano-targeted delivery

systems' effectiveness and safety. To overcome these limitations and effectively use nano-targeted drug delivery in medicine, regulatory authorities, physicians, and scientists must form multidisciplinary collaborations.

**Discussion and Future Directions**

Drug delivery methods based on nanomaterials have revolutionized medicine by enhancing medication control and accuracy. These nanoscale carriers deliver medicinal molecules to specified body locations, improving pharmaceutical effectiveness, side effects, and patient outcomes. This article discusses nanoparticle-based medication delivery technologies and their possibilities. Nanomaterial-based drug delivery methods, which envelop nanoparticles in ligands or antibodies that bind to specific cell receptors, improve the way drugs are administered. This focused strategy enhances the medication concentration at the specific location where it is intended to take effect while reducing the amount of contact with healthy tissues. Pharmaceuticals that are hydrophobic can be encapsulated by nanoparticles, which increases both their solubility and their bioavailability. This feature improves the administration of medication and allows the utilization of medications with low solubility in water for medical purposes. Nanomaterial-based drug carriers enable the gradual and regulated release of drugs over an extended period. The implementation of this regulated release pattern might result in a decrease in the frequency of administration and the maintenance of consistent and steady levels of therapeutic medication within the body. Nanoparticles can transport numerous therapeutic compounds at the same time, which makes it easier to provide combination therapy for complex disorders like cancer. This strategy has the potential to improve the effectiveness of treatment and overcome the development of resistance to drugs.

Nanomaterial-based drug delivery systems might provide individualized therapy depending on patient genetic profiles or sickness phases. For gene therapy, nanoparticles are being developed to transport genetic material like siRNA or CRISPR-Cas9. This offers new molecular illness management and genetic abnormality management options. Immunomodulatory medications like immune checkpoint inhibitors and immunological adjuvants for cancer and infectious disorders may be delivered better using nanomaterials. It may enhance immunotherapies. Pharmaceuticals may be released by nanomaterials in response to pH, temperature, or enzyme activity. This technology accurately controls medicine delivery in location and timing to enhance therapeutic success. Nanoparticles can identify early illness and monitor treatment as imaging contrast agents. Medical imaging and administration allow theragnostic uses. Biodegradable nanomaterials are being developed to clean nanoparticles and avoid buildup. Nanomaterial-based pharmaceutical delivery systems will become increasingly essential in disease treatment and tailored medicine as nanotechnology improves. Nevertheless, in order to effectively employ these groundbreaking technologies in a clinical setting, challenges related to biocompatibility, manufacturing, regulatory approval, and long-term safety need to be addressed.

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Chapter 31

# Nanoparticles in Diagnostic Imaging: Advancements and Challenges

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**ABSTRACT**

Medical imaging technologies facilitate the expeditious identification and assessment of a diverse array of pathological conditions. To enhance their sensitivity and utility, numerous imaging methods, including CT scan and MRI, depend on the administration of contrast chemicals intravenously. Although the present iteration of contrast chemicals has facilitated expedited diagnostic procedures, they continue to exhibit certain unfavorable limitations, such as inadequate tissue selectivity and concerns regarding systemic toxicity. Researchers are currently developing a new type of contrast agent that may overcome many hurdles and offer more precise and sensitive information, thanks to advancements in nanotechnology and materials science. This chapter provides a concise overview of the primary categories of nanotechnology-based contrast agents used in various imaging technologies. It also emphasizes the advancements made in their development, the obstacles that need to overcome, and the biological interactions that influence the behavior of these contrast agents in living organisms. Additionally, it describes significant topics in medical nanotechnology, such as stealth and targeting.

**KEYWORDS**
Multimodal imaging, MRI, CT, PET, Contrast agent, Nanotechnology, and diagnostic imaging

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## INTRODUCTION

In recent years, there has been a notable increase in the field of nanomedicine, which pertains to the progression of medical technology through manipulations at the nano-scale. Significant progress has been made in various domains, including accurate medication delivery (Yao et al., 2014), controlled drug release (Zhou et al., 2014), tissue manipulation (Johnson et al., 2010), and laboratory-based diagnostics (Chen et al., 2015). The domain of contemporary medicine is being considerably influenced by improvements at the nano-scale, such as regulated distribution, increased sensitivity, and the use of multifunctional materials. The field of diagnostic imaging is experiencing a growing recognition of the substantial importance of nanotechnologies. Diagnostic imaging refers to a diverse array of technologies utilized to analyze the internal anatomical components of the human body with the aim of identifying various medical conditions. The utilization of these methods is increasingly playing a vital role in the diagnostic options available to medical professionals. (Jokerst et al., 2011).

Magnetic Resonance Imaging (MRI), Positron Emission Topography (PET) and Computed Tomography (CT) are increasingly being used in hospitals worldwide because of their exceptional ability to generate detailed images of the body. Contrast agents are often utilized by healthcare professionals to aid in the visualization of abnormalities on diagnostic images. These agents are substances that interact with incoming radiation, causing observable alterations in the resultant picture. Contrast chemicals play a crucial role in diagnostic imaging by significantly enhancing the sensitivity of imaging techniques, hence enabling the detection of disorders that were previously undetected (Xu et al., 2021). Nanotechnology is exerting a substantial influence on the area of diagnostic imaging through the advancement of innovative and enhanced contrast agents. Researchers are continuously enhancing the level of sensitivity, biodistribution and biocompatibility profile of different contrast materials by Nano-scale modifications.(Larrañeta et al., 2016).

This chapter aims to provide a comprehensive review of the prominent technologies employed in diagnostic imaging, as well as the utilization of nanotechnology to augment existing methodologies. In addition, we will present a comprehensive examination of the several obstacles that need to be tackled during the development of a novel nanomaterial for medical applications, along with pertinent details on biological interaction.

**Biological Interactions**

To fully understand the obstacles and limitations associated with developing a novel contrast agent using nanoparticles, it is essential to grasp the underlying mechanisms by which nanoparticles interact with the human body. When a large molecule, such a tiny particle of contrast agent, is delivered into the body, it is frequently identified as a foreign substance by the immune system. This identification activates many clearance pathways. The human body employs various processes to effectively eliminate harmful compounds from the bloodstream, including renal filtration and absorption by cells and components of the mononuclear phagocyte system (MPS) found in the liver, spleen, and lymph nodes. Therefore, a notable design obstacle associated with nanoparticle systems pertains to the need for modifications that can extend their circulation duration, while simultaneously preserving their biological compatibility and eventual degradability for removal from circulation once they have successfully achieved their intended therapeutic objective (Dhal et al., 2020). The size and surface features of nanoparticles are the main determinants of their interaction with biological systems. (Weng et al., 2017).

**Immune System and Clearance Routes**

The immune system plays a crucial role in eliminating nanoparticles, particularly through its major histocompatibility complex (MPS) and the complement system. The acronym "MPS" refers to the specialized phagocytes in the immune system that are responsible for the process of phagocytosis and the subsequent removal of foreign substances (Guryev et al., 2020). The complement system refers to a group of plasma proteins that undergo activation when encountering exogenous substances. The initiation of a cascade can lead to the opsonization process, wherein proteins are adsorbed onto the surface of nanoparticles. This process serves to designate the nanoparticles for absorption and subsequent cleaning by the cells of MPS (Wang et al., 2020). Hence, the possession of a surface that hinders the opsonization of complement protein and antibodies confers benefits to nanoparticles, resulting in an extended period of particle circulation. Prior research has indicated that surfaces with hydrophobic properties and high charges are more susceptible to opsonization, whereas surfaces with hydrophilic and neutral properties are less susceptible, making them “stealthier” (Xiong et al., 2009).

Moreover, it has been observed that particle size also has an influence, as bigger particles exhibit a higher rate of clearance compared to smaller particles. However, it is important to make sure that particles are not too tiny, as materials with a hydrodynamic size smaller than 5.5 nm tend to be quickly eliminated by the kidneys (Huang et al., 2020). The size of a particle also affects its capacity to exit the bloodstream and subsequently penetrate different types of tissues, such as tumors. Moreover, it is important to consider that beyond its intended lifespan, the nanoparticle must possess the capability to undergo degradation or be eliminated from the organism in order to mitigate any long-term toxicity (Chan et al., 2015; Zhang et al., 2019).

**Targeting**

The concept of targeting, which refers to the capacity of a nanoparticle to specifically recognize and accumulate within a certain tissue, is frequently highlighted as a prominent advancement in the field of nanotechnology (Liu et al., 2016). Toxic chemotherapeutic medicines can be administered to tumors at far larger dosages by specifically targeting malignant tissues, therefore reducing the adverse side effects commonly associated with chemotherapy (Ovais et al., 2020). By employing targeting, contrast substances can offer far more valuable and intricate data on specific distinctions in tissue types and boundaries, hence facilitating the early detection of malignant or aberrant tissues compared to a systemic contrasting agent (Dash et al., 2018). By providing contrast materials specifically to a targeted tissue, it is now possible to reduce the amount of material used, leading to cost savings and reducing exposure to potentially dangerous compounds (Xiang et al., 2018). There are generally two basic types of targeting methods: passive targeting and active targeting. Passive targeting is an approach that takes advantage of the physiological abnormalities or events that naturally occur in a diseased state to promote the specific accumulation of a substance. The enhanced permeability and retention (EPR) effect found in solid tumors is widely acknowledged as a notable example of this specific targeted phenomena (Yang et al., 2014). The phenomenon of macromolecules being selectively entered and accumulated in tumors can be ascribed to a confluence of factors, including improperly designed and permeable blood arteries, alongside a weak lymphatic drainage system. (Xie et al., 2019).

Concept of contrast agent based on NPs in various modalities of imaging has been shown in Fig. 1. In general, biomarkers that are recognized are those that are either exclusively expressed in the target tissue or significantly more abundant in comparison to healthy tissues. Moreover, in addition to the intrinsic indirect targeting effects, further specificity is incorporated. Moreover, as a consequence of the material's unique affinity for a receptor on the surface of cells, a process of receptor-mediated endocytosis is frequently triggered, leading to the internalization and intracellular buildup of the contrast agent (Fu et al., 2009). The use of active targeting has been investigated for the purpose of

imaging many illnesses, such as cancer (Fig. 1) (Zhang et al., 2021), myocardial infarction (Wang et al., 2019), and inflammatory endothelial cells which may indicate atherosclerosis (Miller et al., 2017). The subject of targeting is extensive and will not be further elaborated upon in this assessment. under more detailed analysis of active and passive targeting under different situations.

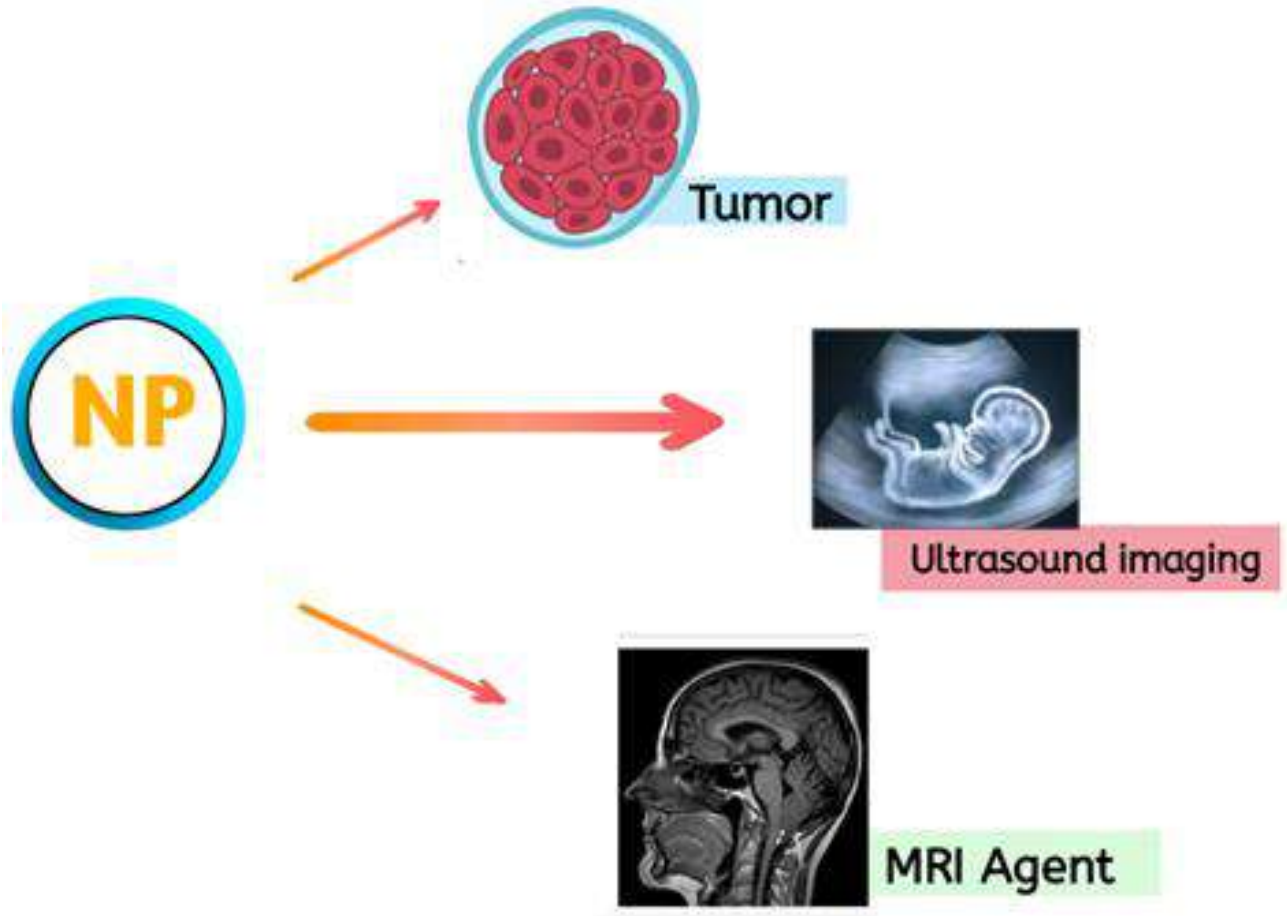


**Fig. 1:** Concept of contrast agent based on NPs in various modalities of imaging.

**Magnetic Resonance Imaging**

In the field of soft-tissue medical imaging and diagnostics, magnetic resonance imaging (MRI) is widely recognized as a potent and indispensable technique. The technology exhibits a notable level of temporal and geographical precision and is often regarded as a secure and non-intrusive method because of the lack of ionizing radiation (Yan et al., 2011).

The current investigation centers on the manufacturing process of altered RBC-UCNPs and their subsequent utilization in mice with triple-negative breast cancer through the use of UCL imaging, MRI and PET imaging techniques. Magnetic Resonance Imaging (MRI) operates by subjecting soft tissue to a robust external magnetic field, which then interacts with the many water protons present in the tissue (Lu et al., 2014). The alignment of magnetic moments in protons is induced by the application of a magnetic field. The tissue is subjected to a radiofrequency (RF) pulse, resulting in the precession of protons in a particular direction and frequency, which is unique to the tissue type in which the proton is located. After the RF pulse is removed, the protons go back to their initial ground state, which is referred to as relaxation (Tian et al., 2019). The relaxation time, which refers to the time it takes for the plasma to return to its ground state, consists of two components: longitudinal relaxation time (T1) and transverse relaxation time (T2). These components correspond to the breakdown of the angular momentum vector of the protons (Lu et al., 2014).

**Computed Tomography Scanning**

Computed tomography (CT) scanning is a medical imaging technique that utilizes X-rays to generate high-resolution cross-sectional pictures of various bodily tissues. The process usually involves capturing many X-rays from various angles to create picture "slices" that may be combined to create a comprehensive collection of cross-sectional images. The use of this technique is prevalent in the imaging of the pelvis, head, abdomen, and chest. At present, contrast agents for CT scans commonly employ iodine or gadolinium-based compounds. Nevertheless, these agents encounter several challenges, such as non-specific biodistribution and short circulation half-lives (Feng et al., 2013). Fig.2 depicts the application of NPs in

breast cancer bearing mice.

**Nanoparticles for CT**

Bi2 S3 nanoparticles coated with polymers emerged as a highly promising and unique contrast agent specifically intended for use in CT imaging. Typically, the production of these nanoparticles entails a two-step procedure. According to Sarkar et al. (2016), the procedure of forming Bi2 S3 cores entails the precipitation of bismuth citrate and sodium sulphide, which is subsequently followed by the application of a polymer coating, such as polyvinylpyrrolidone (PVP), to improve colloidal stability, biocompatibility, and resistance to the MPS. In comparison to contrast agents based on iodine, the particles exhibited a fivefold increase in X-ray absorption. In addition, they exhibited significantly prolonged circulation half-lives, exceeding a duration of two hours. The possible limitations associated with these contrast agents are to challenges in precisely managing the morphology of the Bi2 S3 nanoparticles and a dearth of adequate techniques to change their surface. The utilization of the iodine-based contrast material in the scans did not enable the precise identification of the tumor. A distinct investigation employed 30 nm nanoparticles of gold that were coated with PEG in a mouse model. The study demonstrated the efficacy of these nanoparticles as imaging agents for blood pools and hepatoma (Sui et al., 2012).

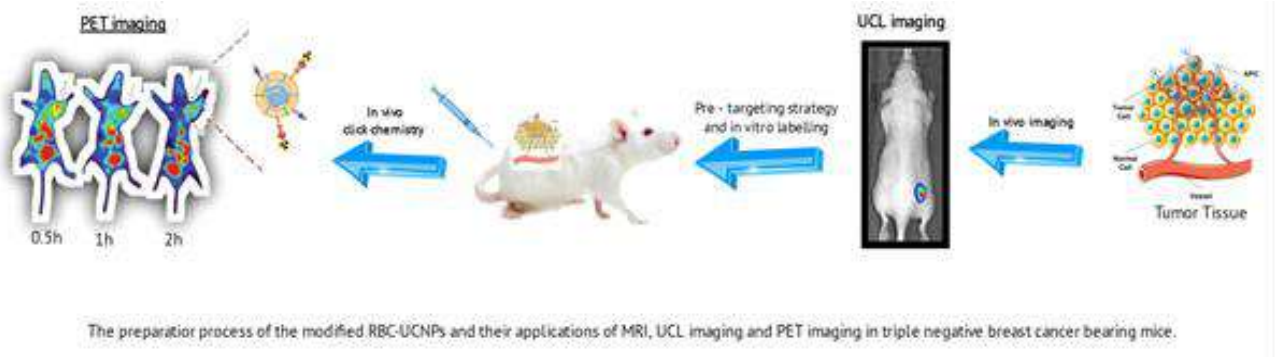


**Fig. 2:** The preparation process of the modified RBC-UCNPs and their applications of MRI, UCL imaging and PET imaging in triple-negative breast cancer-bearing mice.

**Fluorescence Imaging**

Fluorescent proteins and organic fluorophores are commonly used in biological research to investigate a range of cellular and molecular processes. Xu et al. (2020) employ these strategies in vitro and in small animal models to attain a sensitive and non-invasive approach. These minuscule chemical entities can be integrated into different substantial molecules and employed in conjunction with methods like confocal microscopy and flow cytometry to examine the interaction of these substantial molecules with cells in a laboratory environment (such as internalization), as well as their distribution and ultimate destiny in living organisms. Fluorescent chemicals are frequently utilized in cellular labeling and cell tracking applications. Currently, the utilization of common fluorophores for fluorescence-based imaging is constrained by various restrictions. The limitations include inadequate penetration of skin and tissue, broad emission and absorption ranges, low signal intensity, limited imaging time in living organisms, and heightened vulnerability to photobleaching, which is the gradual degradation of the fluorophore caused by exposure to stimulating light energy (He et al., 2011). Consequently, the application of fluorescent imaging methods in living creatures has been restricted, leading to their full omission from clinical utilization.

Recent breakthroughs in fluorescent nanomaterials are addressing the limitations of conventional fluorophores and enabling the application of fluorescence imaging methods for disease diagnosis and a deeper understanding of disease progression at the systemic, molecular and cellular levels (Zhou et at., 2011).

**Semiconductor Quantum Dots**

Semiconductor quantum dots (QDs) are particles of nanoscale size, typically composed of heavy metal combinations such as CdSe, CdTe, InP, InAs, and others (Liu et al., 2014). These QDs possess distinctive optical characteristics that render them highly suitable for substituting traditional organic fluorophores in fluorescence-based imaging (Rao et al., 2014). In contrast to organic fluorophores, QDs possess the capability to absorb light throughout a broad spectral range. However, their emission spectrum remained limited, resulting in a consistent emission of a specific wavelength of light regardless of the input wavelength (Liu et al., 2015). The aforementioned characteristics enable the utilization of QDfluorescent probes in optical multiplexing applications, wherein distinct probes can be illuminated using a common light source while exhibiting varying color emissions as shown in Fig. 3.(Liu et al., 2019).

Moreover, QDs exhibit exceptional luminosity, have remarkable photostability, and feature substantial molar extinction coefficients. These characteristics render them very suitable for in vivo imaging applications due to their reduced sensitivity to tissue depth (Xu et al., 2020). QDs have been employed for the purpose of labeling cellular constituents, monitoring cell migration and differentiation, capturing images of blood vessels and lymph nodes, and visualizing the distribution of

nanomaterials within tissues. A significant limitation of QD fluorescent tags is the presence of significant apprehensions regarding potential hazardous side effects. QDs are often composed of heavy metals, such as Cd, which is recognized for its poisonous properties in its unbound state (Cd2+). Furthermore, there is a lack of comprehensive understanding regarding the degradation mechanisms of QDs that exceed the renal excretion threshold of 5.5 nm hydrodynamic (Gu et al., 2016).

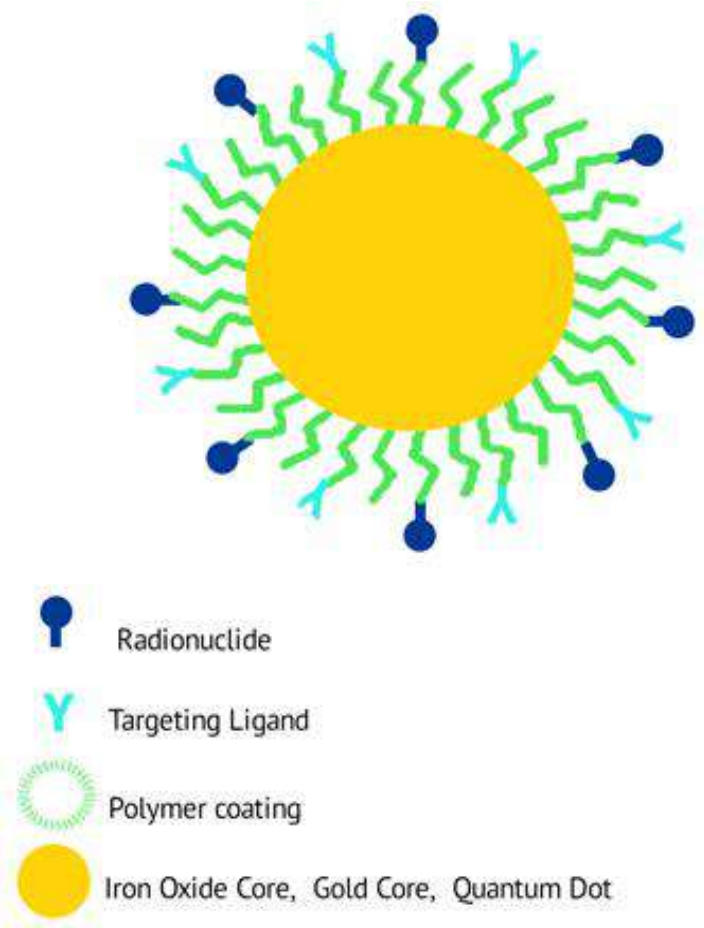


**Fig. 3:** The illustration depicts a hypothetical combination of PET radiotracer with a fluorescent Quantum Dot, X-ray adsorbing Gold, or MRI contrast agent iron oxide core, serving as multimodal imaging contrast agents.

**Discussion and Future Perspectives**

The utilization of diagnostic imaging, coupled with conventional contrast agents, has facilitated the diagnosis of numerous ailments in a way that is minimally invasive (Li et al., 2012). Notwithstanding the considerable achievements, the present iteration of contrast agents continues to encounter numerous challenges that impede their ability to attain even greater levels of clinical efficacy (Yang et al., 2012). One of the primary constraints associated with contemporary contrast technology pertains to its limited tissue selectivity. Most existing agents, such as different iodine or gadolinium formulations for CT and MRI, tend to spread out of the blood vessels and into different types of tissues, making it more difficult to detect abnormalities like plaques or tumors (Di et al., 2008). Nanotechnology presents the potential for more precise regulation of bio-distribution characteristics through the utilization of passive and active targeting methodologies (Qian et al., 2009).

Furthermore, our present contrast materials include inherent drawbacks that restrict their applicability in specific scenarios or patient demographics, in addition to concerns regarding specificity. The prolonged toxicity of gadolinium-based chemicals used in MRI (magnetic resonance imaging) has been a subject of concern, as noted by Li et al. (2008) and Li et al. (2010). Gadolinium should not be given to persons with impaired kidney function since it is associated with the development of nephrogenic systemic fibrosis in these patient populations (Liu et al., 2009). Each imaging approach possesses distinct advantages and constraints: MRI offers favorable spatial resolution in soft tissues, yet it exhibits limited sensitivity (Chen et al., 2012). CT, on the other hand, offers exceptional structural imaging but lacks comprehensive functional information. PET/SPECT, on the other hand, enables quantitative assessment of functional processes but is hindered by inadequate spatial resolution (Wang et al., 2007). Optical/fluorescence imaging, despite its remarkable sensitivity, is constrained by inadequate tissue penetration. To optimize the informational value of a diagnostic image, it is advantageous to integrate numerous modalities for the simultaneous acquisition of various kinds of information (Dong et al., 2011). Multimodal imaging refers to the simultaneous utilization of multiple imaging techniques to gather diverse forms of information, hence enhancing the precision and comprehensiveness of diagnostic assessments. Currently, the integration of CT/PET scanning is a commonly utilized example of multimodal imaging. According to Zhang et al. (2009),

the CT scan provides extensive anatomical and structural information that may be overlaid with the functional PET data. The feasibility of acquiring dual-type data and superimposing them for the diagnosis of various medical diseases is achieved by integrating a positron emission tomography (PET) radiotracer into a computed tomography (CT) contrast agent. Table. 1 explains the advantages and disadvantages of medical imaging modalities.

**Table 1:** Medical imaging modalities, their advantages, disadvantages and effects of nanotechnology

| Modality | Advantages | Disadvantages |
|---|---|---|
| MRI | Noninvasive | Low sensitivity |
| | Evaluating anatomy and function of tissues | Expensive equipment |
| | High spatial resolution | |
| | Provide good soft tissues contrast | |
| CT | High sensitivity anatomical information | Limited functional information |
| | 3D imaging | Poor soft tissue contrast |
| | | Expensive equipment |
| US | Safe | Unable to differentiate between tissues |
| | Low cost | With similar acoustical properties |
| | Real- time measurement | Low sensitivity and resolution |
| NIR | Deep tissues penetration | Low sensitivity |
| | Low tissue absorption and scattering | Problems in aqueous solutions |
| | Minimal auto fluorescence | |
| PET | Provide biochemical information | Limited anatomical information |
| | High sensitivity | Expensive equipment |
| | 3D imaging | |
| SPECT | Detecting multiple probes simultaneously | Low sensitivity |

In addition, there are concerns regarding specific nanoparticle formulations due to the documented or suspected toxicity associated with their degradation byproducts (Wang et al., 2013). Quantum dots, such as those found in quantum dots, include cadmium, a cytotoxic element. Therefore, the release of cadmium, either through the destruction of the particles or the gradual leaching of accumulated quantum dots, is a significant concern. The toxicity of several nanomaterials is now not well comprehended, which will hinder their widespread usage until these inquiries are resolved (Yi et al., 2004). The relationships between nanoparticles and biological medium are intricate and subject to various circumstances, some of which remain inadequately comprehended. Therefore, further research is required to analyze and regulate these interactions in order to create nanoparticles that possess the appropriate pharmacokinetic and degradation characteristics (Mai et al., 2006).

Moreover, a significant portion of the chemical processes employed in the synthesis and modification of nanoparticles necessitates optimization and refinement to ensure the production of consistent and dependable products that are appropriate for clinical applications. Active targeting approaches, although shown considerable success in laboratory settings, encounter numerous obstacles in terms of achieving reproducible and cost-effective synthesis (Shen et al., 2013). Further efforts are required to advance the development of synthesis methods that are both scalable and repeatable. Characterizing the stability of nanotechnologies during storage is a crucial aspect of their commercialization. The high-energy state of nanoparticles is attributed to their significant surface-area-to-volume ratio. (Thwala et al., 2023).

The progress in comprehending the immunological interactions of nanoparticles is expected to assist in creating novel materials that possess specific pharmacokinetic features and improved biocompatibility. Enhancing the understanding of the toxicological impacts of nanoparticles and their breakdown byproducts can improve the knowledge of creating safe materials for healthcare settings. These technological breakthroughs will not only improve our capacity to create efficient nanoparticles for medical purposes, but they will also provide regulatory agencies and the general public with the assurance needed to approve the broader clinical utilization of nanomaterials. The convergence of progress in multiple fields will result in the development of sophisticated nanotechnologies that will have substantial consequences for clinical diagnostics and therapeutics (Wang et al., 2011).

**Conclusion**

The utilization of diagnostic imaging technologies is seeing tremendous growth, hence pushing the limits of our diagnostic capacities. By employing these methodologies, medical professionals are capable of detecting diseases at an earlier stage and with more accuracy, as well as acquiring a diverse range of structural and functional data pertaining to the human body. The contrast agents play a crucial role in these approaches, enabling the acquisition of enhanced spatial resolution and heightened sensitivity in diagnostic pictures. To sustain the advancement of diagnostic imaging technologies, it is imperative to develop novel and improved contrast agents. This is necessary to address the challenges associated with conventional materials and to further enhance the efficacy and precision of these techniques. Nanotechnology presents a promising avenue for the exact manipulation and regulation of the physical and chemical properties of contrast materials, thereby addressing issues related to toxicity, imaging duration, tissue selectivity, and

signal intensity. To develop nanomaterial contrast agents that are both efficient and effective, it is imperative to possess a comprehensive comprehension of the diverse biological interactions that the particles will undergo. By exerting meticulous control over the dimensions of particles and the characteristics of their surfaces, it becomes possible to alter the pharmacokinetic profiles of these nanomaterials. The integration of active and passive targeting approaches has the potential to enhance the precision of imaging modalities, hence enabling timely detection and intervention for critical medical disorders, such as cancer. In addition, nanoparticles enable the integration of diverse functional substances into a unified entity, hence facilitating the development of multimodal contrast agents that can offer concurrent structural and functional insights into the human body. Therefore, advancements in nano-scale contrast agents will be crucial in further improving our diagnostic imaging capabilities in the future.

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Chapter 32

# Infectious Diseases Control: Nanoparticles in Antimicrobial Strategies

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**ABSTRACT**

The development and use of novel antimicrobials with more potent bactericidal properties has traditionally been seen as the suitable approach to combat the escalating menace of antimicrobial-resistant illnesses. However, the period of time from when a new antibiotic is introduced to when bacterial pathogens develop resistance is steadily getting shorter. This implies that alternate approaches should be pursued instead of only focusing on developing more effective antimicrobials. The purpose of this chapter is to explore the following inquiries: (1) Are there any methods available to bypass current antibiotic resistance mechanisms? (2) Can the current status of antibiotic resistance be reversed? (3) What measures may be taken to avoid the development of antimicrobial resistance against novel infection-control techniques, such as nano-antimicrobials? Relying on the natural process of antimicrobial-resistant bacteria being outcompeted and limiting the usage of antibiotics is not a dependable answer to the current antibiotic dilemma we are facing. Promising infection control techniques based on nanotechnology, which are not antibiotics, are being developed. Simultaneously, it is important to closely watch the fast emergence of new resistance mechanisms that may arise when novel techniques are used in global clinical practice. This indicates the need to prioritize research and development efforts towards creating effective combinations of established antibiotics and novel nano-antimicrobials, while ensuring that the emergence of new mechanisms of antimicrobial resistance is prevented. However, the latter proposal necessitates a shift in the research and development priority.

**KEYWORDS**
Infection therapy; Antibiotic-resistance; Combination strategies: Nano-antimicrobials; Reactive oxygen species, Bacterial infections; Infection prophylaxis

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## INTRODUCTION

The proliferation of antibiotics has greatly contributed to the emergence of antibiotic-resistant bacteria posing a growing challenge to our capacity to effectively address bacterial infections (Dolgin, 2010). Bacterial infections that are resistant to antimicrobial agents lead to higher rates of illness and death, impose significant financial burdens on the healthcare system, result in substantial economic damages and are projected to become the leading cause of death by 2050 (Cassini et al., 2019). While the advancement of improved antimicrobials has been widely regarded as the appropriate course of action to avert the realization of this unfavorable forecast, the progress in developing new antibiotics, obtaining regulatory approval, and subsequently translating them into clinical practice is characterized by a sluggish speed (Walsh, 2000). The pharmaceutical industry is shifting its attention from developing new antibiotics to developing drugs for other diseases that have a higher rate of return on investment. This is due to the decreasing duration of their effectiveness before the emergence of the first resistant bacterial strains (Dik et al., 2015), and the growing uncertainty surrounding the return on investment. Regarding novel infection-control techniques, there is a high level of anticipation for nanotechnological advances. Hence, this review initially provides a concise overview of innovative infection-control strategies utilizing nanotechnology. It then delves into the mechanisms underlying antibiotic resistance to look into the following queries:
(1) Can current antibiotic-resistance mechanisms be overcome?
(2) Is it possible to reverse the effects of antibiotic resistance?
(3) How may antibiotic resistance be prevented, especially in view of the latest advancements in infection control technology?

**Emerging new Infection-control Strategies**

Currently, the primary emphasis of research and development is on the implementation of nanotechnology to develop efficient solutions for infection control (Nye et al., 2018). One could argue that the large number of continuously emerging, extremely diversified, unique nano-antimicrobials being produced by nanotechnology are already offering more new treatments than are necessary. Nano-antimicrobials possess surface flexibility and small size, enabling them to effectively infiltrate pathogenic biofilms. The extracellular matrix shields biofilm occupants from antimicrobial assault and the host immune system (Maiden et al., 2018). Antimicrobials must possess the ability to traverse the aqueous channels present in infectious biofilms (Carpenter et al., 2011) without sticking to the walls of these channels. This is required to successfully penetrate the biofilms' entire thickness, which often reaches up to 50 μm (Ruden et al., 2019).

Whether a nano-antimicrobial is made of organic or inorganic components will determine which class it belongs in. Certain inorganic nano-antimicrobials exhibit enzyme mimicry, such as activities resembling peroxidase or deoxyribonuclease. The majority of bacterial strains and species are killed by ROS (Dizaj et al., 2014), yet many of these species have extremely unstable short lives (often ranging from micro- to milli-seconds). Nitric oxide (NO), another reactive nitrogen species produced by nanoparticles, is more persistent than reactive oxygen species (ROS) (Le Ouay and Stellacci, 2015). The bacteria are killed by it because it stops them from replicating their DNA or respirating, and it also stops them from adhering to one another (Busscher et al., 2020). Extracellular DNA (eDNA) plays a vital role in maintaining the structural integrity of a biofilm by serving as a key component of the extracellular matrix. An enzyme with a similar function to deoxyribonuclease is capable of hydrolyzing eDNA (Moriarty et al., 2014).

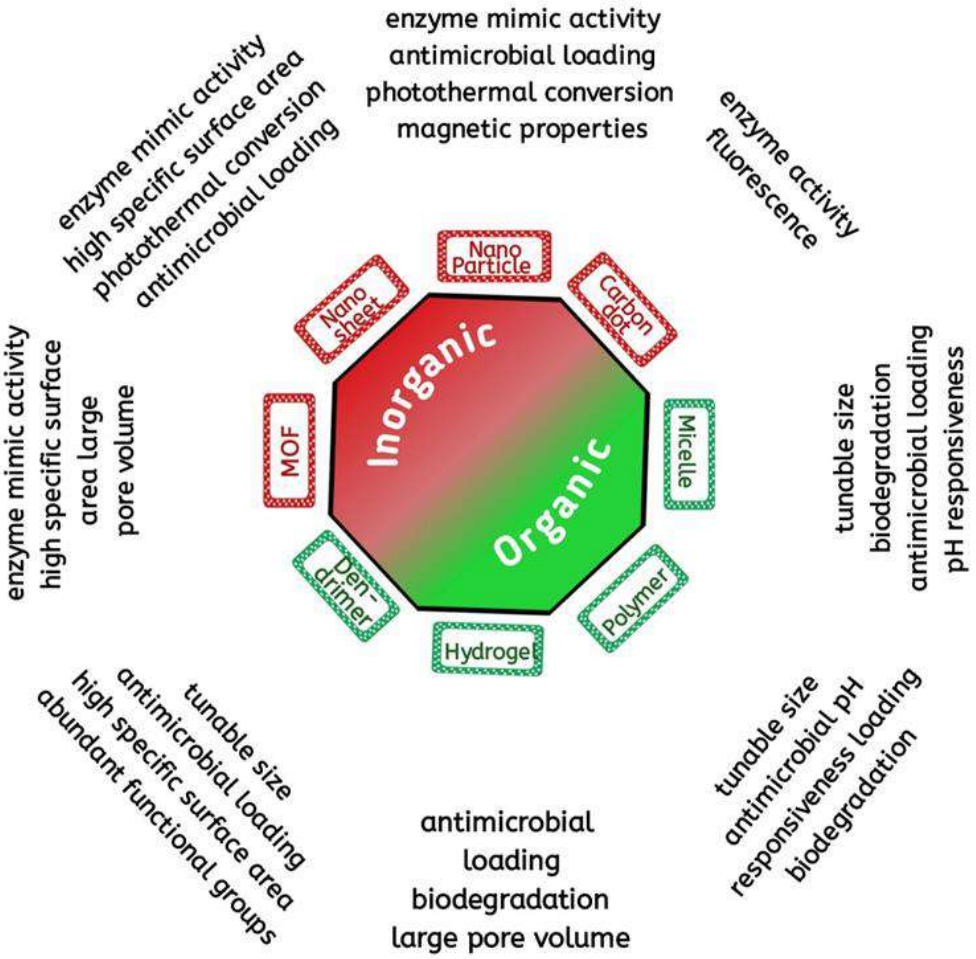


**Fig. 1:** An overview of advanced antimicrobials based on nanotechnology, distinguishing between organic and inorganic materials, and highlighting the potential benefits for infection control.

In the field of infection management, inorganic nano-antimicrobials have emerged as a novel approach due to their composition-dependent photothermal and magnetic capabilities. The local activation of photothermal nanoparticles has been extensively investigated for their potential in tumor suppression (Zhen et al., 2020). However, it is important to note that the heat generated by these nanoparticles may also be lethal to both human cells and bacteria. Photothermal nanoparticles can eliminate infectious bacteria, regardless of their Gram character, strain, or species. Figure 1 depicts an overview of nanotechnology-based enhanced antimicrobials, separating organic and inorganic materials and emphasising infection control advantages (Panáček et al., 2018).

On the other hand, the utilization of photothermal nanoparticles within the human body for the purpose of eliminating bacteria that are infected could potentially result in the loss of heat to neighboring tissues, which could lead to the incidence of collateral damage (Huh and Kwon, 2011).

Anti-tumor magnetic nanoparticles can be directed to a tumor location in a variety of methods, but it can be difficult to precisely target an infectious biofilm that is micrometer-sized and to ensure that the particles are dispersed across the biofilm's whole depth. Numerous nano-antimicrobials possess a substantial specific surface area, enabling them to liberate significant quantities of antibacterial metal ions, including silver ions (Sen et al., 2010). Graphene oxide sheets, with their large specific surface areas, have the potential to bind high quantities of antibiotics. According to Zhao and Drlica (2001), this could potentially be utilized as a method to improve the eradication of bacteria. Similar to a pharmacologist, the sharp edges of two-dimensional nanomaterials have the ability to infiltrate bacterial membranes and extract phospholipids, leading to significant harm to the cell wall. Metal-organic frameworks (MOFs) have a porous structure that offers a multitude of opportunities for the incorporation of antibacterial agents and the deposition of gaseous compounds or enzymes (Gleeson et al., 2013).

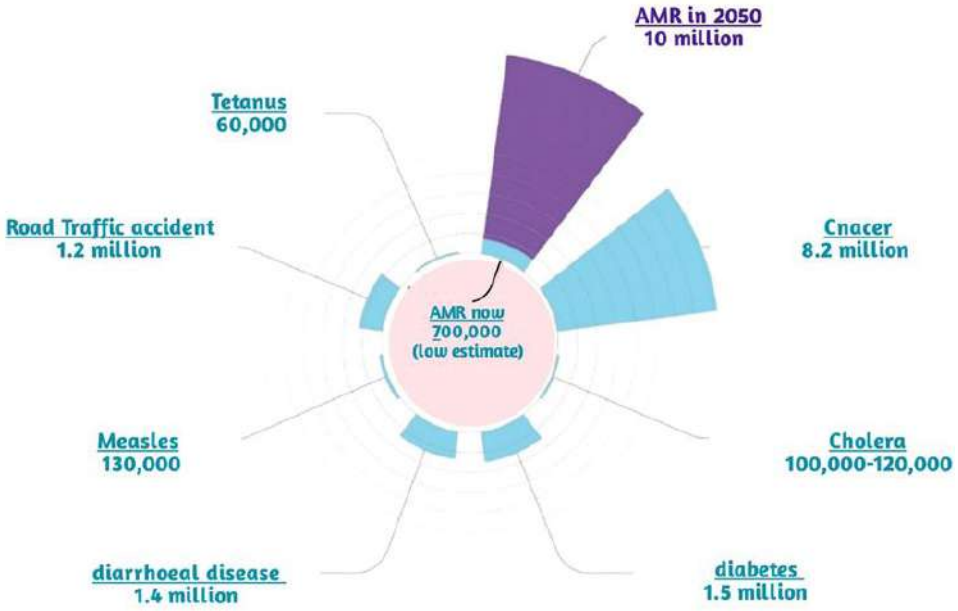


**Fig. 2:** Predictions for 2050 illness death rates, especially antimicrobial-resistant infections. Antimicrobial resistance review i.e., Global drug-resistant disease prevention, findings and recommendations.

The size benefits of organic nano-antimicrobials are comparable to those of inorganic nano-antimicrobials, with the added benefit of being able to be adjusted (Muszanska et al., 2012). Furthermore, organic nano-antimicrobials often possess the ability to undergo biodegradation. For example, chitosan is a naturally occurring organic polymeric material that is both biodegradable and non-toxic (McDougald et al., 2012). Its cationic groups can change the permeability of negatively charged bacteria's cell membranes. Chitosan also binds intracellular DNA, inhibiting mRNA transcription and protein synthesis (Harapanahalli et al., 2015). Antimicrobial-resistant infection mortality predictions are shown in Figure 2. Aloe vera, a natural herbal antibiotic, can suppress *Staphylococcus aureus* and *E. coli* growth. Aloe vera with biodegradable polyvinyl alcohol film coating have the potential to enhance wound healing and avoid surgical site infections (Whitchurch et al., 2002). Thus, nano-antimicrobials could be utilized to overcome resistance and for target specific action (Lee et al., 2018).

**Mechanisms of Antibiotic Resistance**

When discussing antimicrobial resistance, it is crucial to differentiate between the inherent ability of bacteria to resist antimicrobial agents when they are growing individually, and their ability to resist antimicrobial penetration when they are in their protective biofilm mode of growth, which is prevalent in most bacterial infections (Li et al., 2015). Before the term 'biofilm' was created, biofilm formation and the restricted efficacy of antibiotics in combating bacteria within it were recognized. Vinegar was discovered to be an effective antibacterial agent more than three centuries ago by Antoni van Leeuwenhoek. This chemical was specifically effective against bacteria that were on the surface of a bacterial layer, or "biofilm" (Fabbretti et al., 2019). Antibiotic resistance is primarily caused by biofilms and intrinsic processes such as immune bypass, efflux, enzymatic inactivation of antimicrobial agents, target shift, and immunity as shown in Figure 3.

**Biofilm Formation**

Complex microbial populations known as biofilms cling to and modify surfaces. Their structure is comprised of an extracellular polymeric matrix that is self-assembled and comprises extracellular DNA, proteins, lipids, and polysaccharides (Borriello et al., 2004). The process of matrix creation begins th the detection of adhesion forces that originate from the surface of a substratum (Boles and Horswill, 2011). Adhesion forces might vary depending on the specific surfaces from which they originate. Certain strains of biofilms, which aggressively adhere to hydrophobic silicone rubber, formed a large EPS matrix, resulting in reduced susceptibility to gentamicin. Nevertheless, these strains exhibited limited adhesion to hydrophilic silicone rubber covered with polymer brushes, creating the illusion of a planktonic condition (Markley et al., 2019).


**Fig. 3:** Exploring mechanisms and targets of antibiotic resistance. Bacteria gain resistance to antimicrobial agents via several methods, including the production of biofilms and the inherent or acquired capacity to withstand antibiotics due to genetic and resistance mechanisms used by bacterial pathogens.

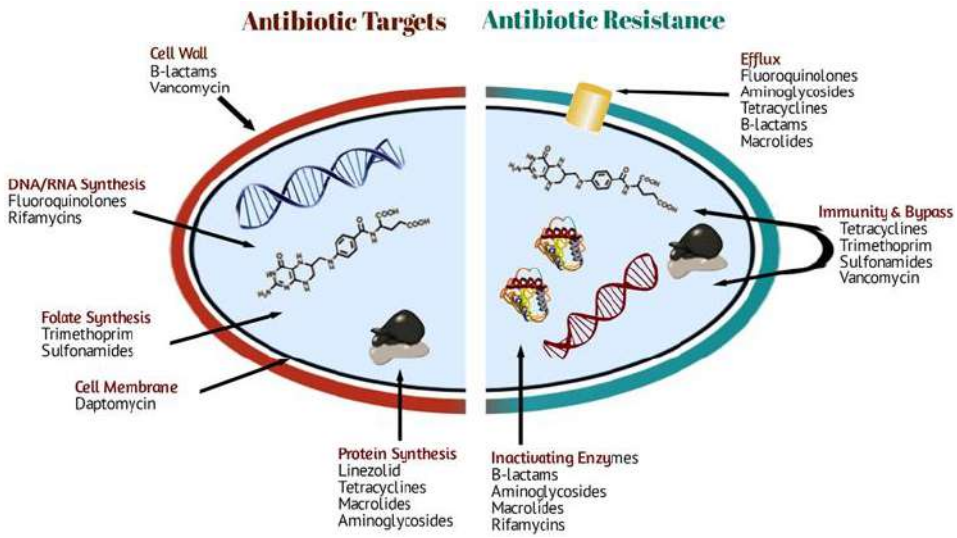


**Fig. 4:** Antibiotic efficacy depends on the antibiotic and the organism's genetics. Bacteria may acquire medication resistance by genetic mutation or gene transfer, notably in biofilms.

As a result, they exhibited a similar level of sensitivity to gentamicin as free-floating bacteria as shown in Figure 4. Within a biofilm, there exist gradients that vary with depth, including differences in the availability of food, pH levels, and oxygen content. These gradients become more pronounced in the lower areas of the biofilm (Wood et al., 2013). These gradients contribute to reduced metabolic activity, which is linked to an increased resistance to antibiotics. These bacteria, which exhibit either low metabolic activity or metabolic inactivity, enter a state of dormancy when they form a biofilm. Dormant bacteria exhibit antibiotic tolerance but lack resistance since they are phenotypic variations without any genetic alterations (Onesti et al., 2018).

**Enzymatic Inactivation of Antimicrobials**

Numerous types of bacteria possess the capacity to release enzymes that offer defense against antibiotics and other antimicrobial therapies. These defense mechanisms usually include an attachment of an acetyl or phosphate group to an

antibiotic's particular site, rendering it inert. Enzymes can render an antibiotic inactive via the obstruction of a particular moleculare site, as well as by instigating changes in the structure of the antibiotic molecule (Cai et al., 2019). β-lactamase has the ability to disrupt the core structure of β-lactam antibiotics, resulting in the inactivation of their active sites, which are either serine residues or Zn2+ (Ge et al., 2018). The presence of structural alterations and subsequent deactivation of active sites might impede the binding of antibiotic molecules to a specific protein due to steric hindrance, resulting in the development of antibiotic resistance. Many enzymes have currently been shown to be able to inactivate different kinds of antibiotics, such as macrolides, β-lactams, rifampicins, and aminoglycosides (Houry et al., 2012). The potential of synthetic anhydrotetracycline analogues as enzymatic tetracycline breakdown inhibitors in E. coli strains harboring tetracycline-destructive enzymes is still being investigated (Stewart, 2012).

**Target Modification**

As shown in Figure 4, different medicines kill bacteria by targeting specific parts inside or outside of cells. The components include genetic material, proteins, the cytoplasmic membrane, and other cell wall materials. Antibiotics have a strong affinity for their targets, hence exerting a detrimental impact on the target's normal physiological processes. Still, it is possible to change target sites so that an antibiotic has less of an effect on a certain target site (Mwangi et al., 2019). This is something that can be done when antibiotic resistance is introduced. It has been determined that the amino acid alterations that took place in the ribosomes of the Streptomyces sp. strain AM-2504 were responsible for the development of resistance from the strain. These substitutions lead to a decreased affinity for the antibiotic azithromycin, which in turn protects the organism (Allen and Cullis (2013).

**Immunity and Bypass**

The makeup of CRISPR spacers and the Cas enzymatic machinery may have an effect on the early stages of research on a nucleic acid-based immune system in bacteria (Friedman et al., 2013). Bacteria may develop antibiotic resistance by generating proteins that identify and bind to medicines or their targets, preventing antibiotics from reaching their targets (Dey et al., 2019). CRISPR/Cas9 or its derivatives may specifically address bacterial antibiotic resistance, increasing their antibiotic vulnerability. MRSA's methicillin resistance is caused by mutant penicillin-binding protein 2a (Ahmad et al., 2015). A CRISPR/dCas9 plasmid system including numerous target sites could serve as a viable alternative to CRISPR-Cas9 for specifically targeting the MRSA mecA gene. This method reduces mecA gene expression to make MRSA lactam-sensitive (Fuda et al., 2005). Alternatively, the CRISPR/Cas machinery can inhibit the transmission of particular nucleic acid sequences into a bacterium, preventing genetic engineering-induced antibiotic resistance. But CRISPR-based bacterial immunity is primarily concerned with preventing viral infections (Barrangou, 2015).

**Circumventing Existing Antibiotic Resistance Mechanisms**

By understanding the mechanisms of antibiotic resistance, we can potentially prolong the effectiveness of antibiotics. The effective encapsulation of current antibiotics into intelligent nanocarriers has been successfully used to mitigate the limited capacity of antibiotics to penetrate pathogenic biofilms. This approach serves as a method for managing antimicrobial resistance (Abed and Couvreur, 2014). The addition of triclosan to smart micellar nanocarriers resulted in the bacterial cell wall membranes inherently serving as efficient barriers against antibiotic absorption, they may also include proteinaceous structures to facilitate the extrusion of antibiotics that have penetrated the cell (Ghosh et al., 2014).

**Antibiotic-antibiotic Combinations**

Administering many medicines simultaneously is seen as a more convenient approach to treating infections (Pihl et al., 2017). It is more advantageous to combine antibiotics with distinct modes of action to enhance effectiveness and overcome bacterial resistance. Significantly, the administration of numerous antibiotics is regarded as having a reduced likelihood of developing antibiotic resistance, especially when the drugs possess distinct target sites (Flemming and Wingender, 2010). This is due to the need for various changes within the same bacteria (Liu et al., 2019). For synergy to occur between two antibiotics, it is necessary for both medicines to simultaneously interact with an infectious bacterium (Huang et al., 2017). A combination of gentamicin and fusidic acid, when added to bone cement used in orthopedic joint arthroplasties, has demonstrated improved effectiveness against a wider range of clinical isolates, including those that are resistant to gentamicin. This combination has shown superior results compared to using gentamicin or clindamycin alone (Espinosa et al., 2016).

**Combination of Antibiotics and Antibacterial Peptides**

Antibiotic peptides have been found to be effective against bacteria that have developed resistance to antibiotics in numerous instances. Antimicrobial peptides consist of a cationic moiety coupled by a hydrophobic appendage (Chen et al., 2016). This combination grants them the capacity to efficiently infiltrate bacterial cell membranes and facilitate the introduction and elimination of antibiotics. It has been found that Pseudomonas aeruginosa, E. coli, and *Acinetobacter baumanii* breach their cell walls when exposed to the peptide-based antibiotic colistin at levels lower than those that stop their growth. The simultaneous treatment of chloramphenicol, tetracycline, linezolid, and vancomycin results in a synergistic effect against microorganisms that are resistant to at least one of these antibiotics (Wrońska et al., 2015).

**Combination of Antibiotics and Synthetic Antimicrobials**

Synthetic antimicrobials are a diverse group of materials. The listed substances include quaternary ammonium compounds, synthetic antimicrobial peptides, and well-known antimicrobials that are frequently used in clinical settings, like triclosan and chlorhexidine (Dai et al., 2018). Antimicrobial peptides and synthetic antimicrobial peptides, as well as quaternary ammonium compounds, demonstrate mechanisms that are comparable to those of antimicrobial peptides. These similar mechanisms allow antibiotics to enter the organism and facilitate the penetration of bacterial cell membranes (Ghafoor et al., 2016).

**Combination of Antibiotics and Nano-antimicrobials**

Several types of nanoparticles, including nitric oxide-releasing nanoparticles (Franci et al., 2015), hydrogels (Singh et al., 2019), chitosan (Blecher et al., 2011), and metal nanoparticles (Pelgrift and Friedman, 2013), specifically silver nanoparticles (Huo et al., 2016), have been employed to combat antimicrobial resistance in various bacterial strains and species. These nanoparticles utilise various methods to eradicate illnesses that are resistant to antimicrobial treatments (Tu et al., 2013). According to the findings of the study, the elimination of vancomycin-resistant *S. aureus* was achieved through the encapsulating of vancomycin within chitosan nanoparticle suspensions. Additionally, the eradication of vancomycin-resistant enterococci and *E. coli* was achieved using the conjugation of gold nanoparticles with vancomycin. Furthermore, the combination of ciprofloxacin with vancomycin nanoparticles exhibited enhanced efficacy in killing vancomycin-resistant enterococci. One important aspect to consider is the prevention of antibiotic resistance (Flemming et al., 2016; Maan et al., 2018).

Undoubtedly, the advancement of innovative antimicrobials that do not trigger resistance may be just as, if not more, crucial than the advancement of more superior antimicrobials. The development of various kinds of antimicrobial resistance necessitates the generation of resistant mutants. However, as a subsequent measure, it is important to selectively enrich a population with the resistant sub-population (Parisi et al., 2017). The methods used to research the initiation of antimicrobial resistance are limited and require a significant amount of time. However, given their possible future significance, we will provide a concise summary below, along with infection control tactics that are now known to be effective in preventing infection (Picca et al., 2017).

**Preventing the Emergence of Antibiotic Resistance**

Undoubtedly, the creation of novel antibiotics that do not lead to resistance might be just as significant as, if not more so than, the creation of antibiotics of superior quality. The development of various kinds of antimicrobial resistance necessitates the generation of resistant mutants. However, as a subsequent measure, it is important to selectively enrich a population with the resistant sub-population (Busscher et al., 2012). However, given their possible future significance, we will provide a concise summary below, along with infection control tactics that are now known to be effective in preventing infection.

**Approaches for Researching the Development of Antibiotic Resistance**

Minimum inhibitory concentrations (MIC) are often measured as part of the assessment process for antimicrobial resistance. It is feasible to move bacteria from a growing culture into a medium containing an antibiotic at a concentration lower than the minimum inhibitory concentration (MIC) in order to perform a phenomenological inquiry on the establishment of antimicrobial resistance (Hampton. 2013). This allows for the sequential transfer of a portion of the bacteria into a new medium that has been supplemented with antimicrobial agents. The MIC is calculated for each serial-passage. Certain methods include the repetitive doubling of antimicrobial concentrations up to half the minimum inhibitory concentration (MIC) after a certain number of serials passes. This reduction may be related to the suppression of growth in wild-type bacteria. The limited metabolic activity of bacteria within the range of the minimum inhibitory concentration (MIC) and maximum bactericidal concentration (MBC) promotes genetic alterations that result in antibiotic resistance. Once the concentration of antibiotics reaches the minimum bactericidal concentration (MBC) within a certain time frame. It is possible to select mutant-resistant strains by using the Mutant Selection Window (MSW), which is located between the Minimum Inhibitory Concentration (MIC) and the Maximum Protective Concentration (MPC) as shown in Fig 5.

The colony-forming units (CFUs) that were obtained from a bacterial culture after it had been exposed to antimicrobial agents were taken into consideration when determining the concentration of antibiotics. Specifically, the colors green and red are used to show microorganisms before and after they have undergone mutation. Combined with the minimum inhibitory concentration (MIC) and MPC, the serum concentration of an antibiotic is plotted against the amount of time that has passed since the administration of the antibiotic. This text will describe the concepts of MSW and MPC. The number of offspring produced by bacteria that develop resistance at a later date is lower than the number of offspring produced by bacteria in Figure 5.

**Nano-antimicrobials not Inducing Resistance: do they exist?**

Nano-antimicrobials typically function through routes that are not related to antibiotics, which means that new bacterial methods are needed to fight their elimination. Bacteria have developed several strategies to counteract antimicrobial effects, and it is anticipated that the introduction of new nano-antimicrobials will inevitably give birth to

other mechanisms of antimicrobial resistance in the future. Heat-tolerant bacteria are already present in many natural habitats such as hot-water springs and industrial settings, such as dairy processing facilities (Hofer. 2019). The unanswered issue pertains to whether the future use of photothermal nanoparticles on a broad scale in clinical settings will likewise confer heat resistance to human pathogenic pathogens. The development of resistance to Ag nanoparticles in E. coli and P. aeruginosa strains has been shown after repeated exposure. This resistance is attributed to the creation of a flagellum protein, which leads to the aggregation of Ag nanoparticles and subsequently renders them inactive (Högberg et al., 2010). The resistance mechanism shown by the nanoparticles could not be effectively addressed with the use of surfactants or polymers for further stabilization.

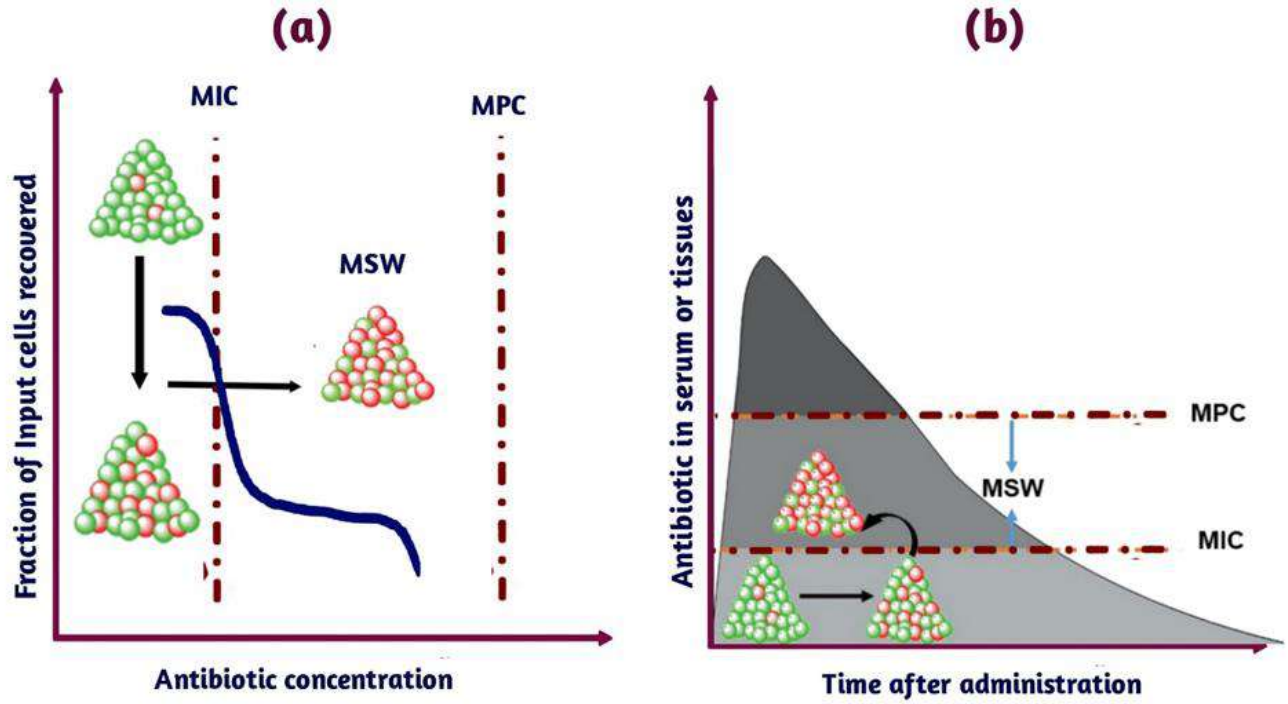


**Fig. 5:** Description of the concepts of mutant selection window (MSW) and mutant prevention concentration (MPC).

**Reverting Existing Antibiotic-resistance**

Some claim that the possibility of antimicrobial-resistant illnesses being the leading cause of death by 2050 will be eliminated if we successfully decrease the global antibiotic pressure. Bacterial strains that have gained resistance by genetic alterations in their DNA or through horizontal transfer of genes must waste a considerable amount of energy in responding to the newly acquired resistance. This waste of energy presents a barrier to their ability to compete with germs that are naturally vulnerable to the infection (Humphreys and Fleck, 2016).

**Expert Opinion**

The widespread and pervasive utilization of antibiotics has resulted in the emergence of bacterial strains and species that exhibit resistance to all currently recognized antibiotics. Hence, it is recommended to concentrate research endeavors not exclusively on developing increasingly potent antimicrobials, but rather on devising appropriate amalgamations of current antibiotics with novel nano-antimicrobials, while ensuring the prevention of the emergence of new antimicrobial-resistance mechanisms.

A last remark on innovative antibacterial methods pertains to the need to do pre-clinical, animal assessments before commencing human clinical trials. There is a widespread recognition that animal research has limited relevance to the treatment outcomes in humans (Llor and Bjerrum, 2014). This is particularly applicable for assessing new infection-control techniques in animals due to a multitude of factors. This might be accomplished by employing novel approaches, instead of solely depending on bioluminescence or CFU enumeration. Utilizing advanced animal infection models that closely replicate the features of severe infectious diseases in humans offers the potential to enhance the precision of animal research in relation to the human setting. Consequently, this could facilitate the implementation of novel antimicrobial therapies in clinical settings (Andersson and Hughes, 2011).

**Conclusion**

In conclusion, the use of nanoparticles into antimicrobial approaches offers a hopeful frontier in the management of infectious illnesses. Nanoparticles possess distinct physicochemical characteristics that provide benefits such as precise distribution, improved availability in biological systems, and the capability to overcome mechanisms of microbial

resistance. Recent breakthroughs have shown their effectiveness in combating a wide range of disease-causing organisms, such as bacteria, viruses, and fungi. Nevertheless, the clinical application of nanoparticle-based antimicrobials encounters obstacles including potential toxicity, environmental consequences, and regulatory barriers. It is essential to tackle these problems by collaborating across different disciplines and doing thorough research in order to effectively utilize nanotechnology in the fight against infectious diseases.

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Chapter 33

# Regenerative Medicine: Nanoparticles for Tissue Engineering

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**ABSTRACT**

Recently, numerous nanotechnological approaches have arisen that show significant promise for biomedical research. The objective of these approaches is to enhance the integration of regenerative medicine and tissue engineering into therapeutic applications. Currently, the utilization of nanomaterials for the goal of revitalizing damaged or injured tissues is widely recognized as beneficial in all fields of medicine. Nanomaterials are being increasingly developed for treating cardiovascular, osteochondral, and neurological defects, as well as for restoring organ functions in various organs including the renal, hepatic, pancreatic, and testicular tissues along with bladder, urethra, and wound healing. These nanoparticles act as support structures, imitate the extracellular matrix, and aid in cell attachment or specialization. The primary objective of this chapter is to examine the most recent advancements in regenerative medicine, specifically highlighting the significant contribution of iron oxide nanoparticles (IONPs) in the fields of tissue engineering and cell treatment. In addition to facilitating non-invasive observation techniques for therapy monitoring, IONPs possess the capability to expedite and augment regeneration processes. This can be attributed to their intrinsic magnetic characteristics or their ability to be altered with bioactive or therapeutic substances, such as medications, enzymes, and growth factors. Furthermore, magnetic fields have the capacity to precisely guide cells labelled with IONPs to a particular location or trigger cell differentiation into a certain cell type by mechanotransduction.

**KEYWORDS**
Magnetic drug delivery, Magnetic particles, MRI, Nanomedicine, Regenerative medicine, Superparamagnetic iron oxide nanoparticles

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## INTRODUCTION

The quantity of scholarly research about the domain of regenerative medicine has experienced a substantial surge throughout the past two decades. The application of nanotechnology in biomedical research has the potential to greatly enhance and expedite tissue regeneration, owing to its adaptability and capacity for functionalization (Dadfar et al., 2019). The materials utilized consist of a diverse array of constituents and display a vast spectrum of forms, encompassing fibrous formations, nanopatterned surfaces, and particulate entities. Nanoparticles (NPs) are used primarily for diagnostic applications because they allow for molecular imaging that is both multimodal and multifunctional (Cicha and Alexiou, 2021). Furthermore, they can be readily altered to function as carriers for the transportation of pharmaceuticals or genetic substances. Furthermore, they are being employed in cellular therapy and tissue engineering methodologies (Mathiasen et al., 2013). Through their advantageous impact on the regeneration process, stem cells can cause particular cell differentiation in the field of stem cell therapy.

Magnetic fields have a diverse and promising function in regenerative medicine (Kumar et al., 2010). These fields are used to control cells, biomaterials, and medicinal agents in novel ways that assist in tissue repair and regeneration (Yamoah et al., 2018). Moreover, they can be employed in tissue engineering as biocompatible architectural components, in conjunction with cells and/or bioactive chemicals, for the purpose of substituting or repairing impaired cells or tissue (Parashurama et al., 2016). Different preclinical *in vitro* and *in vivo* studies have shown that tissue engineering and NP-based cell treatments can effectively regenerate or replace injured or damaged tissue and that MRI monitoring of this process is necessary (Dixon et al., 2016). An overview of the developments in regenerative medicine is intended by this chapter which specifically emphasizes the use of iron oxide nanoparticles in the fields of tissue engineering and cell treatment.

**Methods for the Synthesis, Functionalization and Targeting of Iron Oxide Nanoparticles (IONPs)**

Over the past few decades, a large number of iron oxide nanoparticles (IONPs) have been manufactured for use in the field of biomedicine. Nevertheless, the generation of raw particles mostly relies on a limited number of synthesis processes, encompassing physical, chemical, and biosynthetic approaches (Fig. 1a) (Hu et al., 2016). Although less than 10% of all IONPs are produced by physical and biosynthetic methods, the majority of documented production methods for IONPs are chemical methods, including coprecipitation, microemulsion, and hydrothermal synthesis (Vallabani et al., 2019).

The initial synthesis plays a crucial role in determining the fundamental characteristics of the particles, including their crystalline structure, magnetizability, size, particle distribution, and shape (Saraste et al., 2009). Therefore, it is essential to carefully choose the synthesis method that best aligns with the desired attributes of the IONP (Wen et al., 2015).

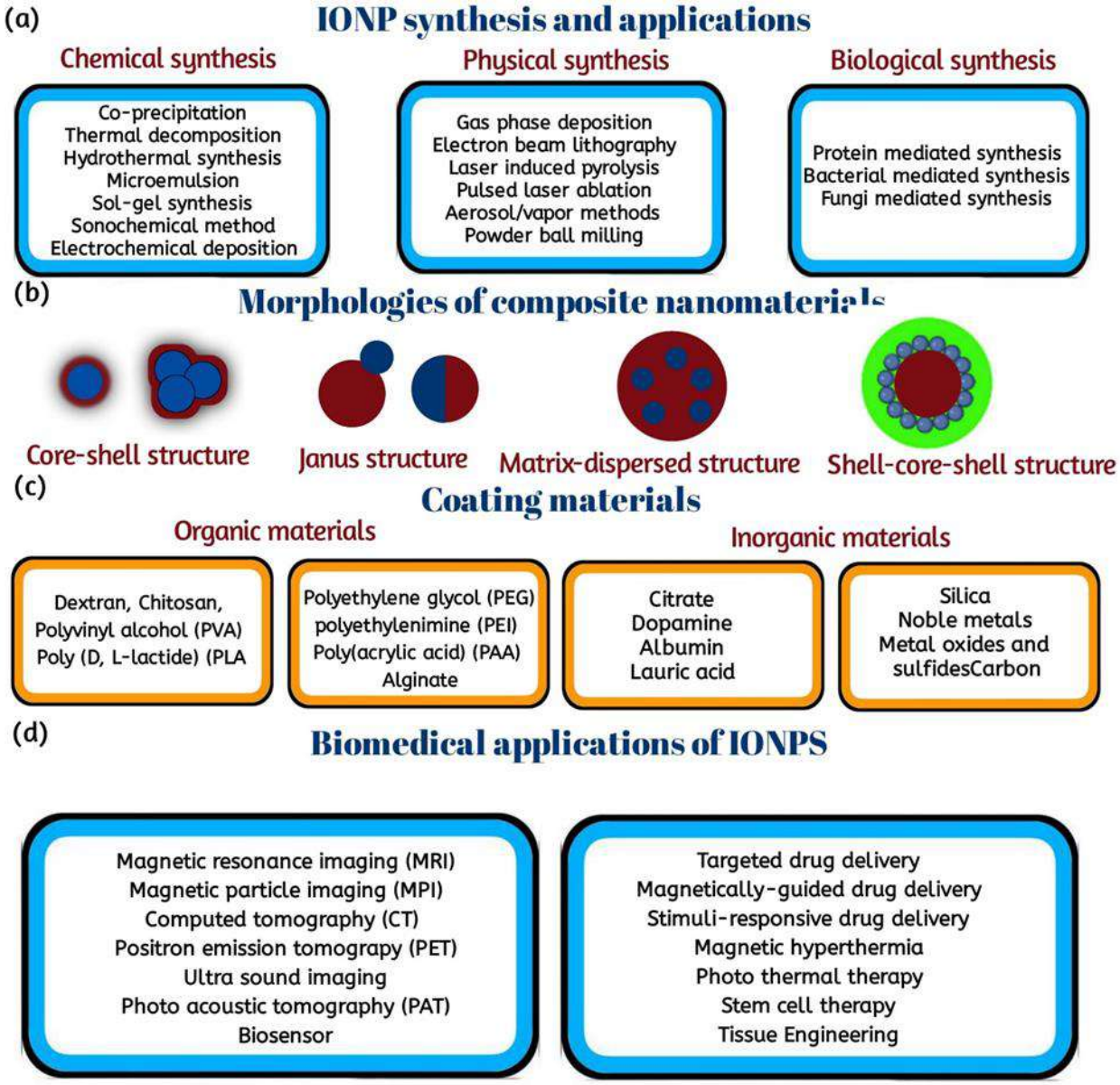


**Fig. 1:** Shows IONP synthesis and use. IONPs may be synthesized chemically, physically, or biologically. The figure shows iron oxide nanoparticle architectures (blue: core; orange and green: coating components). Common organic and inorganic nanoparticle coatings. Therapeutic and diagnostic methods that utilize IONPs are frequently used in biomedical research. Functionalizing nanoparticles allows for their application in several biotechnological and medicinal fields (El-Boubbou, 2018). These materials serve as biosensors and are employed in various diagnostic applications such as magnetic resonance imaging (MRI), ultrasound (US), magnetic particle imaging (MPI), photoacoustic tomography (PAT), positron emission tomography (PET), and computed tomography (CT). They are also used in tissue engineering, regeneration, and disease therapy by targeting or stimulating bioactive drug delivery (Blanco-Andujar et al., 2016). Hyperthermia and magnetically guided drug delivery are feasible due to their magnetic characteristics (Smit et al., 2015).

**Regenerating and Engineering of Cardiac Tissues**

Cardiovascular regeneration chemicals and materials have advanced throughout time. Most blood arteries, heart valves, and myocardium repair or creation are experimental. The progress is illustrated in Fig. 2. Nanotechnology is growing in alternative cardiovascular therapy (Alphandery, 2020). The use of nanoparticle probes for noninvasive imaging of cardiovascular targets, such as myocardial apoptosis, plaques, vascular inflammation, thrombosis and angiogenesis is becoming significant for diagnosis and treatment monitoring (Vangijzegem et al., 2019).

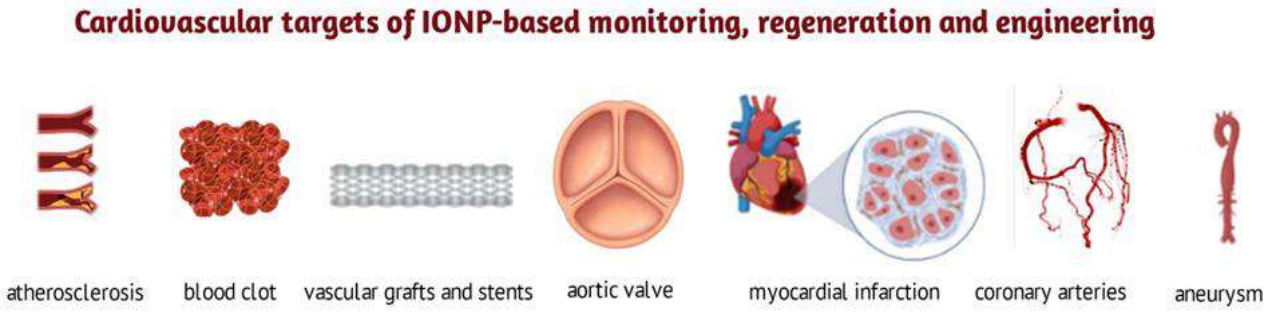


**Fig. 2:** illustrates potential targets for cardiovascular tissue engineering and regeneration with the assistance of IONP

**Stem Cell Therapy**

Stem cell therapy is often regarded as the most promising approach for treating cardiovascular disorders. Essentially, their utilization has the potential to facilitate the regeneration of all damaged and afflicted tissues. Activating angiogenesis in ischemic tissues and organs is difficult in treating cardiovascular and cerebrovascular disorders (Billings et al., 2021). When it comes to successfully restoring blood flow, the restoration of functional collateral networks is necessary. In pursuit of this objective, the majority of tissues possess molecular processes that employ vasodilation, angiogenesis, arteriogenesis, vascular remodeling, and hematopoiesis as means to counterbalance diminished oxygen levels. Nevertheless, the intrinsic regulators of vascular remodeling frequently prove inadequate and necessitate supplementary assistance from proangiogenic and arteriogenic factors, such as fibroblast growth factor-2 (FGF-2) and polarizing growth factor-B (PDGF-B) (Avasthi et al., 2020). In addition, the process of repairing damaged blood arteries or regenerating ischemic tissue can be accomplished through the use of targeted cell treatment including stem cells or endothelial progenitor cells (EPCs) (Hola et al., 2015). Nevertheless, it is essential to highlight the necessity of closely observing the treatment in every circumstance.

**The Rehabilitation and Engineering of Hard and Connective Tissues**

This study reveals a description of relevant research in the field of therapy using stem cells as well as tissue engineering for bone and cartilage abnormalities, wherein IONPs were utilized (Fig. 3). This research deals with the treatment of bone and cartilage deformities. Within the scope of this project, IONPs will be investigated for their potential applications as drug transporters or as instruments for the fabrication of composite scaffolds. Furthermore, it will explore the potential of utilizing the magnetic properties of IONPs to visually examine and regulate treatments conducted on cells and materials. Furthermore, it will investigate the application of IONPs for magneto-mechanical induction, a technique employed to control cellular activity and differentiation. It will also examine how IONPs may enhance nanoparticle-loaded cells.

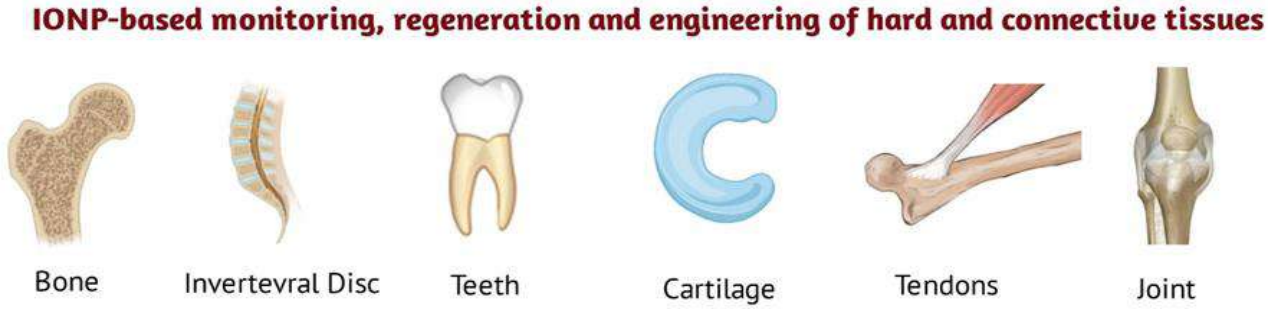


**Fig. 3:** shows IONP-aided tissue engineering and regeneration objectives for hard and connective tissues.

**Cartilage**

**MRI-assisted Stem Cell Treatment for Cartilage Regeneration**

Because IONPs can quickly and effectively label a variety of cells without affecting their growth or differentiation, they are frequently used as labelling agents *in vitro*. This labeling method allows non-invasive monitoring of cartilage-regenerating or cartilage tissue engineering cells (Ganguly et al., 2021). The effects of IONPs (ferucarbotran) on human

BM_MSCs, neonatal chondrocytes, chondrogenic differentiation, viability, morphology, and proliferation are of significant importance (Krasia-Christoforou et al., 2020).

According to the findings, the decrease in chondrogenic genes appears to be influenced by both the passage of time and the kind of cell studied. Additionally, it seems that the amount of ferucarbotran utilized is appropriate for the noninvasive tracking of stem cells and mature chondrocytes (Ganguly and Margel, 2020).

**Magnetically Controlled Cell Therapy for Cartilage Rejuvenation**

Magnetic-based enhancement of IONPs at sick or faulty locations is another potential tissue regeneration method. Mechanical stimulation of an external magnetic field may boost stem cell proliferation, differentiation, and migration (Ahmadi et al., 2021). MSCs are magnetically steered to the defect site, forming a 3D cell sheet (Budde and Frank, 2009). It was found in previous research that magnetically labeled MSCs can be concentrated in rabbit and pig knee joints at the osteochondral defect. Xie et al. (2018) used a magnetic force to enrich. The findings suggested using the least invasive technique for other osteoarthritis or trauma-related cartilage defects. Ferucarbotran magnetized autologous bone marrow-derived mesenchymal stem cells (BM-MSCs) in recent research.

**Drug-Supported Cartilage in Regenerative Medicine**

Magnetic stimulation has the ability to regulate the activity of growth factors and the release of drugs from polymeric systems that respond to stimuli. This can have an impact on the process of cell differentiation (Wu et al., 2015). A magnetic biopolymer nanogel with IONP chitosan and heparin was produced. BMP-2, essential for cartilage and bone formation, is supplied by exact nucleobase pairing in this nanogel. A magnetic field was used to modulate the release of TGF-β1, a heparin-binding domain, promoting chondrogenic growth in the ATDC5 murine cell line (Wahajuddin 2012). Electrogelation created magnetic scaffolding from silk fibroin and bFGF nanoparticles (Hasany et al., 2012). In SaOS-2 osteosarcoma cells, bFGF-IONPs increased mechanical characteristics, viability, and growth. Total protein production, collagen, and alkaline phosphatase enhanced.

**Bone Regeneration**

Within the realm of regenerative medicine, one of the most important goals that can be accomplished is the restoration of bone tissue. These methodologies comprise the purposeful distinction of stem cells and the utilization of a variety of materials for the construction of bone scaffolds. It has been demonstrated by researchers that IONPs can be utilized to visualize the therapy process through the utilization of MRI imaging. Magnetic fields can also be used to induce cell differentiation and increase osteogenesis, which is another benefit of using these devices. Additionally, IONPs have the capability of being utilized to deliver labeled cells or medications to the exact location of interest (Jing et al., 2010).

**Regeneration of the Peripheral Nervous System (PNS) and Central Nervous System (CNS)**

Stem cells, progenitor cells, and differentiated cells, such as adult neurons, Schwann cells, and astrocytes, have been used to treat illnesses that impact the central and peripheral nervous systems. ALS, AD, HD, PD, ischemic stroke, MS, and spinal cord injury are among these neurodegenerative diseases (Cao, 2010). Nanotechnology may enhance brain tissue injury therapy, medication delivery, and tissue engineering (Zhang et al., 2008). IONP labeling allows stem cells, astrocytes, and microglia to be tracked and monitored following transplantation. MRI, PET, magnetic particle imaging, and multiple photon microscopy can accomplish this (Saha et al., 2013).

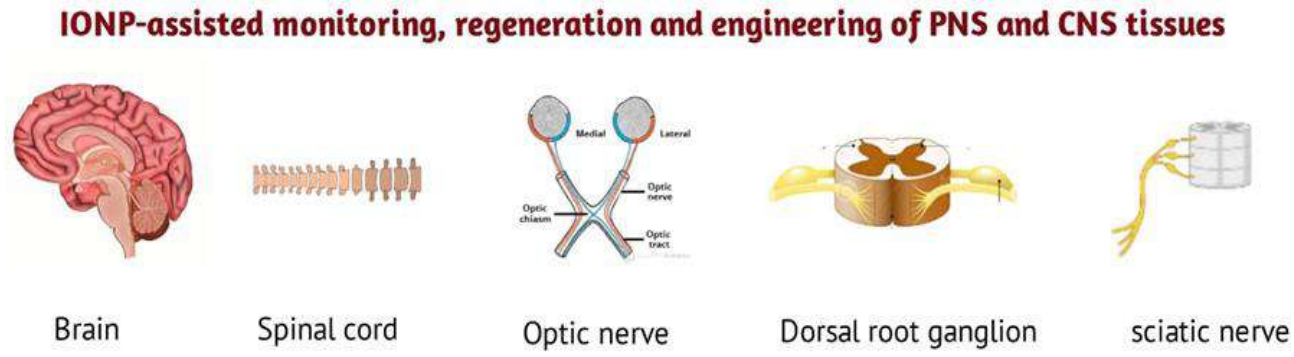


**Fig. 4:** Potential peripheral and central nervous system tissue engineering and regeneration targets with IONP usage

**Additional Methods for the Regeneration and Engineering of Soft Tissues**

In addition to the heart and brain, the utilization of IONPs is seen as a promising approach for the functional restoration of other soft tissues and organs.

**Ear, Eye, Nose, Throat Fold, Glandular Structures**

**Ear**

Mounting data suggests that stem cell transplantation has the potential to restore sensorineural hearing loss. Thus, regular treatment monitoring would be quite beneficial. MRI was utilized to reliably monitor IONP-labeled mesenchymal stem cells (MSCs) transplanted into live mice's cochleae (Santoso and Yang, 2016). In a separate study, it was discovered that magnetic nanoparticles could move via the oval and round windows, which ultimately leads to the production of a T2 contrast effect within the inner ear. Because of this property, MNPs are suitable for diagnostic applications and have the potential to serve as carriers for therapeutic medications that are intended to treat ear injuries. In guinea pigs, the administration of nanocarriers loaded with ferrocene through the intratympanic route did not result in any adverse effects on the animals' hearing. According to this, it appears that these nanocarriers have the potential to be utilized in the field of inner ear theragnostic in the future (Fayol et al., 2013).

**Eye**

Stem cell therapy for degenerative eye illnesses has been studied for a long time (McCarthy and Weissleder, 2008). Additionally, IONP can treat eye diseases. Glaucoma, a major cause of visual impairment, may be treated by stem cells (Zhang and Wu, 2007). Glaucoma causes cell loss in the intraocular pressure-regulating trabecular meshwork (TM). Snider et al.'s *ex vivo* work found that external magnetic fields may move IONP-loaded MSCs to the TM in the anterior chamber of the eye (Noukeu et al., 2018). Edoema, opacification, and vision loss may result from corneal endothelial failure. A feasibility study indicated that IONP labeling marginally affected CECs. In addition, first magnetic exposure improved cell viability (Uppal and Caravan, 2010).

IONP-loaded CECs may treat eye injury, according to the findings. Tissue engineering may treat ocular problems including choroidal neovascularization. Researchers have utilized magnetic force to generate multilayered cell sheets composed of retinal pigment epithelial cells loaded with magnetite liposomes. This approach has the potential to address or manage choroidal neovascularization (CNV) (Waters and Wickline, 2008).

**Nose**

Evaluating the potential applications of magnetically controlled enrichment of IONP-loaded MSCs and intranasal injection as treatments for olfactory impairment is also critical. In a mouse model with olfactory impairment, it is feasible to employ external magnets to induce the migration of magnetized MSCs. This may be accomplished by using magnets applied to the animal (Hou et al., 2020).

**Vocal Fold**

Within the realm of regenerative medicine, one of the most critical challenges that must be overcome is the care of voice abnormalities, such as injuries to the vocal folds. At the moment, there is a restricted selection of surgical or tissue engineering techniques that can be utilized to mend defects of this nature (Dankova et al., 2015). The production of vocal folds by the utilization of cells that are loaded with IONP is continually being pursued. IONPs were incorporated into the vocal fold fibroblasts that were isolated from rabbit laryngeal heads (Brady et al., 2017). This allowed for the magnetization of the vocal fold fibroblasts. In addition, the researchers investigated the effects of IONP absorption on cells and demonstrated that it is feasible to influence magnetic cells and develop three-dimensional structures for the vocal folds through the application of magnetic tissue engineering (Nedopil et al., 2010; Jasmin et al., 2017).

**Salivary Glands**

Stem cell or tissue engineering can replace salivary secretory epithelial cells that degenerate or hypofunction due to autoimmune diseases or cancer radiotherapy (Veloso et al., 2018). In a mouse model, ultra-small IONP-labeled BM-MSCs and acinar-like cells were used to restore salivary gland function. Additionally, in comparison to the BM-MSCs, the acinar-like cells revealed a significantly higher potential for therapeutic application (Son et al., 2015). Another study involved the use of magnetic levitation to produce magnetically labeled primary salivary gland-derived cells within salivary-secreting organoids or mini-glands. According to Karahalilo ˘glu et al. (2017), these cells exhibited secretory activity when activated by cholinergic signals. Furthermore, these cells possessed discrete cellular compartments that were located only within salivary glands. During this interim period, it is possible to generate salivary gland-like epithelial organoids through the use of magnetic bioprinting using NP-labeled stem cells (Fan et al., 2014).

**Discussion and Conclusion**

Over the years, there have been numerous documentations of breakthroughs in the field of nanomaterials for medical applications. Because of their extraordinary modifiability, nanoparticles (NPs) are recommended for use because they enable precise customization of particle properties to fit specific applications. This can be accomplished through the production method, coating, or functionalization that is selected. IONPs can be customized for a wide variety of applications, including the modification of cellular effects through the loading and distribution of bioactive compounds. This is in addition to the fact that IONPs have the potential to be used for scaffold visualization in the field of tissue engineering. When it comes to cell therapy, there are a great many opportunities to have a beneficial impact on the outcome of the treatment. This can be achieved by non-invasively monitoring cells labeled with IONP or by enhancing the

ability to differentiate and regeneration capabilities of the supplied cells through the use of nanoparticles (NPs). IONPs have a broad spectrum of potential uses (Tickle and Chari, 2019).

Additionally, numerous iron oxide nanoparticles (IONP) demonstrate inadequate magnetizability. Therefore, to facilitate the transition to clinical application, it is imperative to carry out the entire process of nanoparticle synthesis by establishing good manufacturing practices and ensuring precise physicochemical characterization. Additionally, it is crucial to conduct thorough *in vitro* and *in vivo* toxicology research, encompassing biodistribution and efficacy assessments (Padmanabhan et al., 2016). Therefore, only a small number of IONPs have effectively completed the process of translating to the clinics up to this point. Notwithstanding this reality, the efficacious utilization of IONPs in several domains of nano-medical investigation engenders optimism about the potential approval of further IONP formulations for clinical implementations in the foreseeable future.

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Chapter 34

# Veterinary Applications of Nanoparticles: Enhancing Animal Health

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**ABSTRACT**

The development of novel methods to alter materials at the nanoscale had a transformative impact on multiple branches of medical science. Currently, numerous nanomaterials can be categorized based on their shape, source, or purpose. Nanotechnology offered innovative answers to longstanding issues. Within the field of medical sciences, these tools are employed for the objectives of diagnosis or treatment. Furthermore, they can be employed in the synthesis of nano adjuvants and nano vaccines. A new era in medical history was marked by their use in gene therapy and the treatment of cancer. In recent times, nanotechnology has begun to be utilized in numerous ways within the veterinary industry. They are gradually intruding into several sectors of animal-related industries, such as animal pharmaceuticals, diagnostics, veterinary vaccine production, farm disinfectants, animal breeding and reproduction, and even animal nutrition. Their replacement of commonly used antibiotics has a direct effect on public health. They minimize the issue of drug resistance in both human and veterinary medicine and solve the problem of medication residues in milk and meat by implementing this adjustment. Additionally, they significantly impact the economy by lowering milk waste and dairy cow calves' culling. Nanotechnology has been employed in the creation of pet care products and various hygiene articles. This chapter reviews the advantages of using nanomaterials compared to their counterparts, explores various types of nanoparticles, and illustrates the applications and importance of nanotechnology in the field of veterinary medicine.

**KEYWORDS**
Nanominerals, Nanotechnology, Nanovaccines, Nanoparticles, Veterinary, Pet care Nanomaterials

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## INTRODUCTION

The word "nanotechnology" was first introduced in 1974 to describe scientific tools used for altering material at the nanoscale. The term "nanoscale" generally denotes materials with at least one dimension ranging from 1 to 100 nanometers. Naturally occurring nanoscale biological components include DNA, with a width of 2.5 nm, and molecules of protein, with an average width of 5 nm. In contrast, the width of a human hair is roughly 80,000 nanometers. Nanobiotechnology refers to the utilization of nanotechnology in biological research (Troncarelli et al., 2013). Nanomedicine involves utilizing a range of nanotechnology tools to create more rapid and effective therapies for medical illnesses and disease management. Not only does it resolve the challenges encountered by conventional medicine, but it also aids in comprehending many pathological and physiological processes (Okamoto et al., 2009). Gaining a profound comprehension of these processes offers fresh opportunities and treatment frameworks for current issues (Mohantya et al., 2014). Furthermore, additional terminology, such as nanotheranostics, is now being utilized. One of the reasons why these formulations are considered novel is because they possess diagnostic as well as therapeutic characteristics in a single application. Their utilization provides vital information regarding the administration of drugs, the location of release, and the effectiveness of interventions. This data enables the modification of treatment procedures according to individual needs (Manuja et al., 2012; Chapman et al., 2013; Rizzo et al., 2013).

The newly constructed nanobiomaterials, which typically range in size from 5 to 20 nanometers, are purposefully engineered to have a structure that is comparable to that of a variety of biological receptors, ligands, proteins, and DNA. The structural similarities that exist among these entities make it possible for them to interact with cell membranes and tissues in a variety of biological contexts (Yi et al., 2016). When it comes to nanomaterials, the nanocarriers that are based

on lipids and are biodegradable are highly preferred. Utilizing them helps to prevent the accumulation of nanoparticles in cells and reduces the toxicity that is caused over a longer period. Macrophages can rapidly absorb and break them (Johansson et al., 2017). The selection of the ideal size for nanoparticles (NPs) is influenced by a significant number of variables (Danesh-Bahreini et al., 2011; Cormode et al., 2013).

When it comes to medical applications, NPs of a particular size are typically acceptable, however, the utilization of bigger NPs is typically avoided because they have the potential to produce embolisms. In addition, they will be absorbed and removed from the bloodstream in a manner that is immediately effective. Conversely, NPs that are minuscule will be cleared by the kidney more efficiently the smaller they are in the first place (Fakhri et al., 2017). Furthermore, when the surface area to volume ratio of a particle increases, it can cause a noticeable increase in its chemical and biological reactivity, making even small particles more dangerous and reactive. Reactive oxygen species (ROS) and free radicals are produced at an increased rate as a result (Venkatesan and Kim 2014). Considerable cellular damage, inflammation, and oxidative stress are brought on by the production of ROS (Jain et al., 2011).

**Table 1:** Benefits of implementation of nanotechnology in the field of medical science the table illustrates several benefits that have been gained through the implementation of nanotechnology in the field of medical science

| The table represents some examples for benefits achieved by the application of nanotechnology in medical sciences | |
|---|---|
| Diagnostic purpose | • Coupling of NPs with tumor-specific antibodies enables early cancer diagnosis which reflects on (A) better survival rates and (B) scanning of the whole body for metastatic lesions<br>• Nanorobotics can be used in investigatory/therapeutic micro-surgeries. They can also carry nanocameras for real-time assist in surgeries<br>• It provides a very rapid screening/diagnostic tool. The use of high-density nano-array chips enables the detection of thousands of proteins, genes, antigens, or disease biomarkers simultaneously |
| Therapeutic purpose | • It is easy to manipulate their physical/chemical properties during manufacturing according to the planned application which provide endless number of variants. This, in turn enables personalization of the therapeutic and diagnostic concept<br>• Their high surface area: volume ratio enables loading of high amounts of payloads<br>• The NPs are characterized by their stability even under high pressure and temperature<br>• Due to their small size, they can cross different physiological barriers as blood-brain barrier (BBB), or even through cell/ nucleus membranes to reach their target sites and avoid their detection and elimination by the reticuloendothelial system<br>• Provide sustained release/long-acting smart drug delivery tools for the delivery of antibiotics, nanominerals, hormones, antioxidants, vitamins, nucleic acids, and imaging agents<br>•The developed micro-robotics can replace RBCs (O2/CO2 gas exchange) and WBCs (entrap circulation pathogens) |
| Prophylaxis | • Provide new concepts for the development of new vaccines and adjuvants which are safer, more efficient and stable to store<br>• Trials to develop wireless sensors which can be implanted under the skin of patients at risk to measure various vital functions and the level of certain target proteins in order to alarm any serious changes in their health situation (real-time monitoring) |

**Types of Nanoparticles**

There are numerous approaches to classifying NPs based on their non-material origins. It is possible to classify inorganic (gold, iron, silver, silica, magnesium, or graphene) and hybrid nanoparticles (NPs) as well as organic (proteins, peptides, or lipids). NPs might be spheres, tubes, or liquid droplets. They can also be characterized by their application and payload as therapeutic, diagnostic, vaccine administration, or nutritional (Thulasi et al., 2013).

**Polymeric NPs**

Polymers can be categorized into two groups: natural polymers, such as chitosan and inulin, which are based on polysaccharides, and synthetic polymers, like polyethylene glycol (PGE) (Torres-Sangiao et al. 2016). PGE is one type of synthetic polymer. During the production process, active molecules are adsorbed onto the surface of NP polymers to form complex spherical formations of dendritic polymers with a tree-like structure (Elgqvist 2017). This is something that happens in the production process. Except for the branches that arise from the central core showing varying numbers of branching points, this structure is similar to a dendrimer (Mohantya et al., 2014). The potential for a dendrimer is what distinguishes this structure (Hamouda et al., 2011; Wu et al., 2018).

They are remarkably adept at loading and conjugating words with ease. Moreover, polymers can be used to create hydrogel nanoparticles, which are well-known for having a large surface area and a large volume of water inside of them (Torres- Sangiao et al., 2016).

**Liposomes: Spherical Biodegradable Non-toxic PEGylated NPS**

Their liquid core is used to transport radioactive materials, pharmaceutical drugs soluble in water, DNA, RNA, and

siRNA (small interference RNA) used in gene therapy. A bilayer phospholipid shell envelops the particles containing fat-soluble (hydrophobic) medicines (Chowdhury et al., 2017). Viral omes, sometimes known as viral envelop glycoproteins, are capable of encasing a variety of antigens (Torres-Sangiao et al. 2016). Particles are shielded from immune system attacks by a protective polyethylene glycol (PEG) coating that envelops the external surface. To immobilize chelated antibodies on an external surface, one may employ them to target and image agents (Thulasi et al., 2013).

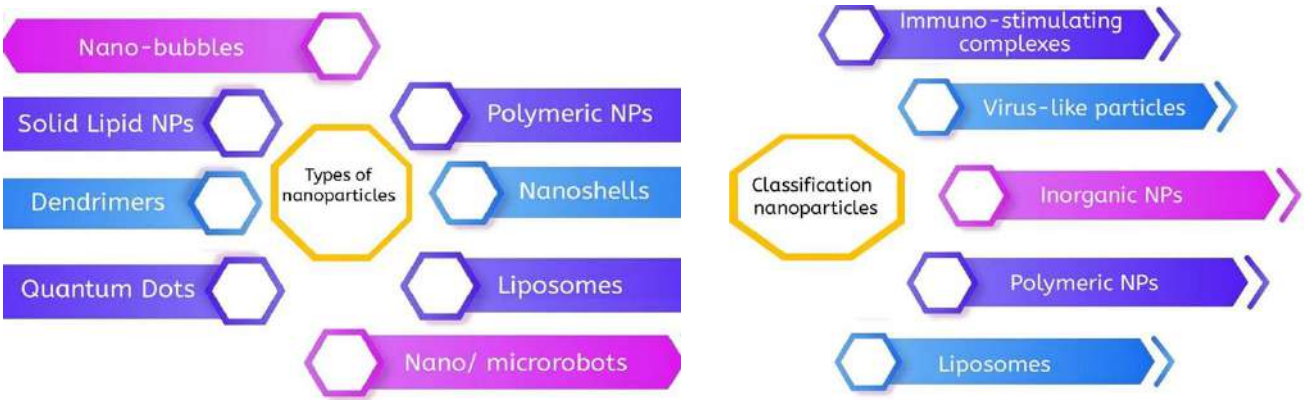


**Fig. 1:** a) and b) showing different types and classifications of NPs

**Buckyballs (fullerenes) and Buckytubes (nanotubes)**

Buckyballs, which have a spherical morphology and are composed of carbon, interact readily with proteins, cells, and microorganisms (Mohantya et al., 2014). A diverse range of fullerene derivatives exists (Meena et al., 2018). The number of carbon atoms in these compounds could range from 20 to 100. Carbon-based nanostructures known as "buckytubes" are distinguished by their unique cylindrical shape. (Elgqvist, 2017) posits that barriers within this particular framework can be classified as singular, dual, or multifaceted. Nanotubes have the potential to be modified for applications as electrochemical DNA hybridization biosensors or as biosensors for detecting glucose, ethanol, and immunoglobulins (Manuja et al., 2012). Because of their small size and chemical inertness, nanotubes have potential applications outside cancer treatment, including gene therapy and DNA transport (Reilly, 2007).

**Nanoshells**

They are spherical with a thin layer of gold covering a dielectric core made of glass or silica.

These items can vary optically, responding to different wavelengths depending on their gold coating thickness (Mohantya et al., 2014). They are usually used to diagnose cancer (Jurj et al., 2017). They have a response to infrared light and can penetrate blood samples. Antibodies and gold nanoshells work together to detect immunoglobulins in blood samples, even at extremely low concentrations (Manuja et al., 2012). Nanoshells accumulate in tumor tissues and have therapeutic potential due to their small size. Infrared radiation from an IR laser passes through healthy tissues without creating thermal energy (Prabhu et al., 2015). However, tumor cell nanoshells absorb infrared light and generate heat. The thermosensitive polymer covering melts, releasing the drug to the desired organ. Over time, cancer cells perish when their temperature reaches 55 °C. Since gold is safe for the body, inert, and biocompatible, but cadmium in quantum dots is hazardous, nanoshells are utilized more frequently than quantum dots (Talukdar et al., 2014).

**Solid Lipid NPs**

Their lipid core, which repels water, can dissolve lipophilic medicines such as radionuclides used in cancer treatment. The surrounding structure consists of dehydrated tails of phosphatidylcholine lipids. The fatty core is coated with a hydrophilic shell, which helps in the conjugation of hydrophilic medicinal medicines or antibodies. The hydrophilic outer layer enhances the stability and dispersion of the medication in the bloodstream, hence enhancing its bioavailability. Cationic solid lipid nanoparticles possess the capacity to immediately adhere to DNA/RNA fragments via electrostatic interactions, rendering them very suitable for gene therapy (Elgqvist 2017). Topical, oral, or injectable treatment allows for gradual release over weeks. As lipophilic substances, they easily adhere to and are absorbed by the mucosa. An important benefit of these drugs is their capacity to cross the blood-brain barrier (BBB) and enter the central nervous system (CNS) (Mishra et al., 2010; Mohanty, 2014). Lipid nanoparticle (NP) formulations in a solid state are colloidal carriers that remain solid both in the body and at room temperature (Krishnan and George 2014). Parallel testing is being done on both liquid and solid lipid nanoparticles (Elgqvist 2017).

**Micelles Polymeric**

Micellar polymers, unlike solid lipid nanoparticles, possess hydrophobic centers that facilitate the transportation of water-insoluble preparations. These compounds are highly soluble in water because they have a hydrophilic shell that

surrounds a hydrophobic core. Micelles made of polymeric materials can be classified into four distinct forms, each of which is distinguished by the composition of their shell: (1) micelles composed of poly amino acids,
(2) micelles made of phospholipids, (3) Micelles composed of biocompatible polyester, and (4) Pluronic micelles have a shell made of a block copolymer with hydrophobic and hydrophilic components (Mohantya et al., 2014).

**Dendrimers**

The nanoparticles possess remarkable water solubility, exhibit biocompatibility, and consist of synthetic polymers that are far smaller than the body's cells (Jurj et al. 2017). Due to their diminutive size and distinctive chemical makeup, they effectively avert any undesired immune responses when supplied intravenously (Chakravarthi and Balaji 2010). These molecules have a complex three-dimensional structure with branching dendrites. The drugs are either encapsulated within the spherical structure or linked to functional groups on the surfaces of the dendrimers (Mohantya et al., 2014). Dendrimers can include a wide range of hydrophobic or hydrophilic therapeutic substances through physical and chemical interactions. They can also encapsulate these molecules within their empty cores using nonbonding loading (Moghaddam et al., 2010).

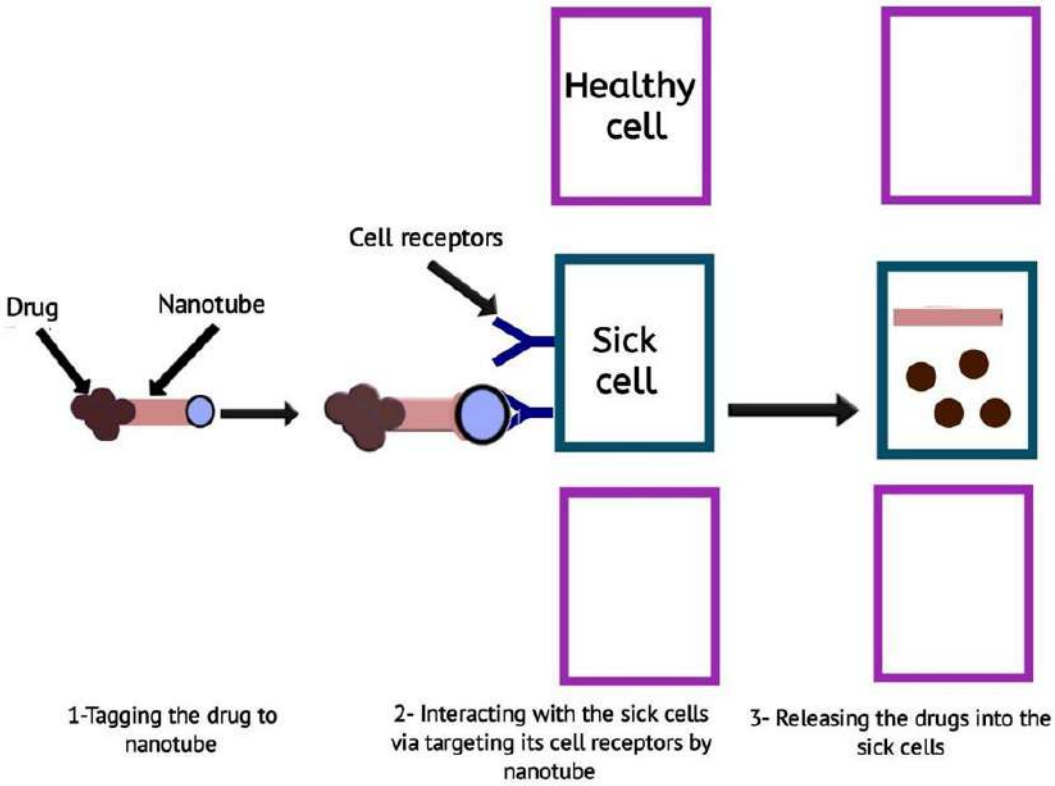


**Fig. 2:** Diagrammatic representation of how nanotechnology is used in an intelligent medication delivery system

**Nanotechnology Applications in Veterinary Medicine**

Nanotechnology offers veterinarians equivalent choices to conventional medical practitioners in the fields of medicines, diagnostics, vaccine manufacture, tissue engineering, and better disinfectants. Figure 3 (Manuja et al., 2012) demonstrates the extensive utilization of nanotechnology in animal health and manufacturing, animal breeding and reproduction, and animal feeding. This applies to all three of these regions. By delivering medications directly to the targeted cells, it becomes possible to administer very small amounts. This, in turn, decreases the number of leftover drugs and the time needed for withdrawal in farm animals (Troncarelli et al., 2013).

**Applications of Nanotechnology in Animal Disease Diagnosis and Treatment**

Veterinarians encounter numerous challenges, including tuberculosis, brucellosis, foot-and-mouth disease (FMD), MRSA, and diseases caused by intracellular or bloodborne pathogens. Nanotechnology provides novel solutions. The efficacy of intramammary drug administration for treating mastitis in cows is being investigated (Kroubi et al., 2010). Nano drugs outperform traditional drugs in many ways (Troncarelli, 2013). Gentamicin binding to a hydrogel via a peptide linker shows their autonomous decision-making ability. Gentamicin stays medically inactive as long as the linker is intact (Greenwood et al., 2008). Only Pseudomonas aeruginosa protease can degrade the peptide linker (Bellocq et al., 2003). Only Pseudomonas aeruginosa can liberate and activate gentamicin. Research has also produced nanoparticles (NPs) that target bacterial toxins and receptors. NPs attach to pathogenic gut bacteria and inhibit their expulsion (Kim et al. in 2010).

When it comes to the cure for prostate cancer in dogs in the United States, surgical intervention has been substituted by the use of quantum dots made of gold. The findings showed that the treatment technique did not harm healthy tissues and used dosages that were 1,000 times lower than chemotherapy (Troncarelli et al., 2013; Casals et al., 2017).

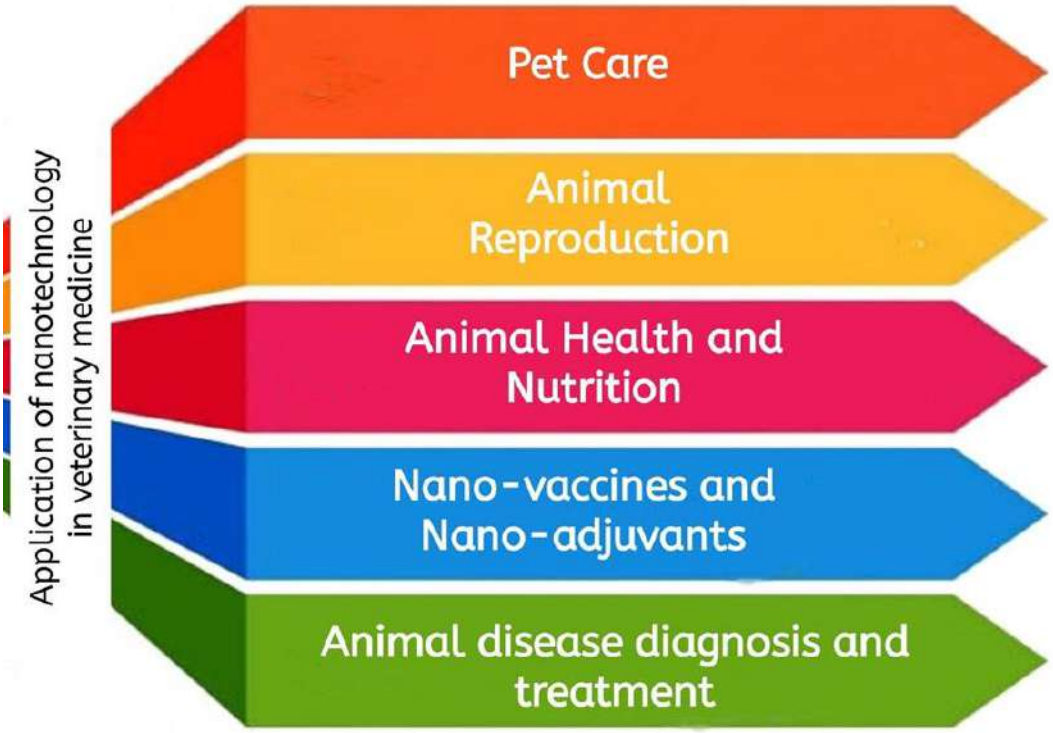


**Fig. 3:** Animal health-related nanotechnology applications depicted schematically

**Nanoadjuvants and Nano Vaccines**

Nanoparticles (NPs) are being utilized in veterinary immunizations. Their immunomodulatory actions enhance the body's immune system responses. They enhance the process of peptide cross-presentation and stimulate the activation of antigen-presenting cells. Additionally, they can postpone the release of antigens as adjuvants, thus enhancing the effectiveness of vaccinations. Nanoparticles (NPs) that carry antigens can selectively direct themselves to lymph nodes, hence enhancing the effectiveness of immunization (Moyer et al., 2016).

Significant advancements have been made in the development of nano vaccines for veterinary purposes (Kanekiyo et al., 2013). The delivery of nano-emulsion vaccines, such as the recombinant Bacillus anthracis spore-based vaccine and the influenza virus vaccine, through the nose promotes the immune response in the mucous membranes. Another significant achievement is the development of PLGA Nanoparticles can be used for vaccinations that target Heliobacter pylori, Rotavirus capsid, Tetanus toxoid, Bordetella pertussis, and Bovine parainfluenza type 3. These oral vaccinations stimulate the production of IgG and IgA antibodies, which are part of the immunological response. Glucosamine biopolymer chitosan nanoparticles are utilized in nano vaccines for veterinary purposes. Recombinant Leishmania SOD vaccines are administered by the subcutaneous route, while pneumococcal antigen A and Streptococci equi vaccines are administered intranasally. Chitosan nanoparticles have also been used in pulmonary administration for tuberculosis vaccination (Bielinska et al., 2007).

**Nanotechnology for Animal Health and Nutrition**

Nanominerals are advantageous to the animal feed business. They are less expensive, require smaller dosages, and strengthen immunity and development. Food pathogens and rumen fermentation are regulated by nano minerals. They could provide solutions for several herd/flock reproductive issues (Swain et al. 2015).

Numerous nanomaterials are used in commerce, such as nano-ZnO. With nano-ZnO, poultry and farm animals grow more quickly, have stronger immune systems, and reproduce more successfully. Furthermore, it reduces piglet diarrhea (Yang and Sun, 2006; Misra et al., 2014). In cows with subclinical mastitis, nano-Zn improves milk yield and reduces the somatic cell count (SCC) (Rajendran, 2013).

Liquid vitamins produced using nanotechnology can improve poultry feed. Because they can pass through the gastrointestinal tract (GIT) and into the bloodstream, nutrients that are nanosized have higher bioavailability. They extend feed nutrients and cover off undesirable flavors. Moreover, they reduce the usage of preservatives (Thulasi et al. 2013).

Feed ingredients are shielded by microencapsulation from light, oxidation, proteases, and other digestive enzymes. According to Meena et al. (2018), they blend well with fat-soluble additives in feed, remain stable at all pH levels and temperatures, and store for a longer period.

A serious illness in both humans and animals is mycotoxicosis (Sridhar et al., 2016). Mycotoxins are present in animal diets at a rate of about 25%, with higher percentages in developing nations. Aflatoxin-binding MgO-SiO2 nanomycotoxin binder was developed via nanotechnology (Moghaddam et al., 2010). Nanomaterials improve packaging by providing strength from nano-titanium dioxide and nitride, antibacterial nano-zinc oxide, and UV protection. Nanosensors can identify even extremely small amounts of chemical or biological contamination (Manuja et al., 2012).

**Animal Reproduction and Nanotechnology**

Numerous applications of nanotechnology exist in animal reproduction. Reproductive success is increased by identifying and treating reproductive illnesses, sifting and freezing sperm, and acting quickly during calving. Reproductive problems such as retained placenta are treated by nanodevices (Swain et al., 2015).

Nanoparticles (NPs) can release reproductive hormones in a controlled and sustained manner. A. Joanitti and P. Silva (2014) state that they inhibit the oxidation and hydrolysis of vitamins, steroid hormones, and gonadotropic hormones. Nanosensors are small, very sensitive devices that use biomolecules as mobile probes (Florindo et al., 2009; Casals et al., 2017). Probes made of nanomaterials are utilized in diagnosis. These tests can identify hormonal, metabolic, and vaginal infections. They can be used to detect estrus (Scott, 2007; Saragusty and Arav 2011). Nanotubes also can sense estrus. Cattle have glowing tubes beneath their skin during estrus. Estradiol sensors are used to test the amounts of hormones in cow blood. These sensors transmit real-time measurements of cow hormone levels to a central computer (Lee et al. 2013). Bull semen nanocapsules can target the ovum during cow fertilization. Nanotechnology can be used to sort oocytes and sperm. Researchers are now developing biochips for determining the sex of a fetus (Patil et al., 2009). Nanosystems can cryopreserve sperm, oocytes, and embryos. Gametes can be frozen quickly and uniformly by laser by microinjecting gold/metal nanoparticle-containing propylene glycol (Saragusty and Arav 2011). Using microfluidics, the entire procedure is carried out on a microchip (Zhao and Fu, 2017). In small quantities, NPs like cadmium can be employed as contraceptives for sterilizing animals if they are toxic (Xu et al., 2011). The reproductive system of animals is the focus of metallic nanoparticles. Researchers can heat gonads utilizing a magnetic field or antibodies attached to poisonous nanoparticles (NPs) as an alternative to using NPs (Wu et al., 2018); Meena et al., 2018).

**Nanotechnology in Pet Care**

At the international level, the pet healthcare industry is undergoing a substantial amount of expansion. In addition, nanotechnology was utilized in the production of innovative items for animals (Knapp et al., 2016). The development of surface deodorizers and disinfectants was made easier by the inherent physical and chemical properties of these substances. The pet care product industry makes use of nanotechnology in a variety of ways, including the creation of shampoos that include nanoparticles of silver (Troncarelli et al., 2013).

**Safety**

While NPs are generally considered harmless, certain NPs can pose a risk to certain individuals.

For instance, employees of pharmaceutical companies may experience reproductive problems as a result of extended exposure to carbon nanotubes. Furthermore, the body's buildup of magnetic iron oxide nanoparticles (NPs) or the unstable binding of therapeutic agents to particles may cause medications to be released into healthy tissues rather than their intended target tissues, which might be harmful to patients. Healthy tissue will be harmed if the preparation is released beyond its intended target (Troncarelli et al., 2013). Moreover, their influence goes beyond the biological domain, impacting the surroundings in many manners (Manuja et al., 2012). The degradation of the ozone layer in the atmosphere, for example, has been linked to the usage of carbon nanofibers and the increasing demand for radionuclides (Mohantya et al., 2014).

**Conclusion**

The significant progress in the design and manipulation of nanomaterials has enabled the production of a wide range of nanoparticles. Consequently, this makes it possible to tailor medicinal therapies. Significant improvements in veterinary medicine have been made thanks to nanotechnology in several areas, including animal reproduction, nutrition, cleanliness, diagnosis, therapy, and immunization.

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Chapter 35

# Beyond Traditional Treatments: Nanoparticles in Cancer Therapy

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**ABSTRACT**

Cancer is one of the most prevalent diseases in the world, causing millions of mortalities per year. It is a metabolic disease which leads to the uncontrolled division of cells in the body. There are various traditional methods for the treatment of this situation including radiotherapy and chemotherapy. But these methods have certain limitations and multiple side effects. In addition to the cancer cells, these methods can also cause toxicity in the normal cells of the body leading to cell death and organ damage. Therefore, new techniques are needed to be developed in order to tackle these issues. Nanotechnology is the latest benchmark of science which is used in the field of medicine for the treatment of various disorders using nano-sized particles. Different kinds of nanoparticles (NPs) are used in cancer treatment such as liposomes, dendrimers, magnetic NPs and quantum dots. NPs can effectively deliver chemotherapeutic drugs to their target sites either by active or passive transport. In addition to targeted drug delivery, NPs can also instigate immune response of the body against cancer cells. They increase the impact of drug via enhancing its stability, retainability and half-life. However, more research and clinical trials are required before applying NPs in clinical practice. This chapter will discuss the applications of nanoparticles in the treatment of cancer and why they should be preferred over traditional therapeutic practices against cancer.

**KEYWORDS**
Cancer; Chemotherapy; Immunotherapy; Nanoparticles; Targeted drug delivery; Traditional therapy

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## INTRODUCTION

Cancer includes a group of diseases in which cells undergo uncontrolled division and possess the ability to spread in different parts of the body. Cancer is a chronic disease and its occurrence and death rate are increasing rapidly throughout the world. According to the reports of 2018, 18.1 million new cancer cases were registered, and approximately 9.6 million cancer related mortalities were reported (Bray et al., 2018). There exist more than 100 types of cancers that are affecting human but the most common among these are: breast, endometrial, bladder, colorectal, thyroid, kidney, lung, prostate and pancreatic cancer, along with leukemia, melanoma, and non-Hodgkin lymphoma (Lyon, 2011; Siegel, 2013). The therapeutic methods against cancer are different and are correlated with the size, depth as well as the stage of cancer. The oncologist observes the condition of patient and the type of malignancy in order to choose a specific treatment technique (Hosseinzadeh et al., 2017). Among these treatment methods, the most commonly applied methods are, surgery (removal of tumor from the body), radiation therapy (radiations are applied on cancer cells that shrink the tumors and kill cancer cells) and chemotherapy (drugs are used to kill cancer cells) (Guidry et al., 1997; National Cancer Institute, 2024). However, treatment methods of cancer can also affect the normal cells, tissues and organs of the body (Zugazagoitia et al., 2016). The most prevalent side effects include appetite loss, anemia, infertility, dirrhea, urinary bladder disorders and memory loss (Raaijmakers et al., 2005; Celaya et al., 2010).

Nanotechnology is a ground-breaking approach in the field of modern technology that focuses on the applications of various materials at nano-meter scale (one billionth of a meter) (Nasrollahzadeh et al., 2019). Therefore, nanoparticles (NPs) can be defined as the particles having size 1-100 nm. NPs are used for a wide range of applications, including medicinal therapies, industrial production of oxide and solar fuel batteries for storing energy as well as integration into commonly used products such as clothes and cosmetics (Dubchak et al., 2010). Nanomedicine is a diverse domain which has brought revolution in the field of medicine. The applications of nanomedicine include gene therapies, targeted drug delivery systems for the treatment of cancer, nano-immunotherapy, cutting edge diagnostics and biosensors (CRISPR/Cas),

personalized nanomedicine (tailored patient specific treatments) especially for cancer, regenerative medicine, as well as modern treatments for neurological disorders (Fuhrmann, 2023). Fig. 1 illustrates the applications of NPs in various fields and also depicts its role in biomedical treatments of diseases.

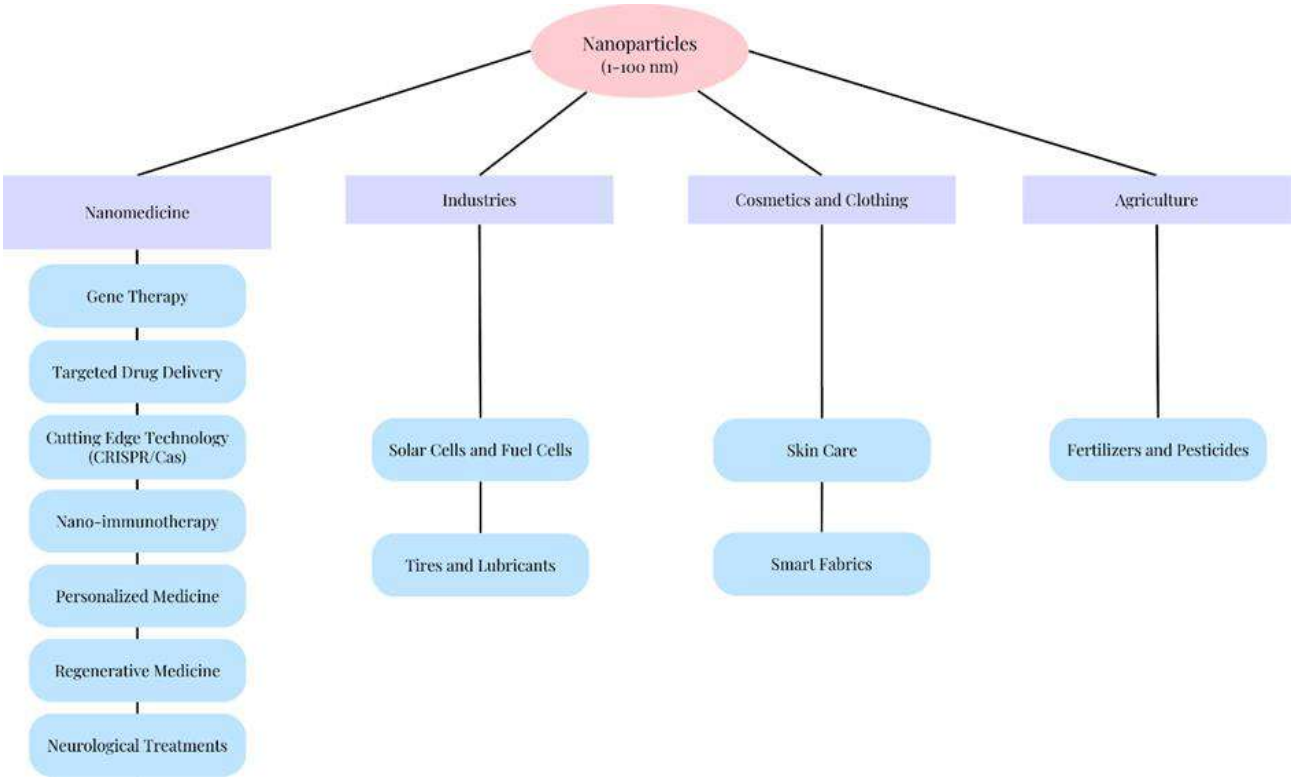


**Fig. 1:** The role of nanoparticles in various fields including nanomedicine, industries, cosmetics and agriculture.

In this chapter, we will discuss about the role of NPs in cancer therapy and its benefits over the traditional treatment methods of cancer. Moreover, we will discuss the challenges to the NPs based treatment of cancer and how they can be tackled.

**Types of Nanoparticles Used in Cancer Therapy**

There are multiple types of NPs that are applied in the therapeutic treatment of cancer. Dendrimers, extracellular vesicles, liposomes, quantum dots, magnetic and silica NPs are discussed here as a potential treatment against cancer. Dendrimers are polymeric macromolecules which are spherical in shape and possess hyperbranched structures (Gavas et al., 2021). The formation of dendrimers is initiated when the reaction between ammonia core and acrylic acid forms a tri-acid molecule. This tri-acid molecule reacts with ethylenediamine to form tri-amine which further reacts with acrylic acid to form hexa-acid. This hexa-acid then forms hexa-amine product and the reaction goes on (Wang et al., 2008). The normal size range of dendrimers is 1-10 nm, nevertheless, it can extend up to 15 nm (Kim et al., 2007).

Extracellular vehicles (EVs) are phospholipid bilayer vesicles. Their size normally ranges for 50-1000 nm. They are secreted in an uninterrupted manner by different types of the cells and vary in size, origin as well as composition. EVs have three types i.e., exosomes, macrovesicles and apoptotic bodies (György et al., 2011). The application of NPs in integration with exosomes is a widely applied technique to fight against cancer as they can easily pass through the immune system. While on the other hand, liposomes possess spherical shape. They consist of either uni- or mutli-lamellar phospholipids that enclose the drug molecules (Gavas et al., 2021). The properties that make liposomes exclusive in medical sciences include their weak immunogenicity, low intrinsic toxicity and biological inertness (Samad et al., 2007). Liposome molecule contains a hydrophobic phospholipid bilayer along with a hydrophilic core which allows it to wrap both hydrophobic and hydrophilic drugs (Allen and Cullis, 2013).

Quantum dots are another type of NPs used in cancer therapy. These are nano-sized semiconductors that possess wide range of absorption, thin emission bands as well as elevated photostability. These properties make quantum dots as suitable candidates for their broad applications in biological imaging (Jamieson et al., 2007). In addition to this, magnetic and silica NPs are also applied in the tumor treatment. Magnetic NPs are metal or metal oxides that are coated with organic substances such as fatty acids and polymers in order to increase the biocompatibility as well as stability (Castaneda et al., 2011). Whereas silica NPs are made up of silicon dioxide ($SiO_2$), a mineral found frequently in rocks and sand. Sol-gel synthesis, chemical vapor deposition and hydrolysis are some methods to synthesize silica NPs (Moulahoum et al., 2023). Their low toxicity and higher biocompatibility make them promising agents for medical and biological applications

(Darwish et al., 2020). Table 1 summarizes the applications and examples of the different types of NPs used in cancer treatment.

**Table 1:** Applications and examples of Nanoparticles applied in cancer therapy.

| Type of Nanoparticles | Applications | Key Examples | References |
|---|---|---|---|
| Dendrimers | Targeting nucleic acids, multidrug resistance management | Polyamidoamine, polyethyleneglycol, triethanolamine | Lim et al., 2013 |
| Extracellular Vesicles | Targeted drug delivery, enhance cytotoxicity in cancer cells | Exosomes loaded with doxorubicin (exoDOX) | Hadla et al., 2016 |
| Liposomes | Drug delivery (paclitaxel, doxorubicin), Higer anti-tumor potential | Doxil®, Myocet® | Zhang et al., 2008; Wang et al., 2017 |
| Quantum Dots | Biological imaging, targeted cancer therapy | Quantum dot aptamers | Bagalkot et al., 2007; Jamieson et al., 2007 |
| Magnetic Nanoparticles | Targeted drug delivery, Magnetic resonance imaging, magnetic hyperthermia | Feridex®, Resovist® | Legge et al., 2019; Gavas et al., 2021 |
| Silica Nanoparticles | Gene delivery, drug carriers, immunotherapy | Amino-silicanes functionalized silica NPs | Katragadda et al., 2010; Gavas et al., 2021 |

**Mechanisms of Nanoparticle Action in Cancer Therapy**

One of the fundamental properties of NPs is their specific targeting of tumor cells. It not only improves the therapeutic effectiveness but also safeguards normal cells of the body from cytotoxicity (Yao et al., 2020). There are various targeting methods that are followed to treat cancer using NPs. Some targeting methods are discussed here.

**Passive Targeting**

Passive targeting is applied to deliver the drugs to their target sites so that they can perform their curative role. Cancer cell proliferation triggers neovascularization along with the production of prominent pores in the vascular wall which leads to the high vascular permeability of tumor vessels as compared to the normal vessels (Akbarian et al., 2022). This frequent angiogenesis allows the macromolecules and NPs to get access and gather inside the tumor tissues by liberating from blood vessels (Yao et al., 2020). Furthermore, the poor drainage of lymphatic system escalates the retention of NPs in the tumor sites, enabling the release of contents carried by NPs into the tumor cells. This leads to enhanced permeability and retention (EPR) effect, which is a key factor for passive targeting (Shinde et al., 2022). EPR effect depends upon the size of NPs, smaller NPs have better penetrability and are less prone to be eliminated by the immune system as compared to larger NPs (Torchilin, 2005; Sykes et al., 2014; Carita et al., 2018).

Tumor microenvironment is another significant factor in the passive transport of medicines using NPs. Warburg's effect (aerobic glycolysis) is the major metabolic characteristic of cancer and a chief energy source for tumor cell proliferation (Pelicano et al., 2006). This glycolysis creates an acidic tumor microenvironment and lowers the pH. In response to acidic environment, some pH-sensitive NPs upon stimulation, release the drugs in the cancer cells (Lim et al., 2018). Therefore, passive targeting has a pivotal role in the targeted drug delivery during cancer. Non-universal existence of EPR effect, varying permeability of blood vessels and non-specific drug distribution are some limitations of passive targeting (Shinde et al., 2022).

**Active Targeting**

Active targeting method is applied to target tumor cells in which ligands and receptors interact directly with each other. The ligands present on the NPs surface are picked to target the specific molecular receptors on surface of tumor cells, that show elevated expression. In this way, NPs are able to differentiate between targeted tumor cells and normal cells (Shi et al., 2011; Kamaly et al., 2012). The ligands on the surface of NPs and the receptor on cancer cell surface interact with each other and instigate receptor mediated endocytosis. This interaction enables the NPs to liberate the medicine present inside them (Farokhzad and Langer, 2009). Hence, active targeting is a preferred method as it can transport macromolecular drugs including, peptides, antibodies, vitamins, carbohydrates and vitamins (Danhier et al., 2010). Ligands are specific for their receptor, some of the widely applied receptors are folate receptor, epidermal growth factor receptor, glycoproteins and transferrin receptor (Yao et al., 2020). Targeting the cancer cells and endothelium via NPs for cancer treatment are some types of active targeting. As described by Xie et al. (2021), Fig. 2 demonstrates the entry of NPs into the cell via active and passive transport.

**Targeting Cancer Cells**

Active transport involves the targeting of specialized receptors present on the tumor cells. Transferrin is a kind of serum glycoprotein which takes part in the transportation of iron into the cells. In most cancer cells, transferrin receptors are overexpressed as compared to the normal cells. Therefore, oncologists use transferrin-conjugated NPs to target cancer cells using active transport method (Amreddy et al., 2015; Liu et al., 2015; Santi et al., 2017). Recent studies have revealed

that transferrin-conjugated NPs are effective against drug resistance provoked by chemotherapy (Nogueira-Librelotto et al., 2017; Soe et al., 2019). In addition to transferrin, active targeting of epidermal growth factor receptor (EGFR) is also effective in cancer therapy. EGFR is a member of tyrosine kinase receptors (ErbB) family. It is hyper-expressed in different kinds of cancer proliferation and used as a target for tumor treatment (Sigismund et al., 2018). In some cases of gastric and breast cancer, EGFR-overexpressed tumor cells are attacked by making modifications in NPs ligands, so they attack only on EGFR receptors (Alexis et al., 2008). Moreover, in order to increase target specificity of NPs during active targeting, two tumor selective ligands are conjugated into a single NP (Balasubramanian et al., 2014).

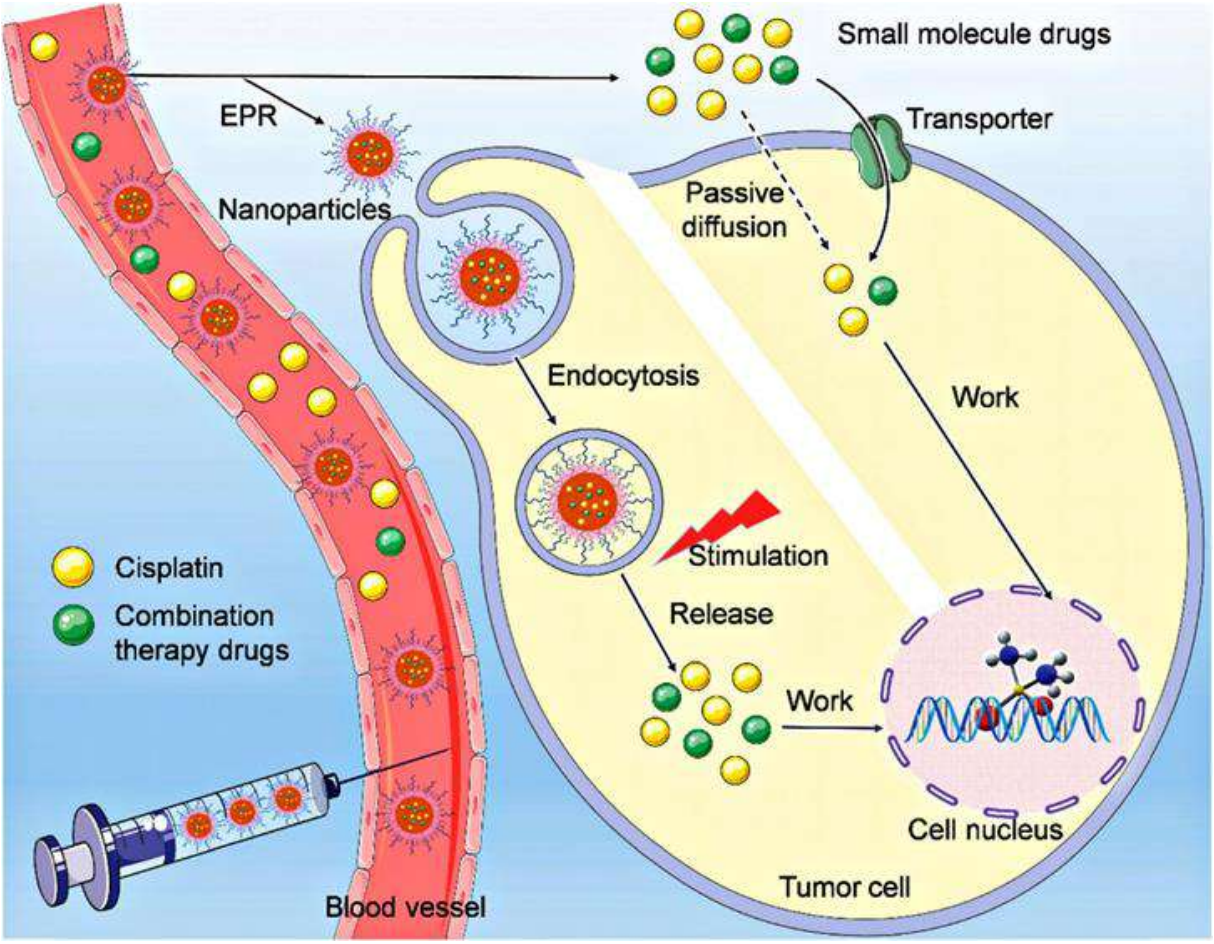


**Fig. 2:** The drug molecules encapsulated inside the NPs are injected into the blood from where they reach the target cells. Drug molecules passing through transporters illustrate the passive targeting using the NPs. Additionally, NPs encapsuled drug entering inside the cell via endocytosis exhibits active targeting. (This figure is reproduced from Xie et al. (2021) under a Creative Commons Attribution-Non-commercial License [CC BY-NC]).

**Targeting Endothelium**

Targeting endothelium is another way of cancer treatment as some NPs produce effect on angiogenesis rather than targeting tumor cells. Vascularization is mainly regulated by vascular endothelial growth factor (VEGF) and VEGF receptors (VEGFRs) (Apte et al., 2019). For instance, targeting VEGFR-3 and VEGFR-2 co-currently using liposomes was found to be effective in cancer therapy (Orleth et al., 2016). One of the methods of targeting endothelium is by attacking on integrins, receptors on the surface of the cell that bind the extracellular matrix proteins. Integrins promote the migration as well as invasion of cancer cells (Desgrosellier and Cheresh, 2010). One of the overexpressed integrins in neovascular endothelial cells is $\alpha_v\beta_3$ integrin which possesses a central role in endothelial cell migration via calcium-dependant pathway (Nisato et al., 2003). Cationic NPs along with $\alpha_v\beta_3$ integrin-targeting ligand was found to be effective in the controlling $\alpha_v\beta_3$ integrin promoted endothelial cancer growth (Hood et al., 2002). Furthermore, $\alpha_v\beta_3$ integrin is also linked with VEGFR-2 signaling which can be reduced by stopping $\alpha_v\beta_3$ integrin-binding. Therefore, targetin $\alpha_v\beta_3$ integrin can enhance the therapeutic efficiency during anti-VEGFR treatment (Ruoslahti, 2002).

**Cancer Immunotherapy**

Immunotherapy has brought novel revelations in the treatment of cancer. In addition to the chemotherapy, NPs also play substantial part in the immunotherapy. Cancer immunotherapy includes the activation of immune response against the tumors (Zang et al., 2017). Targeting the tumor microenvironment and usage of nanovaccines, as well as artificial

antigen presenting cells (aAPCs) are some methods of immunotherapy using NPs (Zang et al., 2017). Nanovaccines take part in the delivery of tumor associated antigens as well as contributing adjuvants to APCs. Furthermore, NPs can itself be adjuvants that elevate antigen presentation of APC and encourage the maturation of dendritic cells. This activates the anti-cancerous activity of cytotoxic T cells (Yang et al., 2018). Various NPs, including gold NPs, dendrimers and liposomes play a crucial role in the cytoplasmic transport of tumor associated antigens into dendritic cells, hereby elevating the immune function in response to cancer cells (Guo et al., 2015). In addition to nanovaccines, immunosuppressive tumor microenvironment is also targeted using myeloid receptor suppressor cells, regulatory T cells as well as tumor-associated macrophages. All of these are essential types of the cells in tumor microenvironment and play important role in the immunotherapy against cancer proliferation (Shao et al., 2015).

**Advantages Over Traditional Treatments**

The applications of nanotechnology in the cancer diagnosis and prognosis have brought a completely new epoch in the cancer therapy. NPs target cancer cells and deliver drugs to their targets either through passive or active targeting without causing any toxicity. NPs can be altered according to the properties of their target. They can either be made temperature or pH sensitive to cope with high temperatures or lower pH in tumor microenvironment respectively. Furthermore, the surface chemistry, size, molecular mass and shape of NPs play a crucial part in the targeted drug delivery system (Gavas et al., 2021).

Traditional cancer treatment methods mostly involve conventional chemotherapy and radiotherapy. Although these methods are effective in cancer treatment but there are several drawbacks associated with them such as cytotoxicity and non-uniform dispersal. The major obstruction from where the chemotherapeutic drugs have to pass is the complex process of drug metabolism present in body. A drug usually has to pass through tumor microenvironment, blood brain barrier, kidney filtration as well as reticuloendothelial system. Reticuloendothelial system contains macrophages, immune cells and blood monocytes (Yona and Gordon, 2015). Whereas microphysiological system present in lungs, spleen and liver react with the drugs and instigate leukocytes that eliminate the drug and reduce the half-life of the drug (Liang et al., 2021). However, surface modified NPs can pass through these mechanisms and effectively target the cancer cells. Nonetheless, infiltration by kidney is an important function in the human body and it is indispensable as it can reduce the NPs induced toxicity (Gavas et al., 2021).

Moreover, blood brain barrier inhibits the toxic drugs from reaching to the brain. This is the reason due to which a very limited number of chemotherapeutic drugs are available for brain tumors (Tran et al., 2017). Nevertheless, NPs can cross the blood brain barrier and target the brain cancer. Gold-NPs are normally applied for transporting drugs to brain tumors where they can provoke apoptosis in cancer cells (Feng et al., 2017). In addition to this, NPs can also enhance the stability of the drugs and prevent them from degradation, as the drugs are mostly encapsulated inside the NPs. NPs are also able to retain themselves in the tumor because of widespread angiogenesis, impaired lymphatic drainage and weak vascular architecture of tumor cells (Brigger et al., 2002). Lastly, NPs can be taken via multiple routes such as intra-ocular, nasal, parenteral and oral and they possess higher surface to volume ratio along with higher intracellular uptake than another drug carries like microparticles (He and Park, 2016). Hence, NPs are more preferable and capable of eliminating cancer than the traditional therapeutic approaches for cancer treatment.

**Current Challenges and Limitations**

Now a days, nanotechnology has made significant advancements and the research about NPs has also upsurged quickly. But only a few NPs based therapeutic methods have reached the stage of clinical trials while most of them was not able to cross in vitro and in vivo stages. Although, application of each NP face different challenges in clinical translation but the most prevalent are biological, technological and study design related restrictions which are faced by almost all of the NPs. Biological problems to NPs involve the challenges in degradation, toxicity, tempering biodistribution, movement across biological barriers and only a few options for dose administration (Ryman-Rasmussen et al., 2006). Intravenous injections are usually used to inject NPs into the body, in this way NPs enter the bloodstream directly and reaching and staying in their target site becomes problematic for them. Consequently, higher does concentration of the drug is applied, which can bring undesired effects (Jia et al., 2018). Besides, magnetic NPs are used to tackle this issue because their movement can be regulated against the bloodstream using 3D magnetic fields. However, it is necessary to do research about the effects of magnetic fields on human body (Gavas et al., 2021). In addition to this, due to the retention and toxicity of NPs they can produce kidney, lung and liver damage. It has been reported that, NPs accumulation has caused inflammation, oxidative stress and cytotoxicity in the lung (Awasthi et al., 2016). NPs can also damage normal cells as they can produce free radicals (Xia et al., 2006). Scale-up synthesis, equal optimization, and performance projections are among the technological problems associated with NPs. Whereas Study design difficulties such as study size, intent, and scheduling of NP treatments during therapy have a substantial impact on clinical studies (Gavas et al., 2021). Most of the studies focus on "cell and animal models," which may not produce intelligible outcomes in human trials. As a result, using a single model makes it difficult to mimic actual human body reactions. Therefore, different models should be tested, and it will be better if any clinical study is performed on those methods of NPs for mitigating cancer.

**Conclusion**

In conclusion, NPs based applications is one of the hallmarks of modern-day science. Cancer is one of the deadliest diseases in the globe. The treatment of the cancer using NPs is found to be very effective and reliable process. NPs can deliver drugs to the target cells either by active or passive transport. They are efficient drug carriers and increase the stability, retainability and half-life of the drug in tumor site which increases the effectiveness of drug against the prognosis of cancer. However, further research and clinical trials are required before applying NPs directly to the humans as their accumulation can itself produce toxicity and free radicals in the normal cells. Therefore, different methods can be sorted out to tackle their toxicity and increase their effectiveness against cancer.

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Chapter 36

# Exploring the Multifaceted Applications of Nanoparticles in Medicine and Beyond

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**ABSTRACT**

The nanotechnology has rapidly expanded over the past decade with nanoparticles finding applications in various industries like medicine, food science and cosmetics. The nanoparticles enables precise delivery to a specific cell or tissue using surface alteration for increasing targeting and controlled release in medicinal therapy. The targeted drug delivery, facilitated by some nano-carriers, which offers specific treatment to a disease sites, minimize side effect and maximizing therapeutic efficacy. The nanoparticles offer potential for targeted cancer therapy but yet face challenges like poor distribution within tumors due to physiological barriers. Ultrasound assisted delivery holds, enhancing nanoparticles absorption also localized drug release offering new avenues for potent cancer treatments.

**KEYWORDS**
Nanoparticles, Nanotechnology, Drug delivery, Cancer

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## INTRODUCTION

The nanotechnology has grown rapidly and several items incorporated nanoparticle are recently used in various industries like medicines, food science and cosmetics (Ranjha et al., 2022). The nanoparticles have diameters ranging between 1 to 100nm and used in various fields such as electronics, cosmetics also in therapeutics and diagnostic medicinal applications which are due to their very small or tiny size and large surface area (Joseph et al., 2023).

The targeted drug delivery is a phenomenon by which nano-carrier transport pharmaceuticals or gene to a specific cell, tissue and organ via blood circulation (Himri and Guaadaui, 2018). By lowering the harmful side effects at non-disease location, increasing therapeutic molecular activities to specific sites, with selective delivery reduces the systematic impact (de Castro et al., 2021). The utility of nanotechnology in medicine has advanced importantly, in the field of gene delivery (Shen et al., 2018). The use of nanoparticles as drug carrier or other bioactive therapeutic compound have maximized the therapeutic efficacy and administration of loaded medications and minimized their adverse effects (Li et al., 2019).

The $Fe_3O_4$ nanoparticles are used in variety of fields such as energy storage, magnetic fluids, biotechnology, catalase and environmental modification (Iqbal et al., 2017). The $Fe_3O_4$ nanoparticles are used in the field of bio-photons, magnetic resonance imaging and hyperthermia reagents, targeted drug delivery, diagnosis and radiation therapy of malignant cells with assistance of a magnetic field (Xie et al., 2018).

The presence of $Fe^{2+}$ state in $Fe_3O_4$ nanoparticles makes them preferable to other magnetic materials because it act as an electron donor (Gerulová et al., 2022). They cause no hysteresis, when external magnetic field is removed and leaves no residual magnetization behind (Mohammed et al., 2017). This lowers the risk of coagulation and also lowers the possibility of *in-vivo* agglomeration. The $Fe_3O_4$ nanoparticles can absorb energy of microwave close to tumors tissue and release drugs, which can quickly transform microwave energy into heat for thermal treatment by using an external magnetic field and microwave radiation (Mohammed et al., 2016).

### Enhanced Drug Delivery to Specific Tissue and Cell

In the developing field of medicinal research which enhanced medication delivery to certain tissue and cells (Sahu et al., 2021). The drug enclosed in nanoparticles can be delivered to specific call and tissue with precision (Mitchell et al.,

2021). The surface modifications of nanoparticles enhance their targeting capabilities by recognizing specific biomarkers on target cells (Sanità et al., 2020). The liposome a lipid-based vesicle is capable of medications delivering to certain tissues or cells (Grimaldi et al., 2016). The drug carriers which release their payload in response to particular stimuli like pH and temperature, makes the targeted and controlled medication distribution possible (Zhang et al., 2018). The therapeutic gene can be delivered to certain cells and tissues by viral or non-viral vectors, potentially providing treatment for hereditary illness and other ailments (Ramamoorth and Narvekar, 2015). The drug molecules are conjugated with small molecules of peptides or aptamers to enhance their selectivity for the targeted tissues or cells (Zhu et al., 2015).

The techniques for delivering controlled medications have a big impact on medicine. The long-term, optimal dosage distribution of pharmaceuticals has been frequently made possible by controlled releasing polymer systems, which also enhance the use of highly unstable and poorly soluble or severely dangerous medications while maximizing the patient compliance and therapeutic efficacy. The nanoscale materials can be used as drug delivery vehicles to produce highly selective and effective therapeutic effects and diagnostic approaches (Adepu and Ramakrishna, 2021).

When passing across the bloodstream, the nanoscale particles do not clog or restrict the microvasculature. The microscopic nanoparticles have the ability to travel throughout the body and penetrate tissues such as tumors. Additionally, cells can absorb nanoparticles by inbuilt mechanisms like endocytosis (Stillman et al., 2020). For cancer therapy, the nanoparticles have been used in the past and imaging agents were used for cancer diagnostics. These special vehicles have been made to know where they should go and help the medicines to reach to the right place (Baetke et al., 2015).

Delivery of drugs to targeted places in the body using NPs through veins and the interactions of the NPs with different cells like macrophages is important to handle it (Polo et al., 2017).

**Overcoming a Biological Barrier with Nanoparticles**

A chemotherapeutic drug encapsulated in the nanoparticles and administered as part of the tailored delivery strategies for diseases such as cancer. They could increase medication delivery's efficacy and specificity, enabling more focused drug delivery (Din et al., 2017). To improve targeting, the nanoparticles can potentially be coated with chemicals that only identify and stick to cancerous cells. The most often used tumor-specific moieties to target are the aberrantly overexpressed tumor receptors (He et al., 2019). The therapeutic drug molecules can be better encapsulated in nanoparticles to increase their bioavailability, bio-distribution, and internalization into the target cell. However, only ~1% of the nanoparticles aggregate even with current developments in the field of nanotechnology such as functionalization with previously mentioned targeted molecules in cancers (Sánchez et al., 2020). A successful malignant tumor treatment plan is still unattainable. The tumor architecture's numerous physiological obstacles may be the cause of the extremely low targeting effectiveness. The first challenge for nanoparticles is their high risk of being removed by blood circulation shortly after intravenous delivery, which occurs well before they enter the tumor microenvironment (Kutova et al., 2019).

This is made possible by the blood proteins' ability to opsonize nanoparticles, which the cells of the mononuclear phagocyte system subsequently recognize and eliminate from the circulation. Eventually, the populations of nanoparticles which the MPS is unable to completely eliminate will get dispersed throughout the population (Gustafson et al., 2015). In order for the nanoparticles to successfully enter the tumor microenvironment, they must congregate at endothelium lining throughout the circulation. A high overexpression and high extracellular matrix density are typical characteristics of the tumor structure; these tissues are distinct from normal tissues. The overabundance of extracellular matrix acts as a crucial barrier to the diffusion of nanoparticles over the interface (Balachander et al., 2021). In other words, the circumstances make it more difficult for the nanoparticles to reach the targeted cells, which cause them to deliver the therapeutic medicine too far from the tumor and its surrounding microenvironment. Due to this distinct clinical features of the tumor, nanoparticles cannot be transported, deposited, or diffused into the tumor effectively, which makes the therapeutic response to the tumor ineffective (Tian et al., 2024).

The constraints noted above are addressed by ultrasound-assisted drug-loaded nanoparticle delivery, which increases the absorption and storage of nanoparticles by cells and stimulates the drug release exclusively at the intended spot. These effects are produced by a number of mechanisms including cavitation and hyperthermia, which follow simultaneous interactions between ultrasonic radiation, nanoparticles and the cells (Canavese et al., 2018).

**Mucosal Barrier**

A quick way to deliver a therapeutic substance to the intestinal mucosa is by low-frequency ultrasound. Initially, the possibility of using low-frequency ultrasound to enhance medication delivery to colon tissues using Franz diffusion cells was investigated. The *ex-vivo* experiment's results showed that administering low-frequency ultrasound (20 kHz) could deliver dextran (3 kDa) tagged with Texas red up to seven times more frequently than the control group (Turcanu, 2022). The images captured using confocal microscopy demonstrated that the tissues had homogeneous diffusion of the fluorescent dextran. However, it hasn't been proven yet that how ultrasound can enhance the transport of drugs in the form of nanoparticles across the lungs mucosal barrier (Chen et al., 2021). The existing restriction of low trans-mucosal nanoparticle/drug delivery for a number of lungs, vaginal, and bladder disorders may be addressed by the possible application of ultrasound in trans-mucosal drug delivery. A barrier to achieving sustained retention, uniform dispersion, and effective medication molecule/nanoparticle administration in the vaginal canal is the mucus-filled vaginal epithelium

(Jicsinszky et al., 2021). The majority of particulate matter including nanoparticle, are captured by the mucus as a result of steric interactions as well as adhesion interactions. The mucous barriers also reduce the efficacy of traditional medication delivery methods in the treatment of urinary tract infections, interstitial cystitis, hyperactive bladder, and bladder cancer. To enhance trans-mucosal medication administration in any of these scenarios where mucous creates a physicochemical barrier preventing the drug or drug carrier from reaching the target, ultrasonic energy may be used (Watchorn et al., 2022).

**Example of Nanoparticles-based Drug Delivery System in Clinical use**

The abraxane, or protein-bound paclitaxel particles, is a well-known illustration of a drug delivery system based on nanoparticles that is now being used in the clinical settings. The chemotherapeutic medication paclitaxel is available in an albumin-bound nanoparticle formulation called abraxane. This is its therapeutic use and how it functions: the strong cytotoxic drugs like paclitaxel cause microtubule malfunction, which causes fast dividing cancer cells to enter a cell cycle arrest and undergo apoptosis (Desai, 2015). However, because of the paclitaxel's limited solubility, typical formulations must include solvents such ethanol and cremophor EL, which can result in toxicities and hypersensitivity responses. By encasing paclitaxel in albumin nanoparticles, abraxane circumvents these drawbacks and does away with the requirement for harmful solvents. The solubility and stability of paclitaxel’s are enhanced by albumin blood nanoparticles, through which drug reaches to the specific cells of tumors (Straubinger, 2021).

**The Therapeutic Application of Nanoparticles**

**Cancer Treatment**

The unchecked cell division caused by mutations in the genes that control cell division and proliferation is the hallmark of cancer. Initially a localized disease, cancer eventually metastasizes that is, spreads to other areas of the body making the condition incurable. Globally, cancer is the second most prevalent cause of death. Every year, more than 10 million individuals are diagnosed with cancer (Matthews et al., 2022). For many years, scientists have been especially interested in effective cancer treatment. One of the most thoroughly studied subjects in life science and technology to date is cancer. The capacity of young cells to multiply and create new ones aids in the body's ability to maintain homeostasis (Sztandera et al., 2018). When defects in the cell that initiate cancer lead to aberrant cell proliferation, tumors are created. The cancer cells proliferated into tumorigenic aggregates of cells by traveling through the blood and lymphatic arteries to various regions of the body. In 2018, cancer claimed the lives of over 9 million people, making it the second most frequent cause of death worldwide (Testa et al., 2018). When cancer is detected early on, there are the best chances of employing appropriate therapeutic intervention strategies. Numerous methods have been employed to efficiently identify cancer in its early stages. These include the use of tumor markers, screening for cytogenetic and cell genetic abnormalities, and imaging techniques such as computed tomography, ultrasound scanning, and endoscopy (Schiffman et al., 2015).

The number of cancer diagnosis and cancer-related deaths is rising yearly, even with the availability of conventional cancer treatment options, new approaches are required to advance the current cancer therapy protocols. The use of nanoparticles and nanocomposites in nanomedicine, in conjunction with thermal therapy, presents new opportunities and significant promise for enhancing the efficacy of cancer treatment (Wang et al., 2019). The nanomaterials can create and, in particular, boost heating capabilities at the tumor site because of their optical and magnetic properties. The nanomaterials have unique properties that allow them to generate heat, which kills cancerous cells. Using the nanomaterials and nanoparticles such as magnetic iron oxide nanoparticles, carbon nanotubes, nanorods, nano-shells, nanocomposites, and other nanoparticles in thermal ablation of cancers, emphasizes the advantages of this approach over the conventional heating methods (Beik et al., 2016).

Numerous therapeutic methods are available, depending on the kind and extent of the malignancy. For instance, surgery helps remove tumors or malignant lumps. The chemotherapy uses drugs to kill specific cancer cells. Immunotherapy, hormone therapy, radiation therapy, targeted drug therapy, bone marrow transplantation, and cryoablation are some other cancer therapies. Although these treatments have been demonstrated to be helpful in a few instances, they also have significant side effects (Debela et al., 2021).

**Nanoparticles Mediated Chemotherapy**

Chemotherapy is a special way of treating cancer by administering chemotherapeutic medications. This technique reduces the toxic and detrimental effects of chemotherapy on particular tissues while increasing its efficacy (Alam et al., 2018). The interactions between chronic inflammation and oxidative stress are part of the pathogenesis of cancer. The most urgent problem of the twenty-first century is still women's breast cancer, even with chemotherapy and magnetic treatment. One major issue is how to handle chemotherapy-induced adverse effects following treatment (Totzeck et al., 2023). Therefore, the scientific community has a challenge in trying to discover target-specific drugs that can treat cancer without having adverse consequences. The production of nanoparticles by mixing medicinally useful plants with metal oxide nanoparticles, which have the greatest therapeutic potential and the least amount of harm, is probably the result of a greater use of green technology (Ward et al., 2020).

In therapeutic contexts, this therapy is sometimes known as drug cocktail treatment. The therapeutic basis for this combination therapy is provided by dose-limiting toxicity of cancer medicines and multidrug resistance (Núñez et al.,

2016). Two and three anticancer medications pose a serious risk not only to cancer cells but also to healthy cells. This dose-dependent deleterious effect is seen in most rapidly growing cells, such as bone marrow, hair follicles, and the endothelial linings of the gastrointestinal system. The anticancer medications can have side effects that can be fatal in many cases (Olatunde et al., 2021). It has been shown that combining multiple anticancer drugs improve therapeutic outcomes and lowers dose-associated toxicity. The anticancer drugs with different toxicity profiles and mechanisms of actions can be combined to achieve synergistic or additive therapeutic benefits. On the other hand, similar therapeutic results are obtained by combining drugs with similar toxicological profiles and modes of action at lower doses, which also reduces toxicity and the potential for multidrug resistance (Shrestha et al., 2019).

Currently, the medical settings frequently employ pharmaceutical cocktail therapy. Different anticancer medication combinations are used to treat cancer patients in order to minimize dose-associated toxicity and prevent the development of multidrug resistance (Kohli and Omray, 2022). It is advised that patients with cancer who are at an advanced stage have this type of combination therapy. Since it has been shown to affect the overall efficacy of cancer therapy, the molar ratio of the drugs being administered is an important statistic in combinational chemotherapy (Shrestha et al., 2019).

**Photothermal and Photodynamic Therapies**

Phototherapy is a modern cancer treatment technique. The phototherapy is a potentially beneficial and less invasive cancer treatment method. The phototherapy is a technique that creates heat for the thermal ablation of cancer tumors with a limited penetration into the surrounding healthy tissues. It works by activating photosensitizing medicines with pulsed laser irradiation in the near-infrared (NIR) band (Bhole et al., 2021). Unlike the phototherapy, photodynamic treatment primarily uses photosensitizers to transform molecular oxygen into harmful reactive oxygen species (ROS), such singlet oxygen, when light at an effective wavelength triggers the photosensitizers. This finally leads to oxidative stress, which kills cancer cells (Zhou et al., 2016).

**Photothermal Therapies**

The thermal therapies have been used to treat cancer cells since the 18$^{th}$ century. In clinical settings, radiation and chemotherapy can work in the concert with hyperthermia. Due to denaturation of proteins and cell membrane breakdown, cancer cells are permanently harmed by heat shock (Beik et al., 2016). However, the normal tissues are frequently impacted by this treatment. Applying the photothermal therapy to cancer therapy can make it easier to use laser radiation treatment for more targeted cancer treatment. Thus, laser-induced hyperthermia appears to be beneficial in the management of choroidal or retinal malignancies (Gupta and Malviya, 2021). The requirement for a powerful laser to kill the tumor cells is a major drawback of this treatment. In the meanwhile, a selective heating photothermal therapy using a photothermal agent has been suggested (Schena et al., 2017). The main prerequisite for photothermal therapy is a biocompatible photothermal agent with a high absorption coefficient, an NIR light source, and an NIR area. The light-excitation coefficient and the absorption of the NIR wavelength determine how much the temperature rises in photothermal therapy.

Following NIR radiation, the gold nanorods primed for combination therapy in metastatic breast cancer were wrapped with DNA loaded with doxorubicin. This combination therapy markedly slowed the development of the breast tumor and lung metastasis (Dykman and Khlebtsov, 2019).

**Photodynamic Therapies**

The introduction of a photosensitizing drug and exposure to light at a certain wavelength is necessary for photodynamic therapy. The advantage of photodynamic treatments is that they may be given even after chemotherapy, radiation, or surgery, and they can be repeated several times without having an immunosuppressive or myelosuppressive impact (Correia et al., 2021). For the past 3,000 years, light has been utilized as a kind of therapy. Light was employed by the ancient Egyptian, Indian, and Chinese cultures to treat a wide range of ailments, such as vitiligo, rickets, psoriasis, and skin cancer (Bhole et al., 2021). A single pure chemical with appropriate stability, cheap production costs, and the capacity to conduct quality assurance studies should be the ideal PS agent. A strong absorption peak in the 600–800 nm (red to dark red) region would be desirable for a PS agent since photon absorption at wavelengths longer than 800 nm lacks the energy necessary to excite oxygen to its single state and provide a noticeable yield (Han et al., 2022). Chlorines, bacteriochlorins, and phthalocyanine, for instance, can enhance the control of tumors. The immune-stimulating effect of photodynamic therapy may depend on a significant inflammatory response and necrotic cell death upon illumination, according to some previous research (Dinakaran and Wilson, 2023). Conversely, it has been proposed that PSs that cause less inflammation and more apoptosis are suitable for uses like brain malignancies, where swelling is desired. The water-soluble porphyrin combination known as the hematoporphyrin derivative (HPD), a refined version of sodium porfimer, was the first PS utilized in cancer therapy. This mixture subsequently became known as photofrin (Almeida-Marrero et al., 2018).

**Infection Disease Management**

**Targeted Delivery Antimicrobial Agents**

The targeted delivery is the effective administration of a therapeutic drug and it predominate accumulation in a desired location. The agent-laden system must escape the immune system, target a particular cell or tissue, and release the

therapeutic agent put into it for an effective targeted delivery. It must also remain in the physiological system for the optimum amount of time (Tamilvanan Shunmugaperumal, 2015).

To get rid of the microorganisms, many antimicrobial agents have been employed. Even though the effectiveness of currently available medications and treatments has been thoroughly demonstrated, ineffective and careless antimicrobial agent delivery may result in an inadequate therapeutic index and unfavorable side effects like nausea, vomiting, rashes, scaling, and a decrease in the microbiota of the stomach (Yaneja and Kaur, 2016). However, changes in the environment, technology, society, and growing microorganisms are also causing new illnesses to emerge and antimicrobial resistance to evolve, in addition to the creation of multidrug-resistant microbial strains. In order to increase the activity against multidrug-resistant bacteria, pharmaceutical companies and the scientific community are constantly developing novel antibacterial drugs, drug targets, and delivery systems due to the persistence of antibiotic resistance in pathogenic and opportunistic microorganisms (Medina and Pieper, 2016). Currently, the development of molecular nanostructures through precise particle size and shape is a major area of study for biological applications, such as the administration of antibiotics. Because of their unique characteristics and high surface area to volume ratio, nanoscale materials and polymers have become innovative antibacterial agents and delivery systems. The development of fresh uses in this sector makes metallic and polymeric nanoparticles an appealing alternative to antibiotics, as they have been shown to exhibit antibacterial capabilities (Mabrouk et al., 2021).

Rapid advances in the nanotechnology will have a significant influence on the tissue engineering, drug and bioactive delivery, and other therapeutic application fields (Kumar et al., 2020). Numerous antimicrobial agents have been employed to eradicate or impede the proliferation of microorganisms, including viruses, fungi, and bacteria. Since these medications' pharmacological properties are widely known, poor delivery and degradation may result in an inadequate therapeutic index and adverse consequences (Khezerlou et al., 2018). The enormous surface area to mass ratio, functionality structure, and tiny, controlled size are only a few of the numerous benefits of nanoparticles.

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Chapter 37

# Role of Nanoparticles in the Treatment of Cancer

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**ABSTRACT**

Cancer is the primary cause of mortality worldwide, with increasing incidence and significant healthcare burden. The traditional cancer therapies like surgery, radiotherapy, and chemotherapy pose challenges due to their impact on healthy cells. The nanoparticles have been developed as innovative methods in cancer therapy, offering targeted drug delivery, enhanced imaging capabilities, and potential therapeutic benefits. The nanoparticles display special physiochemical features which promote them effectively and strike the tumor cells while diminishing system toxicity.

**KEYWORDS**
Cancer, Nanoparticles, Targeted drug delivery, Therapeutics

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## INTRODUCTION

The cancer ranks as the second most prevalent cause of death worldwide. The incidence of cancer has shown a notable rise with time, as in 2018, global deaths from cancer were documented at 8.97 million. It is projected that by 2060, cancer will likely become the primary reason for mortality, resulting in approximately 18.63 million fatalities globally (Mattiuzzi and Lippi, 2019; Hassanpour and Dehghani, 2017; Wu et al., 2016). The WHO (World Health Organization) states that cancer represents the most important global load, accounting for 244.6 million 'Disability Adjusted Life Years' (DALYs), with 137.4 million DALYs in males and 107.1 million DALYs in females. The greatest number of DALYs is notably observed in individuals aged above 60 years, accounting for 124.2 million DALYs, representing 50.8% of the total burden. The most prevalent malignant diseases among those aged fourteen years or younger include leukemia (37%), cancers of the brain and nervous system (16%), and cancer of the lymphatic system (13%). Among individuals aged 15–49 years, breast cancer (13%) stands as the most prevalent malignancy, as compared to liver cancer (12%) and lungs cancer (9%). For individuals aged 50–59 years, lung cancer emerges as the most common malignant disease (18%), compared to liver cancer (11%) and breast cancer (9%). Among those aged 60 years or older, the most common malignancies comprise lung cancer (21%), stomach cancer (9%), liver cancer (9%), and colorectal cancer (9%) (Mattiuzzi and Lippi, 2019). Among men, the most prevalent cancer types are found in the bronchus, lungs, rectum, colon, and prostate, while in women lung, bronchus, breast, rectum colon, thyroid, and uterine corpus are the most prevalent (Siegel et al., 2019). Hence cancer presents a significant health concern that affects people across all human societies. Unfortunately, the illness demonstrates variation within tissues, presenting substantial obstacles to accurate diagnosis and effective treatment outcomes (Meacham and Morrison, 2013).

The surgical, chemotherapeutic, and radiotherapy cancer treatments are readily available and commonly employed. However, their scope isn't limited to cancer cells alone; they also affect healthy cells, posing a major challenge in the current cancer therapy. The NPs, are stable, nontoxic and physiologically compatible substances present naturally, enabling their utilization for efficient drug delivery methods (Ho et al., 2017). The metal nanoparticles offer a potential solution to challenges associated with conventional treatments, playing a key role in the treatment of cancer by enhancing targeting precision, facilitating gene suppression, and improving the delivery of drugs. The metal NPs, when modified with specific binders, improve the precision of energy deposition within tumor cells. Additionally, they serve as diagnostic tools for the

cancer cell imaging. These systems offer simultaneous diagnosis and treatment while allowing controlled and targeted drug release, transforming treatment approaches for cancer (Baranwal et al., 2023).

The word "nano" is derived from the Greek term "nanos," which means "a dwarf." It was adopted in 1947 during the International Union of Pure and Applied Chemistry (fourteen conference) to denote $10^{-9}$ (one billionth) of a unit. The prefix "nano" is employed to characterize entities or procedures that are extremely small, typically at the nanometer scale (Joudeh and Linke, 2022; Qadeer et al., 2022). The nanoscience is a scientific field centered on examining material properties at a small scale, specifically emphasizing the distinctive properties of solid-state materials that vary depending on their size (Mulvaney, 2015). The nanotechnology is the field that involves the production, manipulation, and utilization of materials sized between 1 and100 nanometers, termed nanoparticles (Hasan, 2015).

**Classification of Nanoparticles**

The nanoparticles are classified into three classes i.e. organic NPs, carbon-based NPs, and inorganic NPs (Ealia and Saravanakumar, 2017).

**Organic NPs**

This group of nanoparticles (NPs) is composed of carbohydrates, proteins, lipids, polymers, or alternative organic compounds (Pan and Zhong, 2016). The liposomes, dendritic polymers, micelles, and protein complexes e.g., ferritin is among the most notable examples of this class. These NPs are generally harmless, and biodegradable, and may feature a hollow core in certain cases, such as liposomes. These are susceptible to heat and electromagnetic waves, comprising heat and light (Ealia and Saravanakumar, 2017). Furthermore, these nanoparticles are commonly held together by intermolecular forces, making them inherently less stable and prone to elimination from the body (Ng and Zheng, 2015). Several factors, including composition, surface morphology, stability, and payload capacity, play crucial roles in determining the potential applications of organic NPs. Nowadays, these NPs have been extensively employed in the biochemical industry, especially in targeted medication (Ealia and Saravanakumar, 2017) and the treatment of cancer (Gujrati et al., 2014).

**Carbon-based NPs**

The carbon-based NPs consists of carbon atoms (Ealia and Saravanakumar, 2017). Among this group, bucky-balls, carbonaceous black NPs, and carbon nano-dots are the best examples. The carbon molecules identified as "bucky-balls" are recognized by the symmetric closed-cage structure. For example, C60 bucky-balls are composed of sixty carbon atoms clustered in a football like pattern (Long et al., 2013). Moreover, different types of bucky-balls, such as C70 and C540 have been reported (Dresselhaus et al., 1993). The carbon black NPs are clusters reminiscent of grapes comprised of round particles that have been tightly bonded together (Yuan et al., 2019). The spherical carbon NPs with a diameter of less than 10 nm have been termed as carbon quantum dots (Lu et al., 2016). The carbon-based NPs integrate the fascinating physical and chemical attributes seen at the small scale with the distinctive qualities of trigonal carbon bonds. The extraordinary strength, electrical conduction, optical qualities, electron attraction, sorption capacities, and thermal conductivity of carbon-based nanoparticles (NPs) rendering them beneficial for a wide range of applications, including drug delivery, photovoltaic devices, bio-imaging, energy storage, and environmental sensing applications for evaluating microbial ecology or identifying microbial pathogens. More intricate carbon-based NPs like nano-diamonds and carbon nano-onions, recognized for their minimal toxicity and compatibility with biological systems, are applied in drug delivery and tissue engineering applications (Joudeh and Linke, 2022).

**Inorganic NPs**

This category includes the NPs composed of materials other than carbon or organic substances. The common examples within this category include metallic, semiconductor, and ceramic NPs. The metal nanoparticles consist entirely of metallic starting materials and may occur as monometallic bimetallic (Toshima and Yonezawa, 1998), or polymetallic (Nascimento et al., 2018). The bimetallic nanoparticles can be formed from mixtures or structured with different layers, such as core-shell configurations (Toshima and Yonezawa, 1998). These nanoparticles exhibit distinctive optical and electrical traits because of their localized surface plasmon resonance characteristics (Khan et al., 2019). The semiconductor NPs, made from materials bridging metal and non-metal properties, possess unique wide bandgaps. They experience significant property alterations through bandgap tuning, unlike bulk semiconductor materials (Joudeh and Linke, 2022). The ceramic NPs consist of inorganic materials, including carbides, phosphates, carbonates, and various metalloids and metal oxides like calcium and titanium (Thomas et al., 2015). They are predominantly utilized in biomedical fields due to their remarkable stability and significant ability to bear heavy loads (Joudeh and Linke, 2022). Some of the commonly used NPs along with their use are discussed in Table 1.

**How do Nanoparticles Work to Treat Cancer?**

The nanoparticles are ideal therapeutic instruments for monitoring tumors and administering therapy because of their diminutive dimensions (1–100 nm) and their large surface-area-to-volume ratio (Pedrosa et al., 2015). Coupling NPs with biomolecules enables effective medicine delivery and facilitates both anatomical and functional imaging. The NP delivery

encompasses three essential stages: (a) precise systemic targeting while evading RES capture; (b) migration from blood vessels within tumors; and (c) infiltration into cancer-affected cells. The effectiveness of nanoparticles as anti-cancerous agents stems from their significant accumulation within tumor cells. The microenvironment of a tumor is composed of vascular networks, extracellular matrix lymphocytes, inflammatory cells and signaling molecules (Barar and Omidi, 2013). Compared to healthy tissues, solid tumors exhibit unique features, particularly in their permeable microvascular environments, where capillary pores range from 120 -1200 nm. This permeability enhances the penetration of NPs into the tumor (Omidi and Barar, 2014).

**Table 1:** Different Types of nanoparticles and their applications

| S.NO | TYPES OF NPs | Examples | Applications | References |
|---|---|---|---|---|
| 1 | Inorganic NPs | Gold NPs | Drug delivery, molecular imaging, and bio-sensing | (Baranwal et al., 2023) |
| | | Silver NPs | Inhibit the growth of cancer cells and useful in detecting different cancers such as prostate, lung, cervical and liver cancers | (Baranwal et al., 2023) |
| | | Iron Oxide NPs | Superparamagnetic iron oxide NPs enter cancer cells via the EPR effect and are guided magnetically | (Yu et al., 2012) |
| | | | SPION-based theranostic agents are vital for delivering chemotherapy drugs, genes, and diagnosing cancer | (Baranwal et al., 2023) |
| | | Copper NPs | Generate reactive oxygen species (ROS) within cancer cells, ultimately resulting in cell death, enabling selective targeting while preserving healthy cells | (Das et al., 2015) |
| | | Zinc oxide NPs | Use in Anticancer treatment it triggers cancerous cells to undergo apoptosis | (Akhtar et al., 2012) |
| 2 | Organic NPs | Polymers | Chitosan nanoparticles, widely tested for anticancer purposes, provide controlled drug release without toxicity upon degradation. | (Puluhulawa et al., 2022) |
| | | | Ultrasound-responsive polymeric nanoparticles aid cancer treatment by enhancing drug delivery and lower downside effects | (Thakkar et al., 2012) |
| | | Liposomes | Clinically approved NPs Use for anticancer drug delivery | (Ventola, 2017) |
| | | Micelles | Enhance drug delivery and increase drug concentration on tumor site by using Enhance Permeability and Retention Effect (EPR). | (Kim et al., 2004) |
| 3 | Carbon Metals | Graphene | Extensively studied for drug formulation and delivery purposes, yet its application in medical contexts is constrained by its hydrophobic properties. | (Loh et al., 2018) |
| | | Fullerenes | Used in photodynamic cancer therapy. | (Liu and Tabata, 2011) |
| | | | Examined as carriers for loading cisplatin nanoparticles, aiming to reverse tumor ability to resist cisplatin in cisplatin-resistant human prostate cancer (CP-r) cells | (Liang et al., 2010) |
| | | Carbon nanotubes | Nanostructures designed to trigger the immune response, aimed at suppressing tumor development | (Park et al., 2021) |
| | | | Encourages 5-fluorouracil transportation mechanism effectively suppresses in Vitro MCF-7 breast cancer cells | (Nivethaa et al., 2016) |

**Application of Nanoparticles in the Field of Medicine**

The scientists have persistently explored the application of metal nanoparticles as contrast agents for CT scans, optical imaging, and MRI. A recent breakthrough entails the application of up-conversion NPs infused with lanthanides. These NPs possess the capability to transform near-infrared light into visible light, thereby facilitating the imaging of deep tissue (Du et al., 2022). The researchers have also explored employing metal NPs to detect exosomes, small extracellular vesicles secreted by cancer cells, as an approach for early cancer detection. By integrating gold NPs with surface-enhanced Raman scattering (SERS), they have attained a very sensitive exosome detection (Yang et al., 2022).

The nanotechnology is rapidly advancing in cancer treatment because of its benefits in the drug delivery, diagnosis, vaccine development, and imaging. Some nanomaterials also possess therapeutic properties. The nano therapies with improved blood flow and low toxicity are already being utilized, while others are displaying promising results in ongoing clinical trials. The approved nanoparticle platforms like albumin, liposomes, and polymer-based micelles are used in cancer treatment. Various nanotechnology-based therapeutic approaches such as radiation therapy, chemotherapy, immunotherapy, hyperthermia, and gene or RNA interference (RNAi) therapy, are undergoing clinical studies (Shi et al., 2017).

The NPs are used across different applications because of their distinctive attributes (Machado et al., 2014). The multifunctional nanoparticles can carry lipophilic compounds and selectively target diseased cells, as well as passively reach them, prolong the drug circulation, enhance drug access and aggregation at the site of tumors, surmount resistance to drugs, enhance medication safety and tolerance, and new technologies (Zeineldin and Syoufjy, 2017; Yang et al., 2014). The NPs are used in medical fields due to their different characteristics, including quantum characteristics, which possess a

considerably greater surface-to-mass ratio than larger particles. They can absorb and transport additional compounds like proteins and medications (Baranwal et al., 2023). The non-selectivity of chemotherapy for cancer cells and the rise of side effects have spurred the search for new methods of drug delivery. The fusion of nanotechnology with cell therapy has led to the development of "Nano-engineered" MSCs (mesenchymal stem cells), which can actively home in on tumors and shield drug-loaded nanoparticles from filtration and elimination (Dadwal et al., 2018; Layek et al., 2018).

The nanomaterials used for the treatment of cancer are classified into numerous groups. These nanomaterials target cancerous cells, the microenvironment of tumors, and the defense system. Modification of these NPs done for cancer therapies to decrease issues such as toxicity and absence of specificity, while also improving drug availability and capacity (Cheng et al., 2021). The silver NPs not only possess the capability to stop the growth of tumor cells but also can activate the pathway that impedes cell division making them potentially useful in detecting several cancers such as prostate cancer, lung cancer, cervical cancer, liver cancer etc., (Baranwal et al., 2023). In cancer therapy, the nano-formulated therapeutics have been developed and tested for multiple purposes including boosting the host immune system, silencing oncogenes, inducing tumor-suppressors (suicide-gene therapy), and modulating miRNA processes. Some strategies target challenging cancer-associated proteins like myc, p53, and RAS, termed 'undruggable'. Silencing molecules like siRNAs or shRNAs are used for this purpose (Conde et al., 2015).

The nanoparticle vaccines are under development to enhance T-cell responses against tumors, activate dendritic cells and target specific sites, and deliver antigens to activate the immune reaction against cancer cells (Smith et al., 2013). The NPs are presently undergoing clinical trials as carriers or in combination with immunotherapeutic and immunomodulatory agents, including vaccines, cytokines, and adoptive cellular therapies (Liu et al., 2023; Gonçalves et al., 2020). In a study on melanoma, particles based on liposome containing antigen of tumor and a Toll-like receptor (TLR) agonist demonstrated enhanced immune responses compared to traditional vaccination methods. The clinical trials have also investigated dendritic cell nano-therapy for the skin cancer, cancer of the prostate, liver cancer, and cancer of renal cells, but with relatively decreased efficiency outcomes (Sanaei et al., 2021; Shang et al., 2017).

Around 50–60% of cancer patients undergo radiation therapy during treatment. However, its high toxicity to both cancerous and healthy cells significantly limits its application (Miller et al., 2022).

The nanotechnology enhances radiotherapy by leveraging X-ray interaction with NPs, exploiting their atomic properties. The high atomic numbers (Z) NPs amplify X-ray effects (photoelectric, Compton effects) boosting tumor cell destruction. They can also deliver drugs at tumor sites, further improving radiotherapy efficacy. The high-Z metal NPs have shown potential in animal models for sensitizing radiotherapy, offering promise for clinical glioblastoma treatment (Choi et al., 2020). Recently, interest in cancer gene therapy has risen, with studies investigating therapeutic delivery for DNA and RNA using nanomaterial, including genes, siRNAs, microRNAs, and oligonucleotides, both internally and externally. Utilizing the NPs for gene delivery provides benefits like high loading capacity, precise tumor targeting, and immune system avoidance (Roma-Rodrigues et al., 2020).

**Conclusion**

The NPs hold tremendous promise in revolutionizing cancer treatment and diagnosis. The unique properties of NPs enable precise drug delivery, targeted therapy, and enhanced imaging techniques that can improve patient outcomes and reduce side effects associated with conventional cancer treatment.

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Chapter 38

# Nanoparticle-based Targeted Drug Delivery

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## ABSTRACT

Nanoparticle-based drug delivery systems represent a significant innovation in contemporary medicine, providing enhanced precision in targeting diseased cells and reducing adverse side effects. Leveraging their unique size and surface characteristics, nanoparticles can be engineered to deliver therapeutic agents specifically to targeted cells or tissues, thus overcoming the limitations associated with conventional drug delivery approaches. This paper explores recent advancements in the design of various nanoparticles, including liposomes, polymeric nanoparticles, dendrimers, and inorganic nanoparticles, and their roles in targeted drug delivery. The discussion focuses on targeting mechanisms, such as passive targeting via the enhanced permeability and retention (EPR) effect, and active targeting through ligand-receptor interactions. The clinical potential of these systems is highlighted through case studies demonstrating increased efficacy and decreased toxicity in treating cancer, cardiovascular diseases, and infectious diseases. Despite the promising outcomes, challenges such as nanoparticle stability, production scalability, and potential toxicity are addressed. Future directions suggest the integration of personalized medicine with nanotechnology, paving the way for more effective and safer therapeutic interventions.

**KEYWORDS**
Targeted drug Delivery, Nano-Particles, Nano-medicines, Active Targeting, Passive Targeting

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## INTRODUCTION

### *a) Nanoparticles*

The nano-fluid was first discovered by Choi. These are manipulated colloids comprising the base fluid and nanoparticles. These may be organic fluid, oil and other lubricants, water etc.

As the name indicates the nanoparticle are so small i.e., less than 100nm. The main constituent of nanoparticles are oxides, metals and carbon nanotube (Hussain et al., 2024).

### *b)* Nano particle-Based Targeted Drug Delivery

The nanoparticle drug delivery system gained attention in multiple filed due to its characteristics. Its small size and ability to penetrate different barriers, elevate the limit of drug in target cells/tissues. They are designed as controlled drug release system i.e., they control the property of particles by lowering their side-effects and increasing their efficiency. The nanoparticle is synthesized from biodegradable materials which are safe for the body use. Coating of the drugs in nanoparticles carriers guard them from environmental damage i.e., pH, Temp etc. They are shaped to ignore the immune system clearance and increase the drugs circulation time. Targeted and controlled release of the nanoparticle lower the effect of drugs on non-targeted tissues (Shang et al., 2024).

### *c)* Nanotechnology and their use

The technology or science permitting to regulate, study, manipulate and build the devices and structures of nanometer size. The Nanoparticle's small size, customized coating, well developed solubility and many more functions assist them in creating innovative biomedical application. Through nanotechnology we can develop new tools that helps to diagnose, target and treat many notorious disease like neurodegenerative disease, cancer and tumor, develop single dose vaccine, and oral delivery of therapeutic protein (Singh et al., 2009).

***d)*** **Scope and Advantages**

- Rapid human culture, economy, and medical advancements have led to increased focus on combined therapy. Innovative therapeutic policies show effectiveness in treating various diseases, boosting nanotechnology development.
- Nanomaterials are used for disease diagnosis, targeting, and treatment, like nerve injury and kidney disease targeted therapy. (Shang et al., 2024).
- Different nanoparticles i.e., inorganic, organic, polymer base, hydrogels, etc. (Yu et al., 2020; Yuan et al., 2009), are used as carrier of drugs for their controlled and targeting release.
- The bio-compatible and biodegradable nature of nanoparticle made them suitable for biomedical application (Tang et al., 2012).

**Nanoparticle Carriers**

**a) Active Targeting**

The nanoparticle carrier or therapeutic drug in active targeting attach and bind itself to the specific tissue or cell. In the active targeting, the targeted molecules bind their-self to the drug delivery system to be reached directly to targeted site i.e., tumor tissue, intracellular organ, cancer cell or specific molecule in it. The carrier drugs through this system attach to the surface of tissue specific antigen, carbohydrate and receptor to treat tumor (Hsu et al., 2023)

**b) Passive Targeting**

Passive targeting, together with the drugs carriers inactively reach the specific target site. The mechanism in which the vessels leak the drug and gather in the cell by increasing the retention and permeability (Muhamad et al., 2018) when the tumor size increases up to $2mm^3$ the absorption ability of the cell becomes weakened and start angiogenesis to fight with the problem. Due to this, abnormalities in the basement membrane lead to leaking of vessels of the pore size 100 to1200nm. Through which carrier molecule with drugs leak out (Hsu et al., 2023).

**Types of Nanoparticles Used in Targeted Drug Delivery**

**Classification of NPs**

On the base of size, structure and chemical properties nanoparticles are divided into different categories which are as follow (Khan et al., 2019).

**a) Organic NPs**

The organic nanoparticle are alternative to metallic nanoparticle because of their biocompatible nature, they are use as imaging agent because of their ability to deliver drug to target site, e.g. in cancer cell they deliver drug to tumor cell and disease site (Venkatraman et al., 2014) Moreover, with the passage of time the use of organic nanoparticle in medical field increased worries about the potential accumulation inside the body. The accumulation leads to unintentional concern and adverse reactions (Puri et al., 2023).

**b) In-Organic NPs**

Inorganic nanoparticles coated with biological membranes are promising in personalized medicine, integrating synthetic and bionic nanocarriers for disease treatment. These nanoparticles have been widely used in tumor treatment, toxin removal, and antibacterial applications, achieving excellent results compared to traditional drugs. (Zhang *et al*., 2022).

**c) Carbon-based NPs**

The two major groups, carbon nanotube and fullerenes represent carbon-base nanoparticles. Fullerenes are made up of allotropic carbon. Due to their properties like electrical conductivity, electron affinity, high strength, versatile nature and structure they gain commercial importance (Astefanei et al., 2015).

Carbon nanotube have tubular elongated structure having diameter 1-2 nm (Ibrahim, 2013). It resembles graphite structurally.

**d) Metal NPs**

The metal nanoparticles are synthesized from the precursors of metals. The nanoparticles of noble and alkali metals such as copper, gold, silver, have a large band of absorption in the visible portion of electromagnetic solar spectrum (Dreaden et al., 2012). Gold nanoparticle is use in SEM i.e., to obtain high resolution image.

**e) Ceramics NPs**

They are nonmetallic inorganic solid form through heat and successive cooling. In atmosphere they can be present in different forms i.e., porous, dense and hollow form (Sigmund et al., 2006). Due to their application in various field of research they attract greater attention of researchers (Thomas et al., 2015).

**f) Semiconductor NPs**

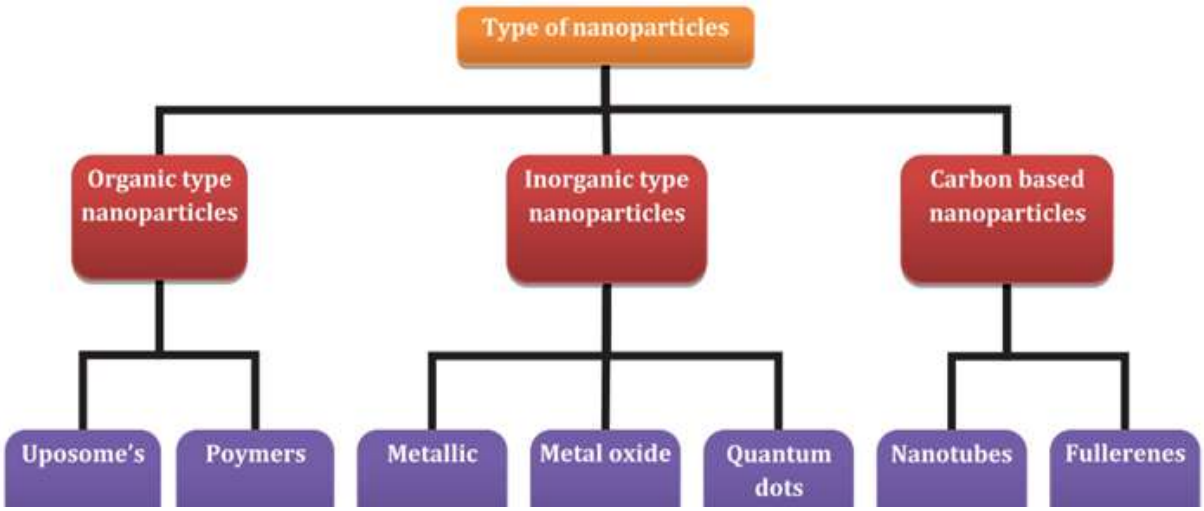
They have characteristics between nonmetal and metal and because of this they have great importance (Ali et al., 2017; Khan et al., 2017). Semiconductor have bad gap and band proper therefore they are rarely effective in water splitting application (Hisatomi et al., 2014).

**g) Polymeric NPs**

Nano-capsular and nano-sphere shape organic nanoparticles (Mansha et al., 2017). In nanocapsule the solid material is covered completely within the particle while in the nanosphere the solid material is absorb on the outer boundary of the sphere (Rao and Geckeler, 2011).

**h) Lipid-based NPs.**

Lipid nanoparticles are 10-100nm in diameter and are spherical in shape. They are generally used in many biomedical fields. Through lipid nanotechnology. The lipid nanoparticle are synthesized and design for multiple applications i.e., drug delivery and carriers (Puri et al., 2009), and in cancer therapy the release of RNA (Khan et al., 2019).




**Characteristics Importance of Nanoparticles in Drug Delivery**

Nanotechnology have a great use in the delivery of the drugs, improve their availability and solubility. Through this process the therapeutic drug is protected from degradation. Various research studies reveal that nanoparticles have great benefits over microparticles. Nanoparticles are ideal for intravenous delivery due to their small size and biodegradable nature, while microparticles are slightly larger. The smallest capillaries are 5-6um, so particles smaller than 5um are better suited for penetration and avoid embolism. (Singh et al., 2009).

**a) Size of the Particles**

The most important characteristics of nanoparticles are the distribution and the size of the particle. Due to the size of particles, biological fate, the ability of targeting in the system and its toxicity is determined. It also has impact on loading of drugs, their stability and their release. The nanoparticle passes from the endothelium tight junction opening of the BBB through hyperosmotic mannitol, which make the delivery of therapeutic easier for brain tumor and other complex disease (Hsu et al., 2023).

**b) Surface Properties of Nanoparticles**

The intravenously injected nanoparticle is recognized by immune system of the host and removed by phagocytes from the circulation. The hydrophobicity of nanoparticals investigate the component level of the blood and also help in determining the *in-vivo* fate of the nanoparticles (Hsu et al., 2023).

**c) Drug Loading**

An effective delivery system has a well develop drug loading ability which decreases the amount of matrix materials for administration. Two possible ways for drug administration are, the adsorption and incorporation method. The absorption occurs after the formation of nanoparticle while the incorporation occur at the time of formation of nanoparticle (Singh et al., 2009).

**d) Drug Release**

The rate of drug release is proportional to a) absorption of the drugs which are bound to the surface; b) solubility of the drugs; c) degradation of the nanoparticle; d) the diffusion of the drugs by the matrix of nanoparticles; e) the combination of two process i.e., diffusion and erosion. However, the release of drugs are governed by diffusion, solubility, the particle biodegradation ability (Hsu et al., 2023).

**e) Surface Load**

The most important feature that can affect the drug delivery is the surface charge which penetrates the endosomes (Vergote et al., 2013). After entering into endosomes gold nanoparticle load to the surface with cytosole and glycol, after that nanoparticle depart itself from endometrium and go into cytoplasm (Majoros et al., 2006).

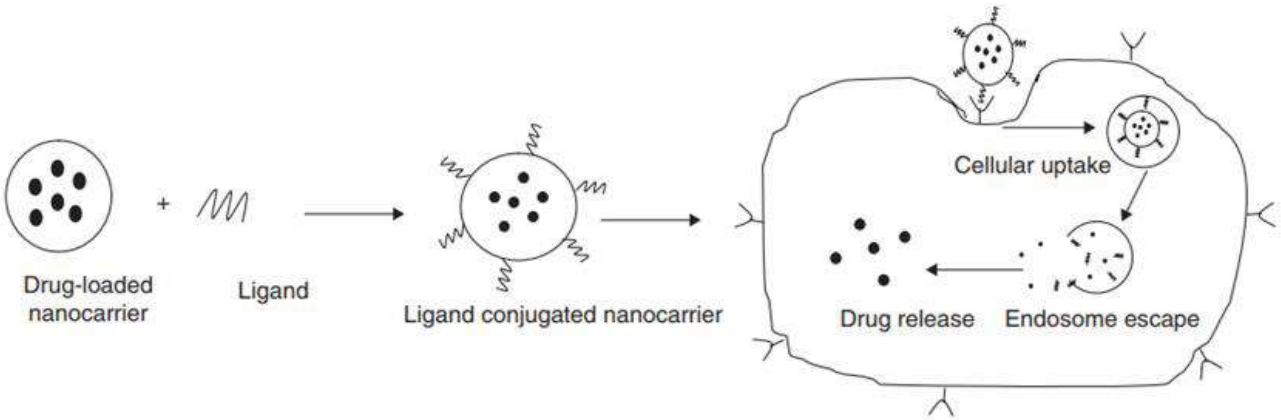
## 5. Principles of Targeted Drug Delivery

In the early years most of the people were treated with conventional method of chemotherapy with serious side effects. Several years ago for the enhancing of the chemotherapy drugs efficacy nanoparticles were developed (Muhamad et al., 2018). Nanoparticles have the ability to mimic or change the biological processes, due to this property they are widely used in tissue engineering, infection etc. (Singh et al., 2009). Both the active and passive targeting mechanisms are functional in cancer cells.

## 6. Strategies for Targeted Drug Delivery Using Nanoparticles

### a) Ligand-based Targeting

Numerous nanoparticle-based drug delivery and drug targeting systems have been developed or are currently under development. Their use aims to minimize drug degradation, prevent side effects, and increase drug bioavailability. These systems use ligands, which recognize and bind to target antigens by specific cells or tissue components. Ideal ligands for targeted delivery have high avidity, specificity, internalization of polymeric particles, compatibility with chemical modification, and sufficient quantity. Low ligand density is necessary for effective interaction with the receptor. Ligands also impact drug release kinetics. Targeted drug delivery is non-toxic and safe to normal tissue, as the expression of the receptor to its specific ligand is limited. This precise and selective binding allows for tumor specificity and limited toxicity, potentially overcoming cytotoxic chemotherapy challenges. (Das et al., 2009).




### b) Antibody-based Targeting

Immunotherapy has been enhanced by the discovery of antibodies' structure and hybridoma technology, enabling targeting tumors both in vitro and in vivo. Modern antibody technology involves designing and preparing fragments against various tumor antigens. Cancer cells overexpress known protein biomarkers or tumor-associated antigens (TAA), and these TTA's provides insights for antibody-mediated targeting. Antibody-coupled nanocarriers are attractive drug targeting systems due to their specificity and stability. The FDA has approved several targeted cancer treatments using antibodies for specific cancer types. Receptors, particularly growth factor receptors, are increasingly used for antibody-mediated targeting. The use of antibody fragments can reduce immunogenicity and improve nanocarrier pharmacokinetic profiles. (Das et al., 2009).

### c) Magnetic Targeting

Due to magnetic field and the moment of network units these nanoparticle are activated and when the magnetic field is absent they act as an inactive particles. The magnetism and nanotechnology combination seek attention for many years due to their wide use in different field i.e. catalysts, magnetic fluids, magnetic resonance, water treatment, biomedicine, magnetic resonance imaging, biotechnology etc. (Kianfar, 2021).

### d) pH-responsive Targeting

Due to the intracellular and extracellular stimuli of the pH responsive nanoparticles against tumor increase the glycolysis which in turn increases the production of lactic acid through which tumor gain energy (Amoozgar et al., 2012). The external pH of tumor is between 6.5 to 7 which is slightly acidic while the internal pH is slightly higher than the other body fluids and tissues. Meanwhile some internal parts of the cells are more acidic such as lysosomes have pH 4.5 to 5 and endosomes have pH 5 to 6.5 (Farr et al., 2011). These two insights explain the mechanism of pH responsive nanoparticle which is; a) chemical bonds cleavage b) protonation of nanocarrier (von Erlach et al., 2011). At high pH the chemical bond break and the drug is release to the targeted environment. The linkers which are sensitive to drugs allow the conjugation of drugs through conformational changes and deliver the drug smartly to target site by avoiding the systematic toxicity. The pH responsive nanoparticle changes the charge of their surface at varied pH by the process of charge reversal strategy. Different pH linkers are amides, vinylesters, hydrazones, orthoesters etc. (Kianfar, 2021).

**Challenges and Limitations of Nanoparticle-Based Targeted Drug Delivery**

a) Biological barriers

b) The degradation of endosomes and discharge of lysosomes. The lysosomes sensitive therapeutic agents such as DNA are stopping to get into lysosomes by alleviating the escape procedure of endosomes (Kianfar 2021).

c) The fission of biological barriers (Yang et al., 2019).

d) The failure of physiological constituent like blood flow, body weight, cascular source, distance from target, route of injection and tumor volume (Kianfar 2021).

c) Toxicity concerns

Nanostructured materials are comparable with typical cellular components and proteins in size therefore can cross the human body's natural mechanical barriers via various routes including inhalation, intravenous, dermal, subcutaneous, oral, and intraperitoneal routes, causing adverse health effects. Absorption occurs when nanomaterials interact with proteins or cells, affecting organs like the liver, brain, blood, kidney, heart, colon, and bone. Studies have reported potential harmful effects in in-vitro and in-vivo experimental models, identifying key physicochemical properties influencing nanoparticle toxicity, including particle size, composition, charge, surface area, agglomeration, and dispensability. (Sharma et al., 2012).

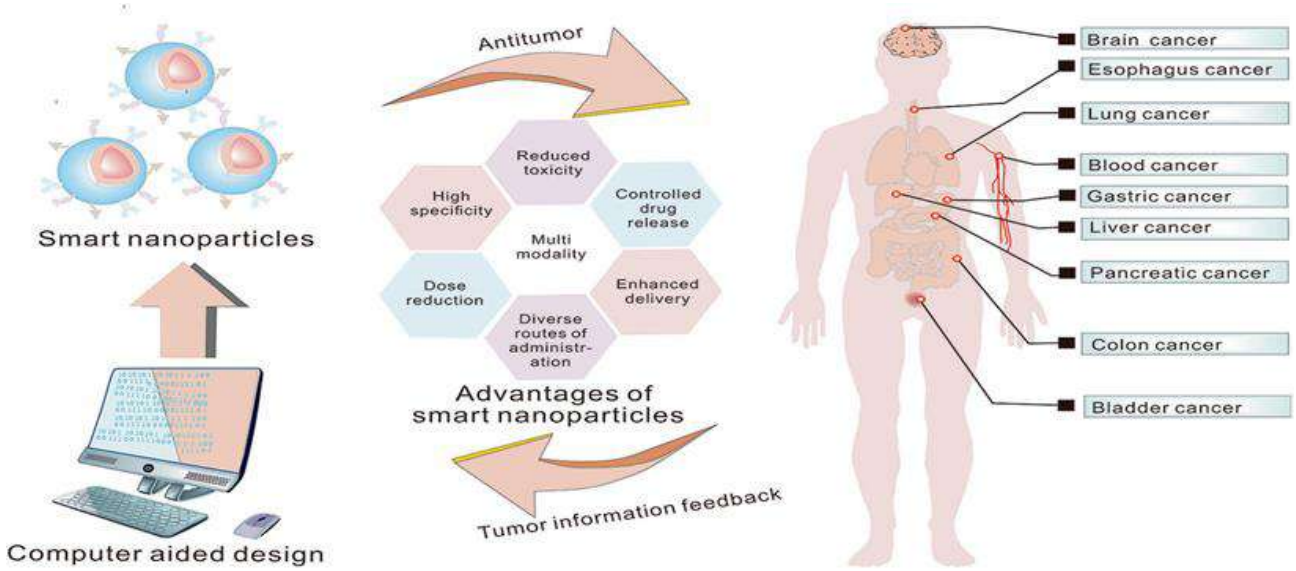
**Applications of Nanoparticle-Based Targeted Drug Delivery**

Nanoparticles apply to many diseases as a targeted drug.

**a) Nanoparticles use in Cancer Therapy**

Around the world cancer is considered one of the major causes of mortality (Sung et al., 2021). In the present time due to high efficiency chemotherapy is one of the common treatments of cancer (Hosomi et al., 2020; Messinger et al., 2011). Due to the lack of selectivity targeting of different challenges they face some of the practical limitations. The nanoparticles targeted confined site for attack with large amount of drug delivery (Gupta et al., 1990). After activation these nanoparticles are accumulate at specific site and unload their materials and develop a smart mode of treatment (Sun et al., 2023).

- Nanoparticles having different size and shape are selected with the accordance of delivery of drug and treatment. For example, liposomes are used to increase the cellular intake of different drugs, for the delivery of water insoluble drugs the carrier micelles are suitable (Joy et al, 2022; Cabral et al., 2018).
- One of the most important example of the nanoparticles are tumor targeting characteristics by activate their surface by tumor specific ligands.
- The conventional nanoparticles deliver chemotherapeutic drugs, while the new generation nanoparticle deliver various type of drugs.




**b. Nanoparticles for Immunotherapy**

The checkpoint inhibitors of immune system help to treat cancer still face a lot of challenges like patient's variability, efficiency of the drug etc. Few immunotherapeutic like proteins have a lower potential as compared to other nanoparticle (Wu et al., 2020).

**c. Nanoparticles for Genome Editing**

Recently researchers brought many advances to engineer the genome for vast use in biomedical field, like advancement in CRISPR, TALEN, ZFN technologies. According to an estimate over 3000 genes of humans are connected to

mendelian diseases but less the 5 % are treatable, which are now treated with advanced genome editing. Furthermore, the safe delivery to the specified tissue and organ without causing toxicity is still needed (Huang et al., 2021). The PNPs and LNPs deliver different types of nucleic acids *in-vivo* at multiple stages in clinical development such as lipid nanoparticles siRNA drug known as Onpattro which is used for the treatment of amyloidosis approved by FDA (Alkhawaldeh et al., 2023; Yin et al., 2017).

**B. Neurological Disorders**

The understanding of mechanisms accounting for neurological diseases has extended opportunities for NP technology to treat these diseases. Potential future direction for NP-based systems may involve refining the particle to reduce toxicity and enable increased target specificity (Kanwar et al., 2012).

**Future Perspectives and Emerging Trends of Nanoparticles**

- Nanoparticles improve delivery efficacy in therapeutics through charge, shape, size, responsiveness, and surface charge.
- Nanoparticles with exact medicine therapy increase patient hierarchy methods and total therapeutic efficiency.
- Study of nanoparticle size and shapes in biological state identifies new trends for intelligent nanoparticle design.
- Study of nanoparticle design and their relation with the human body is necessary to modify specificity.
- Continuous use of nanoparticles in laboratories allows data analysis and addition to nanomedicine library.
- Nanoparticles enable cellular targeting, tailored therapy, and several off treated effect become possible to treat.

All of these can be possible with nanoparticle engineering, increasing the accumulation, reducing toxicity, increase responsiveness on demanded drug (Sun et al., 2023).

**a) Personalized Medicine**

Nanoparticle have lots of uses in biomedicine (Wagner et al., 2006). Nanoparticle is also useful in investigation, such as optical mediator, in drugs delivery, photoacoustic, increasing cancer disclosure to therapeutics, prevent the deterioration of drugs during transport, increase tumor integration (Hussain et al., 2024).

**b) Multi-functional Nanoparticle**

In active targeting the targeted particle move to the delivery system to target specific site such as tumor, intracellular organs etc. (Naumann and Coleman, 2011). Through this method the drugs transfer to carbohydrate surface, surface receptors, specific antigens of tissues, and into the primary tumor which was not yet metastasized (Gu et al., 2007)

**i. Targeting against Carbohydrates**

The carbohydrate lectin system is the example of active targeting carbohydrate, cell surface carbohydrate influence the healthy cells which are connected to tumor cells. These associations are connected by lection proteins, which have the ability of binding with carbohydrates (Armstrong et al., 2013). Few lectin protein trigger both acquired and innate immunity by this means (National Cancer Institute 2014).

**ii. Targeting against Antigen and Receptor**

Drugs enter into cancer cell by antigens and receptors through endocytosis. Drugs attach their self to carrier polymers and get into the cell in this way, their separation occur in the extracellular space and specifically in the lysosomes through the lysosomal enzymes (Naumann and Coleman, 2011)

**iii. Monoclonal Antibody**

This is considered as the first and most important group of targeting particles that have the ability of binding to tumor specific antigen. The anticancer antibody development is dependent on the identification of specific antigens. The suitable antigen is found in all cancer cells but not in healthy cells (Carroll et al., 2011; Kelemen et al., 2013).

**iv. Aptamer**

Aptamers are DNA or RNA oligonucleotides, new group of targeting molecules, have the ability to bind with the surface of ligands (Zhao et al., 2009). They have the power of detection of antinomies of multiple particles, like theophylline and caffeine through a method called 'evolution of ligand system' (Kelemen et al., 2013).

**v. Oligopeptides**

Recently, the peptides are used as targeting particles. Peptides have advantages over antibodies because of their lower immunization, smaller size, easier production and high stability (Ramamoorthy et al., 2007; Yuan et al., 2009).

**vi. Folate**

Folate such as folic acid have wide uses as targeted particles, the receptor of folic acid is expressed in different types of cancers like lungs, breast, uterine, colon and brain cancer. This particle attaches to different types of drugs like polymer nanoparticle, liposomes, and dendrites (Gonen and Assaraf, 2012).

**c. Combination Therapies**

Therapy of single drug based procedure of single chemotherapy is not perfect for clinical use. Different complications in cancer make it compulsory to develop combine scheme of two or more drugs to achieve better results (Sun et al., 2023). Moreover, it is a hard task to develop combine treatment having low toxicity. Such as gemcitabine monotherapy is the treatment of choice for breast cancer patient. Samir et al 2018 developed a combination therapy using gemcitabine and imiquimod, based on hyaluronic acid, to stimulate immune activity against cancer cells. (Singh et al., 2022). In clinical application the synergistic drug combination therapy for cancer have multiple uses which are delivered to a system through smart nanoparticles (Zhang et al., 2016).

Traditional combinations of drugs which are injected intravenously fail to keep constant ratio of drugs. To fight with cancer cell multidrug resistant, smart nanoparticle deliver combination synergistic drug to cancer cells (Wang and Huang 2020). The EPR and receptor mediated extravasation, nanomaterials are accumulate, bind and enter to tumor cell by endocytosis and unpack the drug materials there (Nastiuk and Krolewski, 2016). The drug enters into the nucleus by exerting synergistic effects, break the DNA double strand in large amount there (Jaaks et al., 2022).

**d. Nanotechnology Advancements**

The relation between patients and the drugs attract the core focus AI application in chemotherapy. The principal application contains the management of chemotherapeutic drug use, their tolerance of prediction and optimization of chemotherapeutic programs (Pham et al., 2020). AI has a role in the early recognition of cancer. For example, they translate and review the mammograms quicker with high accuracy, by reducing the need of biopsies for the detection of cancer (Greish, 2010). The physicians uses AI for immunotherapy for the adjustment of treatment plan for the cancer patients (Yang et al., 2018). To identify the margin of cancer exactly during surgery with the help of AI.Effective reality recreation and AI can train oncologists and surgeons of the near future (Kobayashi 2011: Yu et al., 2016). Furthermore, through AI human cancer research and their treatment is on peak, by concreting the way for the production of anticancer treatment and also elevate the discovery of new medicine and materials (Hou et al., 2020).

**e. Future Scope**

For future personalized medicine, nanoparticles will play a certain, from identification to monitoring. Nanoparticles enhance biomarker sensitivity, aiding early disease identification and treatment. After diagnosing, nanoparticle effectively use in disease condition with decrease harms and side effects. Future challenges include drug loading and unloading, and advancements in diagnosing and treating nanoparticles. Nanomedicines have numerous uses and risks, with potential applications in medical expertise and probabilistic diagnostic algorithms. (Verma et al., 2023).

**Limitations**

The nanoparticle quality control and problems of reproductive system creating scalability and elevate their production rates and the management of unwanted nano-engineer byproduct are important problem both scientifically and technological barriers. The rates remain high, while without knowing their importance regarding health and environmental implications

**Conclusion**

Nanoparticle-based targeted drug delivery marks a major breakthrough in medical treatments, offering the potential to enhance effectiveness and reduce side effects. By employing nanoparticles, medications can be precisely delivered to specific cells or tissues, improving therapeutic results and minimizing harm to healthy cells. This approach allows for controlled drug release and accurate targeting, leading to better patient outcomes, particularly in cancer treatment. Nonetheless, challenges persist in understanding long-term safety, achieving large-scale manufacturing, and ensuring reliable and targeted delivery. Ongoing research and development are essential to fully harness the potential of nanoparticle-based drug delivery systems in clinical practice.

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Chapter 39

# Nano-delivery and Anti-breast Cancer Potential of Limonoids

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## ABSTRACT

Breast cancer, a global health issue, demands innovative therapeutic approaches due to metastasis, drug resistance and high toxicity associated with conventional treatments. Nimbolide; a limonoide, a compound from *Azadirachta indica*, having anti-breast cancer potential covers broad range of pharmacological activities, like anti-oxidant, anti-inflammatory, and anti-cancer effects. Moreover, in vitro and in vivo studies also reveal the nimbolide's molecular targets and action mechanisms across various cancer types. Notably, nimbolide influences pivotal signalling pathways such as EGF, VEGF, Wnt/β-catenin, MAPK/JNK, PI3K/AKT, TNF-α/NF-κB, and DR5. Broad availability and minimal side effects makes nimbolide as an ideal therapeutic agent against breast cancer. Additionally, integration of nano-delivery systems enhances the efficacy of limonoids. This particular compound combats multidrug resistance, and influences oncogenic signaling pathways, which results in prevention of cancer. Through the integration of knowledge from cancer chemoprevention, molecular targets, and nano-delivery methods, this investigation will offer a comprehensive viewpoint on the use of limonoids for improved treatment of breast cancer.

**KEYWORDS**
Limonoid, Breast cancer therapy, Nano-delivery systems, Anticancer effects, Molecular targets

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## INTRODUCTION

### Overview of Nano-delivery Systems

Nano-delivery systems encompass a diverse array of technologies designed to improve the delivery of medicinal substances to target locations within the body. These systems utilize nanoparticles, typically ranging in size from 1 to 1000 nanometers, to encapsulate drugs and prevent them from degradation in the bloodstream. The primary purpose of nanoparticles in drug delivery systems is to enhance the therapeutic drugs effectiveness while minimizing their harmful effects on healthy tissues (Patra et al., 2018a).

### Definition and Purpose of Nano-delivery Systems

Nano-delivery systems are defined as platforms that employ nanoparticles to transport drugs, genes, or imaging agents to specific cells or tissues within the body. The key purpose of these systems is to overcome biological barriers, such as rapid removal, low bioavailability, and inadequate solubility, which often hinder the effectiveness of conventional drug delivery methods (Yusuf et al., 2023).

### Importance of Nano-delivery Systems in Drug Delivery

The significance of nano-delivery systems in drug delivery lies in their potential to improve the pharmacokinetic and pharmacodynamics properties of therapeutic agents. By encapsulating drugs within nanoparticles, it is feasible to accomplish controlled release kinetics, sustained drug concentrations at the target site, and enhanced cellular uptake (Patra et al., 2018b) (Yetisgin et al., 2020). By keeping the encapsulated cargo from degrading, these carriers help improve drug stability. Drug activity can be preserved by incorporating relatively substantial amounts of the medicines without the requirement for a chemical reaction. Making dosage forms that are dry and solid is a common way to increase the chemical stability of medications. Compared with nano liquid products, these are more stable (Yusuf et al., 2023; Wu et al., 2011).

Several drug delivery methods, including parenteral, intraocular, nasal, and oral, can be used to deliver nanoparticles. Nanoparticles exhibit a comparatively elevated intracellular uptake and surface-to-volume ratio in contrast to their larger counterparts, microparticles (Yusuf et al., 2023; Panyam and Labhasetwar, 2003).

According to reports, on comparison of nanoparticles with micrparticles (>1 µm), nanoparticles are much more effective as carriers of drug delivery because of their better cellular absorption. Better control over drug targeting and release in malignant tissue is made possible by these features. This can lead to improved therapeutic outcomes, reduced drug dosages, and fewer adverse effects compared to conventional drug formulations (Yusuf et al., 2023; Hsu et al., 2023).

**Limonoids: Introduction and Background**

Limonoids are a class of naturally occurring compounds primarily found in citrus fruits and some other plants of the Rutaceae family. These compounds have drawn interest recently because of their wide range of pharmacological characteristics, which include antioxidant, anti-inflammatory, and anticancer effects (Indriyani et al., 2023)

The term "limonoids" comes from the bitterness of lemons and other citrus fruits. Tetranortriterpenoids, another name for limonoids, are created structurally when four terminal carbons of the side chain in the apoeuphane or apotirucallane skeleton are lost (Fan et al., 2022). This is followed by cyclization to generate the 17β-furan ring. Within the kingdom of plants, limonoids are primarily found in the Rutaceae and Meliaceae families, with a lesser frequency in the Cneoraceae (Sun et al., 2018;Tan and Luo, 2011).

**Definition and Types**

The Rutaceae and Meliaceae plant families include oxygenated triterpenoids known as limonoids (Durán-Peña et al., 2023). Limonoids are characterized by their triterpenoid structure, which consists of a polycyclic framework with oxygen-containing functional groups. They can be classified into various subclasses based on their chemical structure, including limonin, nomilin, and obacunone (Shi et al., 2020).

**Natural Sources**

Limonoids are abundant in citrus fruits i.e. oranges, lemons, and grapefruits, as well as in seeds, leaves, and peels of certain plants. These substances give citrus fruits their distinct bitter flavor and are produced by plants as a defensive mechanism against pests and diseases. A class of highly oxygenated terpenoid secondary metabolites known as citrus limonoids (CLs) are mostly present in the peel tissues, seeds, and fruits of citrus fruits, including mandarins, pumellos, oranges, lemons, limes, and grapefruits. The aglycones and glycosides of CLs, which are represented by limonin, have been demonstrated to exhibit a wide range of pharmacological properties, including insecticidal, antidiabetic, anticancer, and antibacterial properties (Gualdani et al., 2016).

**Bioactive Properties**

Limonoids show a wide range of bioactive properties, including anticancer, antimicrobial, antiviral, and insecticidal activities (Fig. 1). Several studies have demonstrated the potential of limonoids as chemopreventive and therapeutic agents against various types of cancer, including breast cancer (Shi et al., 2020; Roy and Saraf, 2006a).

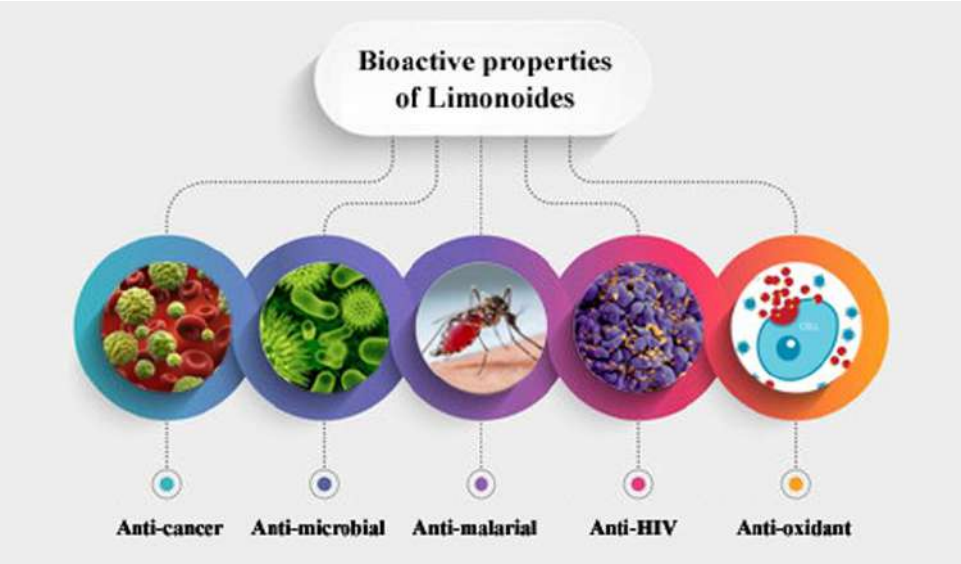


**Fig. 1:** Bioactive Properties of limonoids including anti-cancer, anti-microbial, anti-malarial, anti-HIV and anti-oxidant properties

**Anti-cancer Activity**

Numerous experimental studies have demonstrated the chemopreventive properties of limonoids, which are found in citrus fruits and their juice. In addition to their ability to target and stop neuroblastoma cells, limonoids have also been demonstrated to reduce the proliferation of breast cancer cells in human that are positive and negative for estrogen receptor in culture (Zhou et al., 2022).

With exceptional cytotoxic activity against lung, colon, oral, and skin cancer in in vivo systems as well as human breast cancer cells, the citrus limonoids obacunone, limonin, nomilin, and their glucosides as well as certain aglycones prevent the

development of chemically caused cancer and many cancer cell lines in humans. In addition, Guthrie et al. were granted a patent recently for their composition and techniques of using limonoids in conjunction with flavonoids and tocotrienols to treat neoplastic disorders (Koolaji et al., 2020; Roy and Saraf, 2006b).

**Anti-malarial Activity**

Limonoids, like gedunin, nimbin, nimbolide (Fig. 2), and many more were isolated from *Azadirachta indica*, *Cedrela odorata*, *Guarea mltiflora*, and *Khaya grandifoliola* have been found to have in-vitro antimalarial action against P. falciparum (Braga et al., 2020; Kayser et al., 2003). After a veterinarian and self-medication behavioral investigation of Uganda's wild chimpanzees, novel antimalarial limonoids were identified from *Trichilia rubescens* leaves (Krief et al., 2004).

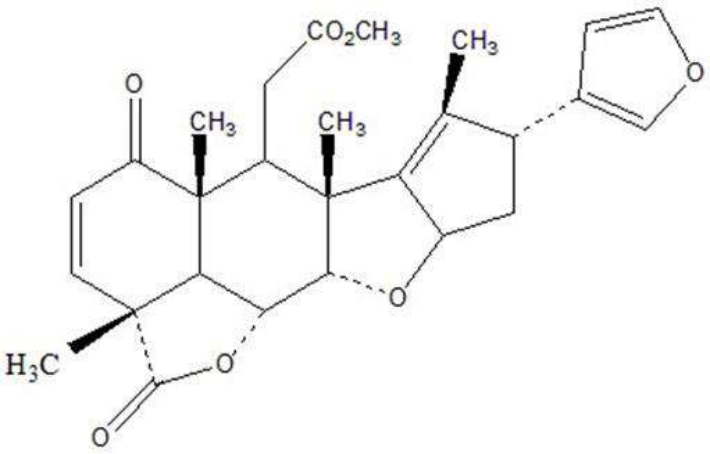


**Fig. 2:** Chemical structure of nimbolide; a limonoide

**Anti-microbial Activity**

According to a recent study by Germano et al., *Trichilia emetica* contains limonoids, which may be the cause of its ability to inhibit a variety of clinically isolated bacterial strains ("Phytochemical Composition and Biological Investigation of Trichilia Emetica Vahl. Seed Extracts," 2020; Germanò et al., 2005). Certain Khaya species yielded limonoids, which exhibited strong antifungal and antibacterial properties (Tan et al., 2021; Abdelgaleil et al., 2004). Significant antifungal action was discovered for limonoids from numerous plants in the rutaceae and meliaceae families in a different investigation (Braga et al., 2020; Abdelgaleil et al., 2005).

**Anti-HIV Activity**

The anti-HIV-1 replication of nomilin and limonin has been demonstrated in several cellular systems. A new limonoid that was extracted from Clausena excavata has been found to exhibit HIV-1 inhibitory properties (Kaur et al., 2020; Sunthitikawinsakul et al., 2003).

**Other Miscellaneous Activities**

It has been demonstrated that several limonoids have antioxidant and radical scavenging properties, which is thought to contribute to their anti-proliferative properties (Saini et al., 2022; Yu et al., 2005). Nomilin has been shown to have immunomodulatory properties by Raphael and Kuttan (Zhou et al., 2022; Raphael and Kuttan, 2003). Limonin is thought to be very beneficial for protecting the lungs and removing congested mucous (Agarwal et al., 2022; Roy and Saraf, 2006c).

**Nano-delivery Systems for Drug Delivery**

**Introduction to Nanoparticles**

Nanoparticles are solid, colloidal microscopic particles that range in size from 1 to 100 nanometers. Since most biological molecules exist and function at the nanoscale, where nanoparticles can be utilized to encapsulate medications, shield them from degradation, and deliver them to precise target sites in the body, nanotechnology aims to solve disease-related issues at this scale (Khan et al., 2019; Wang and Thanou, 2010). Nanoparticles' sub-micron size allows for deep tissue penetration, epithelial fenestrations, and effective uptake by target cells, leading to increased bioavailability of therapeutic components. The unique nanoparticle drug delivery technology makes use of the properties of the tumor and its surrounding tumor environment (Gavas et al., 2021a; Sharma et al., 2023). Nanoparticles overcome multidrug resistance in addition to the drawbacks of traditional cancer treatment. Additionally, nanoparticles are the subject of increased research as new mechanisms of multidrug resistance are discovered and examined (Gavas et al., 2021b).

**Types and Characteristics**

Various types of nanoparticles such as, carbon nanotubes, dendrimers, liposomes, silica, magnetic naoparticles, polymeric micells, SLNs (Solid lipid nano-particles) have been investigated as drug delivery carriers in cancer therapy (Afzal et al., 2022).

**Dendrimer-based Nanoparticles**

Dendrimers are spherical polymeric particles (< 5 nm diameter) having a large surface area for the encapsulation. Dendrimer formations are characterized by their highly branched structure. An ammonia core and acrylic acid react to form a tri-acid, which is subsequently reacted with ethylenediamine to produce a tri-amine known as generation 0 (G0) product. This process is the first step in the production of dendrimers. Generation 0 (G0) product on reaction with ethylenediamine further give rise to hexa-acid (G1), and so on .Reaction continue until desire product is achieved (Gavas et al., 2021c).

These are utilized to target nucleic acids because of their unique characteristics, which include defined molecular weight, customized branching, bioavailability, and charge. The widely used dendimers include polyamidoamine (PAMAM), PEG (poly(ethyleneglycol)), PPI (polypropylenimine), and TEA (triethanolamine) (Gavas et al., 2021d).

**Polymeric Nanoparticles**

Polymeric nanoparticles are the most extensively investigated nanotechnology platform for targeted delivery of anticancer medication. It generally consists of acrylates, polylactic acid, or polyglycolic acid (Yousefi Rizi et al., 2022). Abraxane, albumin-bound nanoparticle, has been recommended for the control of metastasized or proliferated breast cancer. This field is expanding with over 10 polymeric nanoparticles containing anticancer medicines (Sharifi-Rad et al., 2021; Zhao et al., 2015).

**Magnetic Nanoparticles**

Magnetic nanoparticle takes advantage of charge interactions and energetic processes. These systems combine therapeutic or reporter genes with magnetic nanoparticles and subsequently directed to the target region (Rarokar et al., 2024) (Dobson, 2006). Luteinizing hormone-releasing hormone-conjugated superparamagnetic iron oxide nanoparticles are excellent for diagnosing and detecting breast cancer cells (Nian et al., 2019; Meng et al., 2009).

**Silica-based Nanoparticles**

Silica is a major element in many natural things, including sand and glass, and has been used for decades. Silica has recently found applications in biomedicine, owing to its versatility in serving specialized purposes. One common method for employing silica in gene delivery is to alter the surface of silica nanoparticles using aminosilanes. The combination of N-(6-aminohexyl)-3-aminopropyltrimethoxysilane and readily commercially available silica particles produces effective results with little toxicity (Hoang et al., 2022; Kneuer et al., 2000).

**Liposomes based Nanoparticles**

**Structure and Composition**

Liposomes, natural phospholipids, are spherical vesicles recognized for their biological inertness, low immunogenicity, and low intrinsic toxicity. Because of their encapsulating properties, these lipid bilayer structures serve as excellent carriers for a wide range of hydrophobic and hydrophilic medicines (Fan et al., 2019c; Malam et al., 2009).

Liposomes typically have a "hydrophilic core" surrounded by a "hydrophobic phospholipid bilayer". This particular arrangement allows liposomes to encapsulate both hydrophilic and hydrophobic medicines, efficiently preventing environmental deterioration of the medication enclosed while it circulates in the body (Allen and Cullis, 2013). Doxil®, Myocet®, and DaunoXome® are approved liposome formulations with daunorubicin for treating metastatic breast cancer (Bulbake et al., 2017; Hofheinz et al., 2005).

**Role of Liposomes in Nano-delivery**

It has been shown that liposomes are effective at delivering medication. These devices do "contact-facilitated drug delivery," which comprises adhering to or interacting with the intended cell membrane. This promotes better lipid–lipid exchange with the lipid monolayer of the nanoparticles, speeding up the convective flux of lipophilic drugs (such as paclitaxel) to diffuse through the outer lipid membrane of the nanoparticles and enter the targeted cells. These nanosystems can function as drug depots with long-term persistence at the target location and extended release kinetics (Singh and Lillard, 2009a).

**Micelles**

**Formation and Structure**

As a proof of concept for the delivery of two or more drugs, “prodrug-based” nanomedicines are created. Therefore, it makes sense to create nanocarriers that might incorporate the benefits of prodrugs with nanoscale delivery methods like micelles (Wei et al., 2021). The hydrophilic drug is combined with an amphiphilic lipid or synthetic biodegradable polymer molecule for this purpose. These molecules have the ability to self-assemble to create micelles, which enclose the hydrophobic drug or medicines in the micellar core (Hanafy et al., 2018) (Negut and Bita, 2023). Therefore, prodrug micelle-based nanomedicine blends the advantages of both prodrug and micelle technologies for drug formulation, such as effective drug loading and maximum cellular absorption, as well as structural stability and technical simplicity (Wang et al., 2023; Zhang et al., 2021; Mi et al., 2013).

**Advantages in Drug Delivery**

Recently, there has been considerable attention given to micelles—nanosized delivery systems—due to their remarkable achievements in terms of in vivo stability, drug entrapment protection, release kinetics, ease of cellular penetration, and ultimately, greater therapeutic efficacy. One method of accumulating nanosized micellar drug formulations is passive targeting (Wang et al., 2023). Numerous research teams in academia and industry concentrate on creating novel approaches to enhance the therapeutic effectiveness of micellar systems (e.g., prodrug conjugate-formed micelles, active targeting to the tumor site, multidrug delivery systems designed to overcome multidrug resistance, etc.). In South Korea, there is just a single micellar medication formulation that has been put into clinical use. Nonetheless, a number of micellar formulations loaded with untargeted anticancer drugs are undergoing clinical studies and may find application in clinical settings. In the near future, a large number of new items are anticipated to be released (Keskin and Tezcaner, 2018).

**Applications in Medicine**

- It has the potential to minimize the systemic toxicity through production of the functionalized particles for targeted treatments (Alexis et al., 2008a).
- It also provides an alternate way to combat multidrug resistance since they can bypass the drug efflux mechanism associated with this trait (Awasthi et al., 2018).
- By altering the polymer's characteristics and surface chemistry, it can be tailored to provide both disease-specific localization and controlled drug release (Singh and Lillard, 2009).

**Anti-Breast Cancer Potential of Limonoids**

Limonoids, naturally occurring compounds found in citrus fruits and other plants, have shown significant potential in the prevention and treatment of breast cancer. These compounds exhibit anti-cancer properties by inducing apoptosis, inhibiting cell proliferation, and blocking metastasis in breast cancer cells (Aly et al., 2024; Ejaz et al., 2006).

**Mechanisms of Action**

Utilizing the estrogen-dependent MCF-7 and estrogen-independent human breast cancer cell lines MDA-MB-231, investigators investigated the molecular pathways underlying the apoptotic response triggered by the nimbolide; a limonoid (Table 1). MTT assay was used to evaluate nimbolide's growth-inhibiting impact. JC-1 mitochondrial membrane potential labeling, cytochrome c release, caspase activation, cleavage of PARP, and AO/EtBr dual staining were used to test if nimbolide therapy induced apoptosis (Razak et al., 2019).

Real-time PCR analysis and Western blot were utilized to look at how apoptotic proteins were altered. (Apoptotic proteins are for intrinsic pathway: TRAIL, FasL, FADDR, and Caspase-8; Apoptotic proteins for extrinsic pathway: Bax, bad, Bcl-2, Bcl-xL, Mcl-1, XIAP-1, and caspase-3, 9). MCF-7 and MDA-MB-231 cell growth was dose- and time-dependently inhibited by nimbolide treatment. The presence of programmed cell death known as apoptosis in these cells was demonstrated by the modulation of JC-1 staining (Elumalai et al., 2012a).

**Table 1:** Table showing mechanism of action of nimbolide; a limonoide through various cell lines in breast cancer (Nagini et al., 2021a)

| Study type | Cancer Type | Cell Line/Animal model | Mechanisms | References |
|---|---|---|---|---|
| In vitro | Breast Cancer | MCF-7 and MDA-MB-231 | Induces autophagy mediated apoptosis (Increased Beclin 1 and LC3B; decreased p62, mTOR and BCL-2). Modulates HDAC-2 and H3K27Ac expression. | (Pooladanda et al., 2018) |
| | | MCF-7 and MDA-MB-231 | Inhibits P13K/Akt and MAPK signaling mediated by IGF-I. | (Elumalai et al., 2014a) |
| | | MCF-7 and MDA-MB-231 | Inhibits invasion and migration by downregulating expression of uPAR chemokine genes, VEGF, MMPs and NF-kB. | (Elumalai et al., 2014b) |
| | | MCF-7 and MDA-MB-231 | Through intrinsic and extrinsic pathways induces apoptosis. | (Elumalai et al., 2012b) |

Limonoids have been known to exhibit anti-oxidant and anti-inflammatory activities. Limonoids have been shown to trigger programmed cell death called as apoptosis, in cancer cells, so aiding in the elimination of aberrant or damaged cells. They can also limit cell proliferation by interrupting the cell cycle at different stages, such as the G1 phase, S phase, or G2/M phase, stopping cancer cells from reproducing uncontrolled. influence signaling pathways implicated in cancer formation and progression, including the MAPK and PI3K/Akt signalling pathways (Fan et al., 2019; Chidambara et al., 2021; Tian et al., 2001a).

**Cell Cycle Regulation**

Limonoids induce cell cycle arrest at various stages including the G1 phase, S phase, and G2/M phase by targeting the key regulators of cell cycle progression. They can affect the expression and activity of proteins that govern the cell cycle,

such as cyclins, cyclin-dependent kinases (CDKs), and cyclin-dependent kinase inhibitors (CKIs) (Ngabire et al., 2018). By altering all above mentioned proteins, limonoids can disturb normal course of the cell cycle, causing cell cycle arrest at various checkpoints such as the G1, S, or G2/M phases. This stops the unregulated division of cancer cells, which can eventually lead to their death by apoptosis or prevent further tumor development (Huang et al., 2024).

**Apoptosis Induction**

Limonoids can exert cytotoxic effects in human. By inducing cell cycle arrest in the G0/G1 phase, p53-dependent p21 accumulation, and down-regulation of the cell cycle regulatory proteins cyclin B, cyclin D1, and PCNA, it significantly decreased the survival of malignant cells in a dose-dependent manner. Annexin-V staining, the presence of a subdiploid peak, and distinctive changes in nuclear morphology all suggested that apoptosis (programmed cell death) was the mode of death (Doan et al., 2019; Chidambara et al., 2021).

The induction of intrinsic apoptotic pathways is one important method. Cytochrome C and other pro-apoptotic substances may be released from the mitochondria into the cytoplasm when limonoids interfere with the potential of the mitochondrial membrane. Caspases are key players in the apoptotic process, which ultimately results in cell death, and this activates them (Yadav et al., 2016; Chidambara et al., 2021).

Additionally, by controlling the expression of Bcl-2 family members, limonoids can cause apoptosis. Apoptosis is crucially regulated by these proteins, some of which (like Bcl-2) encourage cell survival and others (like Bax) encourage cell death. Limonoids have the ability to tip the scales in favor of apoptosis by expression upregulation of protein that can induce apoptosis in cancerous cells like Bax and downregulating the expression of proteins that do not induce apoptosis like Bcl-2 (Qian et al., 2022; Chidambara et al., 2021).

Overall, the apoptotic effects of limonoids in cancerous body cells involve the initiation of multiple pathways and also the modulation of key regulatory proteins, ultimately leading to the elimination of cancerous cells (Chaudhry et al., 2022; Nagini et al., 2024).

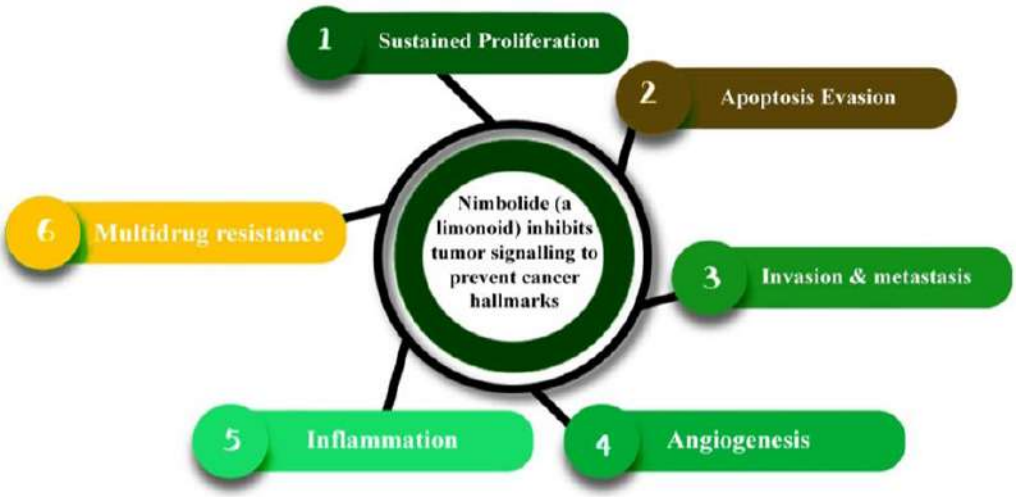


**Fig. 3:** Nimbolide inhibiting oncogenic signalling in the prevention of cancer hallmarks, including sustained proliferation, apoptosis evasion, invasion, angiogenesis, metastasis and inflammation

**Experimental Evidence**

**In vitro Studies**

In vitro studies have shown that Limonoids have demonstrated encouraging antitumor properties against breast cancer cells. These investigations have demonstrated that limonoids have the ability to stop breast cancer cells from proliferating and spreading, cause programmed cell death, or apoptosis, and interfere with several signaling pathways essential for cancer development (Salim et al., 2024; Saini et al., 2022).

For example, investigated the mechanism by which polymethoxyflavones from citrus peels, a kind of limonoid, slowed down the growth of breast cancer cells by triggering apoptosis and cell cycle arrest (El-Kersh et al., 2021). In a similar vein, discovered that the limonoid D-limonene, which is present in citrus fruits, inhibited the growth of breast cancer cells through ERK-1/2 pathway-mediated apoptosis. These in vitro results demonstrate limonoids' potential as natural breast cancer-fighting agents. However, additional research, such as studies on animals and clinical trials, is the efficacy and safety of limonoids for breast cancer treatment (Saini et al., 2022; Klimek-Szczykutowicz et al., 2020; Wang et al., 2014).

**In vivo Studies**

In vivo investigations have shown that limonoids have the potential to be highly effective anticancer treatments for breast cancer. These investigations employ animal models to look at the consequences of limonoids on tumor proliferation, metastasis, and overall survival rates. For instance, looked into how limonin affected mouse breast cancer cells. The results showed that limonin downregulated genes linked to the advancement of cancer, hence suppressing tumor development and metastasis (Fan et al., 2019).

A rat model of breast cancer was used by Liu et al. (2019) to study the anticancer effects of limonin. According to their

findings, limonin prevented the growth of tumors by reducing the expression of angiogenic factors, which in turn prevented the tumors from forming new blood vessels (Fan et al., 2019).

These and other in vivo studies offer substantial support in favor of limonoids' potential as an all-natural breast cancer treatment. To properly comprehend the safety and effectiveness of limonoids as a therapy for breast cancer, more investigation is required, including clinical evaluations (Chavda et al., 2023; Park et al., 2022).

**Comparative Analysis of limnoids with Conventional Treatments**

The comparative analysis of limonoids with conventional treatments for breast cancer can provide valuable insights into their potential as alternative or adjunct therapies.

**Efficacy**

Natural substances called limonoids, which are present in citrus fruits, have demonstrated promise in halting the growth of tumors and lowering the spread of breast cancer (Tian et al., 2001b). In contrast, regular treatment for breast cancer involves conventional treatments including radiation therapy, hormone therapy, and chemotherapy, all of which have undergone significant studies (Łukasiewicz et al., 2021). Chemotherapy is effective in killing rapidly dividing cancer cells, but it can also damage healthy cells and have unfavorable side effects like nausea and hair loss (Anand et al., 2023). In radiation therapy, high-energy radiation is used to eliminate cancer cells, but adjacent tissues may be harmed (Baskar et al., 2012). Hormonal therapy is a very effective treatment for hormone receptor-positive breast cancer, however it is only appropriate for certain types of breast cancer (Puhalla et al., 2012; Tundis et al., 2014).

**Side Effects of Limonoids**

When it comes to negative effects, limonoids may be less harmful than other medicines because they are natural substances. They might potentially increase the effectiveness of current medicines by working in concert with them. Though strong intervention is frequently necessary in advanced stages of breast cancer, limonoids might not be as effective as traditional treatments in these cases (Fan et al., 2019).

**Challenges and Opportunities in Nano-delivery of Limonoids**

Nano-delivery of limonoids for anti-cancer applications presents various challenges and opportunities.

**Bioavailability Issues**

Bioavailability refers to the extent and rate at which a substance, i.e. limonoids, is absorbed into the bloodstream and is made available to the target tissues or cells (Price and Patel, 2024). Numerous phytopharmaceuticals have low bioavailability due to a variety of factors, including quick first pass, type I and type II metabolism, microbial degradation in the gut, drug efflux pumps in the GIT, insolubility in water, and poor stability in gastric conditions. However, limonoids, like many phytochemicals, have poor bioavailability when consumed orally. This is often due to their low solubility in water. Through nano-delivery systems, their effectiveness as anti-breast cancer agents by increasing their concentration at the target site can be enhanced (Lim et al., 2022; Manners, 2007).

**Strategies to Enhance Bioavailability**

With promising results, modified drugs and drug delivery systems based on nanotechnology are currently being investigated and introduced into the market for improved cancer therapy and management. Nanoparticulate drug carriers can overcome a number of challenges, such as improving the drug's stability and solubility, extending its blood half-lives, reducing adverse effects on undesirable organs, and raising medication concentration at the intended spot (Upaganlawar et al., 2022). The technique is based on building nanostructures in which the natural prodrug is attached to or enclosed in submicron-sized capsules or nanoparticles that guarantee their regulated distribution, solubility in water, and targeting characteristics. Overall, the growing demand for citrus fruit-based nutritional supplements and edibles is ushered in a new era of diet-based medicine, which is being facilitated by these sophisticated encapsulation techniques (AbouAitah and Lojkowski, 2022).

**Safety Concerns**

Although the combination of nanotechnology and biology has been heralded as a breakthrough technological advancement, safety considerations must also be taken into consideration. Due to limitations including nonspecific distribution, multistep production, low bioaffinity, decreased light immovability, and toxicity of nanoparticles inside living systems, the wide range of applications of nanomaterials remain difficult to achieve (Kashyap et al., 2023).

**Toxicity Assessment**

Assessing the safety of limonoids, particularly as anti-cancer drugs, involves a thorough series of tests and studies to assess a variety of potential harm. These include acute, subchronic, and chronic toxicity searches, genotoxicity, and carcinogenicity assessments (Mugale et al., 2024). Critics of herbal medicine frequently express concern about the lack of clearly defined therapeutic dosages, which can lead to user abuse and overdoses. As a result, clinical research on

animals is critical for fully understanding the potential detrimental effects of diverse medicinal plant extracts on people (Alexis et al., 2008b).

**Mitigation Strategies**

Designing formulations that improve limonoids' solubility, stability, and bioavailability along with regular monitoring of patients receiving limonoid medication can assist in discovering and managing any potential adverse reactions.

**Regulatory Considerations**

Cancer nanotechnology strives to develop safer and more effective technologies for diagnosing and treating cancer. One interesting method is to encapsulate medications with targeted nanoparticles, which could improve the efficacy and safety of existing treatments. Furthermore, nanotechnology allows for the improvement of wholly new cancer medications. A rational design of nanocarriers results in selective targeting, intracellular uptake, and controlled therapeutic delivery of a payload (Zhao et al., 2018a; Alexis et al., 2008c).

**Future Perspectives**

Nimbolide cannot be commercialized until pharmacological, pharmacokinetic, toxicological, and clinical researches have been validated. Before being approved for clinical trials, limonoids needs to be thoroughly evaluated for safety, effectiveness, and druggability standards in preclinical settings (Zhao et al., 2018b). Limonoids evaluation for drug development can be expedited by high-throughput screening employing a variety of pertinent cell-based and molecular bioassays, target validation, and investigation of effects on particular physiological pathways (Blay et al., 2020).

**Conclusion**

Limonoid is considered a complementary drug because to its impressive antiproliferative actions against a variety of human cancerous cells, especially breast cancer, in vitro as well as in in vivo tumor models. Nimbolide has an impact on every stage of multistage carcinogenesis, including metastasis, apoptosis evasion, angiogenesis, invasion, genotoxicity, carcinogen metabolism, and epigenetic changes. In the last few decades, single-target agents—drugs that specifically target a single biological molecule, typically a protein—have been the focus of anticancer drug design and discovery. Nevertheless, there are a number of issues with this strategy, such as resistance and poor therapeutic efficacy.

In light of the intricate and multifaceted nature of cancer, the utilization of multitarget medications has become a more practical approach. This has led to the well-thought-out development of numerous innovative small molecule multitarget therapies with promising clinical outcomes in recent times. It is a difficult task to integrate pharmacophores during the drug development process (Nagini et al., 2021b).

Natural compounds having broad-spectrum, multitargeted activity, such as nimbolide, are appealing candidates for the creation of drugs that can fight against breast cancer. Still, before bringing preclinical findings to the clinic, much research is needed on soluble nimbolide analogues, effective delivery methods, and nanoformulations that can increase bioavailability. It is also essential to conduct research on acute, chronic, and subacute toxicity.

The significance of incorporating nano-delivery methods for enhanced drug delivery has been shown by the prospective applications of limonoids, specifically nimbolide, in breast cancer treatment. To confirm the effectiveness, safety, and commercial viability of limonoids as anticancer agents and to clear the path for advancements in breast cancer treatment, more investigation and clinical studies are required.

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Chapter 40

# The role of Nanoparticle Formulations in Pulmonary Drug Delivery

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**ABSTRACT**

Global health faces a significant threat from pulmonary diseases, prompting recent nano therapeutic developments. Innovations involve creating nano medicine for pulmonary admiration of pharmaceutical components, modified drugs, and unconventional molecules like antimicrobial peptides. Pulmonary delivery challenges arise from nano medicine's poor aerodynamic properties during inhalation, hindering lung deposition. However, inhaling aerosolized or dry powder Nano drugs can act deeply on lung tissues for therapeutic results. Particulate-based drug delivery systems complement conventional inhaled drugs, produced through various methods detailed in reviews of therapeutics for inhalation. Inhalable formulations, developed for delivery via advanced inhalation tool ensure efficacious therapeutic compound administration. Particle engineering proves crucial for enhancing drug admiration, improving therapeutics and achieving by enhanced targeting in inhalable formulations. Technological advancements in inhalation devices contribute to more efficient lung-based drug delivery mechanisms. This chapter highlights pulmonary drug delivery mechanism along with barriers and most common infectious disease.

**KEYWORDS**
Nano Particle, Pulmonary Drug, Therapeutic results

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## INTRODUCTION

Lung targeted the drug delivery systems, offering promising treatment options for conditions like lung cancer, COPD, asthma, tuberculosis, cystic fibrosis, and respiratory allergies. In the present time, the pulmonary drug administration system has attracted substantial biomedical attention, presenting advantages such as avoiding systemic side effects, reducing drug degradation, enhancing efficacy, and improving patient compliance. Nano therapeutics play a pivotal role by addressing gaps in current treatments and focusing on constructing, regulating, and using nanotechnology-based therapeutic drugs and devices. Many nano therapeutics are assessed for pulmonary drug administration, aiming for more focused distribution, improved solubility, reduced toxicity, controlled release, and deposition kinetics. Examples include inhaled liposomal amikacin for pulmonary infections and PEGylated nano-liposome DOX for lung cancer. While direct inhalation of nanoparticles faces challenges, researchers are advancing pulmonary drug delivery technologies through new inhaler technology and particle design. Formulating NPs allows better control over powder morphology, enabling deep lung delivery with improved drug absorption and dissolution. This chapter lays out crucial perceptions of nano medicines, techniques for preparation, advancements in inhaler devices, and evaluations emphasizing their applications in pulmonary delivery (Kole et al., 2023).

### Lung Anatomy

The lungs play a vital role in supplying oxygen to cells through gas exchange. They are made up of five lobes, three in

the right lung and two in the left. It contains lymph tissue, alveoli, bronchi, and tiny blood arteries. Alveoli and bronchioles are reached by the bronchi's branches into primary and secondary bronchi. With over 300 million alveoli, each inclined with pulmonary capillaries, the lungs form an extensive network of 280 billion capillaries. It offers a surface area of 70 $m^2$ for the blood gas barrier. Alveolar gas exchange primarily occurs at interstitial cell layers, endothelium, and alveolar epithelium. The alveolar epithelial cells of Type I and Type II make up the alveolar wall, facilitating gas exchange through a thin barrier of about 0.5µm. A layer of alveolar fluids and mucus, rich in phospholipids and surface proteins, coats the alveoli, reducing surface tension for effective gas swapping. The connective tissue layer that supports the distal respiratory airways contains lymph vessels, fibroblasts, and macrophages providing an optimal location for drug administration with access to the lymphatic and pulmonary systems.

**Pulmonary Infections**

**Bacterial Infections**

Pulmonary diseases related to bacterial infections involve lower respiratory tract infections, encompassing various pathogen infections with distinct pathogeneses clinical manifestations, and epidemiologists. To effectively treat these diseases, effective pulmonary medication delivery strategies based on nanoparticles must take into account a number of aspects, including the kind of bacteria that affected the respiratory tract area, the local microenvironment and the pathophysiological process associated with the disease.

**Cystic Fibrosis and Chronic Infections**

Cystic fibrosis, prevalent among Caucasians, results from 230kb of gene mutations on chromosome 7. (Troeger and Blacker et al., 2018) This mutation leads to impaired chloride ion transport and thickened mucosal secretions, fostering colonization of microorganisms in the respiratory system. *Haemophilus influenza* and *Staphylococcus aureus* are the most common in the early stages, progressing to *Stenotrophomonas maltophilia*, *Burkholderia cenocepacia*, *Achromobacter xylosoxidans*, and *Pseudomonas aeruginosa*.

**Chronic Obstructive Pulmonary Disease (COPD)**

COPD is the third most common cause of death globally, as it affects over 174 million people. Characterized by chronic inflammation in respiratory ducts and impaired lung defense. (Lozano,Naghavi et al., 2012) COPD is exacerbated by bacterial infections from *Moraxella catarrhalis*, *P. aeruginosa*, *Streptococcus pneumoniae*, and *H. influenza*. These infections, linked to acute exacerbations and high mortality, occasionally lead to recalcitrant biofilms similar to those observed in cystic fibrosis. (S.Sethi, 2010) Current antibiotic treatments focus on brief treatments for moderate-to-severe exacerbations because of worries about the emergence of antibiotic resistance. (Staykova, Black, et al., 2003)

**Tuberculosis (TB)**

Tuberculosis, due to Mycobacterium tuberculosis, is still present. The emergence of multidrug-resistant strains adds to the challenge. Standard TB treatments involve long-term oral administration of complex drug regimens, leading to poor compliance and adverse effects that could be fatal. The TB of M. persistence in lung macrophages poses additional difficulties, as systemic administration often fails to efficiently penetrate alveolar macrophages. (Dartois, Barry., 2010)

**Other Respiratory Infections**

Non-CF bronchiectasis-related infections cause respiratory tract injury, inflammation, and persistent widening of the airways. These infections have been linked to pathogens such as *Neisseria* spp., *Prevotella* spp., and *Veilonella* spp. Ventilator-associated pneumonia (VAP), occurring 48 hours after tracheal intubation, is caused by antibiotic-sensitive bacteria initially, progressing to multidrug-resistant pathogens (Priftis, Litt, et al., 2013).

**Barriers for Pulmonary Drug Delivery**

In pulmonary inhalation, drugs encounter various clearance mechanisms that act as primary barriers to absorption after administration through the lungs.

**1. Mucociliary Barrier**

Mucociliary clearance, a crucial lung defense mechanism, eliminates inhaled particles and substances. This system, comprising the periciliary layer and mucous layer, utilizes cilia on airway epithelial cells and mucin rich mucus. (Araújo, Martins, et al., 2018)

Inhaled drugs often face interception by viscoelastic mucus and the PCL, impeding their diffusion and interaction with cells. The mucus layer, which is made up of MUC5AC and MUC5B mucin, presents a barrier affecting drug dissolution and hindering epithelial diffusion. (Kurbatova et al., 2015). Modulating particle size and charge properties plays a crucial role in optimizing the interaction with the mucus layer for efficient drug delivery. (García-Díaz et al., 2018).

**2. Pulmonary Surfactant**

Pulmonary surfactant (SP) is a lipoprotein tissue with bipolar produced by alveolar type II cells (AT-II) in alveolar

epithelium. They consist of phospholipids and surfactant proteins (García-Díaz et al., 2018). Pulmonary surfactant (SP) also associated with the removal of drug carriers and therapeutic drugs (Parra, 2015).

**3. Immune Cell Barriers**

Alveolar macrophages, the major phagocytic cell that protects the lungs from inhaled particles, might come into touch with medication particles that haven't dissolved. (Labiris and Dolovich, 2003). An immune barrier made up of alveolar macrophages does not differentiate between chemicals that could be hazardous and potentially advantageous ones. The macrophages may take up drug particles and remove them from the respiratory system by the lymphatic system or by moving them to the mucociliary escalator's foot.

**4. Enzyme**

Other than the metabolic enzymes and biological barriers in pulmonary epithelial cells, maintaining the functions of barriers. For example, antitrypsin and tripsin. (Greene CM, 2009).

**5. Biofilm**

As a physical barrier for pulmonary inhalation, biofilm is important. Microbial cells produce complex structures called biofilms, which are encased in their extracellular matrix. They adhered to the biological or inert surface. Extracellular polymer, lipid, polysaccharides, and DNA make up biofilm, which is a significant obstacle to the effective penetration of antimicrobial drugs.

**Pulmonary Drug Delivery**

**Aerosol Medication**

Aerosol medication carried inhalation way is the best choice, which is a trendy treatment method of respiratory. The two essential steps in inhalation for a good delivery of the medicine are production of medicine carriers (aerosol particles) and reproducible dosage levels during the patient administration. Thus, these technical difficulties are resolved by several classifications of inhalation devices (inhalers) as well as formulations which are neatly adjusted so that they can be easily aerosolized. Generally unremarked harmful influence which cannot be ignored because of the requirement to ensure stability and reproducibility of inhalable drugs administration.

**Inhalation Local Delivery**

Most of the time, when we talk about inhalational administration, we mean the process of administering medications or other exogenous substances directly into the lungs through specific lung regions or cells, where they preferentially accumulate and have limited penetration into the bloodstream.

Treatment for a variety of respiratory conditions benefits greatly from this kind of inhalation therapy. It is anticipated that this mode of delivery will move the active component or components to the site of action and hold them there for the necessary amount of time. Particle size, breathing circumstances, lung aerodynamics, inhalation techniques, and devices employed all affect how effective inhalation delivery is. A given liquid or solid must produce an aerosol by suspending it in a gaseous medium before inhalation.

**Dispersions of Colloids**

For the purpose of drug delivery, a colloidal system, also known as colloidal dispersion, is a heterogeneous system made up of a dispersed phase (a liquid or solid medicine) that is uniformly distributed throughout the dispersion medium (typically water). The solid or liquid hydrophobic medicine in water using probe sonication is the most straightforward method for creating aerosols of water impermeable pharmaceuticals. Several techniques can be employed to create aerosols from the resultant colloidal dispersion, which can then be inhaled to enter the lungs. Tacrolimus dispersion, intended for nebulization and inhaled delivery to the lung transplanted animal model, is an illustration of such a system.

**What Aerosols Need to be Directed into the Respiratory Tract?**

The variability of diseases and variances in respiratory systems (affected by age, gender, and disease) posed obstacles for appropriate inhalation therapy planning. Inhalation therapy is also regulated by the location of receptors in the airways. Coarser particles may target receptors in the major airways (M3), while small particles may target receptors in the peripheral (beta-2). The lung can also be used as an entrance point for numerous systemically active medicines (hormones, vaccinations, cytostatic drugs, etc). In such circumstances, it will be necessary to administer agents to certain areas of the lungs (alveoli and alveolar sacs) while avoiding delivery to other locations. The fundamental logic is to provide the maximum dose at the desired site while reducing the undesirable effects at other sites.

**PEGylation**

PEGylation is a popular technique for increasing the therapeutic effect of a drug by extending its body residence time. It includes attaching one or more PEG chains to an object, which can include proteins, peptides, particles, tiny compounds, and cells. Several PEGylated proteins have been approved for therapeutic usage as injections. Polyethylene glycol is a

neutral linear polyether with a variety of molecular weights.

Its safety is guaranteed by its widespread use in foods, cosmetics, and pharmaceuticals. PEGylated proteins, once in the bloodstream, would offer sustained plasma quantities of the protein for which they were administered. PEGylated rhG-CSF and PEGylated insulin were both absorbed via the lungs. However, systemic absorption decreased after PEGylation on many protein sites and the use of large PEGs (5-12 kDa). PEGylation has also been utilized to protect peptides against localized proteolysis in the lungs, increasing systemic absorption of the undamaged molecule after pulmonary administration (Loira-Pastoriza et al., 2014).

**Liposomes and Lipid Nanoparticles in Pulmonary Drug Delivery**

Liposomes and lipid nanoparticles (NPs) have received a lot of interest in recent years for their versatility in pulmonary medication delivery. The utilization of liposomes and lipid NPs in pulmonary drug delivery, highlighting their formulation strategies, mechanisms of action, and therapeutic applications. Liposomes, sphere-shaped vesicles made up of bilayers of lipids, and lipid NPs, such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), provide distinct benefits for lung drug delivery. These advantages stem from their biocompatibility, tunable physicochemical properties, and capacity to encapsulate a variety of medicines, including tiny compounds, proteins, and nucleic acids.

**Nanoparticles**

Nanoparticles (NPs) are particles with a single dimension ranging between 1 and 100 nm. The characteristics of nanoparticles vary based on their surface functions and size (Gwinn and Vallyathan, 2006). NPs are employed as pharmaceutical drug carriers for therapeutic and diagnostic purposes. It is proposed that these NPs, which include solid NPs, liposomes, polymeric NPs, and nano-emulsions, may find use in medicine. Many factors, including their physical and chemical characteristics, drug loading effectiveness, drug release, and most importantly low or non-toxicity of the carrier itself, determine their therapeutic application (Puri et al., 2009).

**Nanoparticles for Pulmonary Medication Delivery**

The ability to combine pharmaceuticals into nanoparticles opens up the possibility of cell-targeted delivery of drugs, demonstrating an innovative approach in pharmacology. In vivo transporters have been developed using non-targeted nanoparticles, such as solid lipid particles and polymer-coated nano-carriers. These days, however, medications can be attached to nano-carriers that are particular to certain cells or organs. It is therefore possible to deliver medications specifically to organs, tumors, and cells by either depositing them in subsurface oil layers or trapping them inside the carriers.

**Nanoparticles Formulation in the Pulmonary Drug Delivery**

**Pure Drug Nano-assembly**

PDNAs are designed to contain only active drug molecules—no chemicals or inert molecules of any kind. PDNAs can be classified into three categories based on the makeup of the drug modules: (i) single pure drug nano-assemblies (SPDNAs), which comprise only one type of drug module; (ii) dual pure drug nano-assemblies (DPDNAs), which comprise two types of drug modules co-assembling; and (iii) multiple pure drug nano-assemblies (MPDNAs), which comprise more than two types of drug modules co-assembling. Drug modules are assembled primarily through non-covalent bond interactions such as hydrophobic contacts, the intermolecular π–π stacking, bonding with hydrogen, and electrostatic attraction.

**Polymeric Nanoparticles**

The use of polymers in pulmonary medication delivery is becoming more and more important. Many different kinds of polymers have been researched and investigated for this purpose. They offer a number of benefits, including modified surface characteristics, efficient encapsulation combined with degradation protection, long-term drug delivery capability, and longer shelf life when stored (Menon et al., 2014). Polymers made from nature, including gelatin, chitosan, and alginate, as well as artificial polymers like poloxamer, poly (lactic-co-glycolic) acid (PLGA), and poly (ethylene glycol) (PEG), are commonly used as nano-carriers in pulmonary drug administration (Sung et al., 2007). As stated by the U.S. Food and Drug Administration, PLGA is a synthetic polymer that is both biocompatible and biodegradable. Depending on the monomer ratio used, it can provide

**Drug-loaded Liposomes**

When amphiphilic lipids are spread in water, they spontaneously form liposomes, which are spherical vesicles with aqueous interior cores surrounded by lipid bilayer shells (Manna et al., 2019).

The term "drug to lipid ratio" refers to the ratio of the mole fraction of the entrapped drug to the moles of lipid; it is often represented as "moles of drug per moles of phospholipid" (Bulbake et al., 2017). The flexibility of the lipid bilayer helps to create stable drug-loaded liposomes by modifying the integrity of the bilayer (He et al., 2019). Since then, over ten more lipid-based formulations have been developed (Roberts et al., 2020).

**Pulmonary Drug Administration Mechanisms**

Experiments on both humans and animals have examined the systemic uptake of a wide variety of medicinal medicinal products by pulmonary medication delivery. The medication administered by the pulmonary route can be split into two main modes: intranasal and oral inhalative. There are physiological restrictions associated with intranasal administration, such as a restricted airway lumen. It is preferable to provide oral inhalatively, which permits minute particles with a 20% concentration loss as opposed to an 85% concentration loss via the nose route.

Intratracheal instillation and intratracheal inhalation are further classifications for oral inhalative delivery. Intratracheal installation is the most often utilized technique in laboratories. A tiny amount of medication solution and dispersion are injected into the lungs by an intratracheal instillation, which delivers the medication to the lungs quickly and quantitatively using a specialized syringe.

Aerosol drug delivery in the pulmonary airway occurred through the following processes: gravitational sedimentation, inertial impaction, and diffusion. The lung morphology and respiratory characteristics, such as particle size, droplets, and geometrical shape, are crucial.

**Nanoparticle Delivery Systems for Pulmonary Applications**

Nanoparticles are important for biomedical applications which play an important role in pulmonary diseases of respiratory cell types. Asthma, cystic fibrosis, and ciliopathies are linked to ciliated cells in respiratory disorders. (Yaghi A, 2016; Aghapour M, 2018) Numerous respiratory cell types, such as lung epithelium, lung endothelium, and fibroblasts, are necessary for cell repair following lung injury (Whitsett JA, 2019). Long-term respiratory conditions such pulmonary arterial hypertension, chronic obstructive pulmonary disease, and idiopathic fibrosis of the lungs are caused by defects in lung repair (Huertas A, 2018; Jandl K, 2020). Crucially, it is creating novel medication delivery methods using nanoparticles that can target particular lung cell types.

Both intravenously and intratracheally delivered nanoparticle delivery methods encounter unique challenges. Intratracheal injection is the preferred method for targeting airway epithelial cells because of its ability to directly transport nanoparticles to lung tissues without being obstructed by first-pass metabolism (Kan S, 2020). The passage of nanoparticles toward target cells, bronchoalveolar fluid, and phagocytes across the walls of the alveoli and passageways can all disrupt the mucosal layer. (Lee WH, 2015; Guagliardo R, 2018)

**Fundamental Research on Inhalable Nano Formulations**

Four siRNA medications—Zhang, Bahal, Rasmussen, Manautou, and Zhong—have been approved for usage, and numerous other siRNA therapeutics are presently undergoing evaluation in local or disease-specific clinical trials as a result of ongoing research into RNA interference technology (Table 5). These authorized siRNA medications are all given intravenously. Although siRNA inhalation formulations are not currently authorized, a number of medications are undergoing clinical studies.

Using RNA interference (RNAi) technology, Alnylam Pharms created the injectable siRNA ALNRSV01 to treat respiratory syncytial virus (RSV) infection. With the exception of two nucleotide overhangs at the duplex's third end, ALNRSV01 contains 19 pairs of nucleotides overall without any chemical alteration or distribution. (Alvarez et al.). RSV subtypes A and B were both resistant to intranasal spray that targeted the mRNA of the RSV nucleocapsid protein (N protein) (Xing and Proesmans). Four clinical trials have been conducted on ALN-RSV01, and the results of the Phase I trials in healthy adult men have confirmed the drug's safety.

Then, in phase II clinical research, 88 healthy participants were injected with RSV and given with ALN-RSV01 nasal spray or a placebo to see if ALN-RSV01 had antiviral activity. It was discovered that ALN-RSV01 could considerably lower RSV activity (DeVincenzo, et al.). Patients who had received a lung transplant and had an RSV infection were the subjects of phase IIa and IIb clinical trials. Nevertheless, the initiative has not advanced any further because the final outcomes fell short of the clinical trial's end aim. ExcellairTM, a siRNA inhalation formulation created by ZaBeCor Pharmaceuticals, is another one that is going through clinical studies.

It targets spleen tyrosine kinase (Syk) mRNA to reduce inflammation and treat asthma; it has no delivery mechanism. The results of the Phase I clinical research showed that ExcellairTM considerably improved asthmatics' ability to breathe while posing no appreciable side effects, and that patients tolerated it well. Regretfully, ExcellairTM's Phase II clinical research was stopped in 2015 for undisclosed reasons (Burnett, Rossi, and Tiemann). Furthermore, one option for successfully battling SARS-CoV-2 infections, which are currently endangering human health, is to use siRNA inhalation formulations. Globally, academic institutions and pharmaceutical firms are actively creating and testing siRNA medications against the virus's primary genomic sequences.

After screening compounds from 15 anti-new coronavirus siRNAs, the NRC Institute of Immunology FMBA of Russia discovered that siR-7 was one of the best at preventing virus reproduction in vitro and may target the virus-dependent RNA polymerase (RdRp). The researchers created siR-7-EM by adding LNA to the 3′-end of the SS and AS of siR-7. They then combined siR-7-EM with the cationic peptide dendrimer KK-46 to create the siR-7-EM/KK-46 aerosol, which improved the antiviral and in vivo stability of siR-7. Preclinical experiments have demonstrated that local delivery of siR-7-EM/KK-46 to the lungs efficiently counteracts SARS-CoV-2 and greatly reduces inflammation in the lungs.

The medicine is now authorized for research trials by the Ministry of Health of the Russian Federation. In parallel,

hundreds of siRNAs were screened against highly conserved areas of the SARS-CoV-2 genome by Alnylam Pharms and Vir Biotechnology. VIR-2703 is one of the most potent medications against SARS-CoV-2 to date; in an in vitro viral model, it was reported to have an EC50 < 100 pmol and an EC95 < 1 nmol. It might be applied as an inhalation remedy to cure or stop the infection. The two businesses plan to apply for an IND to treat COVID-19 with VIR-2703 at the US FDA.

**Diverse Inhalation Devices**

Because inhalers may instantly administer medication to the lungs, they are a valuable tool in the treatment of respiratory illnesses. Those gadgets are available in many types, every designed to satisfy patients' desires and alternatives. Many inhalation gadgets facilitate the transport of medication, from metered dose inhalers (MDI) and dry powder inhalers (DPI) to new products, which include nebulizers and Respimat. Information about the opportunities and variations between those gadgets is vital to make sure suitable treatment outcomes and improve respiratory for doctors and sufferers.

**1. Pressurized Metered Dose Inhaler (MDI)**

Commonly speaking, the pMDI production technique includes combining (i.e., grouping) method additives and then transferring (i.e., filling) the product into packing containers (Vallorz). The accuracy and precision of the fabric is especially vital all through the compounding system, as this ratio will decide the attention of lively pharmaceutical aspect (API) and components within the finished product (1990and1:34061)

The process is complex via the inclusion of non-toxic chemical substances, 1,1,1,2-tetrafluoroethane (HFA134a or norfluorane) or 1,1,1,2,3,3, three-Heptafluoropropane (HFA 227ea), additionally referred to as apaflurane). Propellers are liquefied gases at standard operating pressure, which means that they always maintain a constant vapor stress at a specific temperature. This makes the reduction process ineffective.

Leaks because of detailed quantity ranges or stress adjustments. The weight of the product packaging and the product is a degree to determine the leakage and packaging degree at some point of manufacturing.

The use of propellants needs to be cautiously taken into consideration. Gaseous propellant can input the pump or fill strains, growing protection troubles, disrupting drift charges, and causing malfunctions. It is particularly proper in sizable extent vegetation wherein the feed and system are very huge, or the scale is huge while the batch is discharged, causing a huge part of the propellant to transition into the fuel segment. Moreover, the navigation and inspection location of the ship is critical for getting entry to the liquid phase for meting out and doling out.

**2. Dry Powder Inhalers (DPIs)**

Dry powder inhalers (DPIs) are a broadly widespread approach to breathing in medicinal drugs, particularly in Europe, now utilized by many patients to supply medicine for medical functions: allergies and pneumonia. After the advent of Serevent Diskus within the 1990s, DPI adoption inside the US slowed, largely due to the success of Advair Diskus in the recent years. This approach of mixing gently acquired pills in a simple and easy use tool creates a diagnosed model for the delivery and remedy of lung illnesses that might have been unthinkable within the previous few years. DPI no longer most effective affords excellent comfort to patients, especially in mixture therapy, but also ends in higher overall performance. Currently, there are more than 20 dry powder inhalers (DPIs) available for gifting, and at least every other thirty are substandard. Although clinicians are aware that DPIs can be a good alternative to pressurized metered dosage inhalers (pMDIs) for certain patients, they frequently question the devices' relative efficacy.

**3. Nebulizers**

Nebulizers are the oldest contemporary method of delivering aerosols to the lungs for the reason of breathing drug delivery. At the same time as the use of nebulizers remains giant in the health center and domestic setting, sure more recent nebulization technology has enabled more portable use. Numerous essential strategies of droplet formation and breakup are used in cutting-edge nebulizers, and these procedures affect tool performance and suitability for nebulization of various formulations.

It is essential that both patients and pharmacists understand how to utilize the device correctly because the majority of asthma patients take nebulized medications at home.

**a) Jet Atomizers**

Jet atomizers are powered via a transportable compressor or relevant air supply. Essentially, excessive air pace emitted from the nozzle entrains the liquid and disperses it into droplets (first generation) through instabilities as a result of viscosity.

**b) Ultrasonic Nebulizer**

The ultrasonic atomizer makes use of a piezoelectric sensor to create droplets from an open reservoir. The strain waves emitted through the piezoelectric vibrator at the bottom of the reservoir unfold to the floor, developing a fountain at the wave consciousness.

The droplets shape because of excessive-power surface instabilities at the bottom of the fountain. This technique can't effectively aerosolize the drug in suspension because most of the suspension is in the reservoir. For the reason that energy

is transmitted through the liquid container, it's far clean that viscosity has a fantastic impact on aerosol particle length and output, and high viscosity liquid may also perform negatively.

**c) Passive Mesh Piezoelectric Atomizer**

Omron U1 atomizer makes use of an ultrasonic transducer to create lengthy vibrations on the capillary tube. This motion lets the liquid come back into touch with the liquid. The decrease cease of the tube expels the liquid through the ceramic mesh subsequent to the other end. The grid has micropores with diameters within the range of 5 Âμm. The benefit in comparison to jets and traditional ultrasonic nebulizers is that there's much less residue in the medication chamber.

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Chapter 41

# Nano-oncology: The Future Perspectives

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**ABSTRACT**

Nano-oncology has exhibited promising advancements in treatment and diagnostics of cancer. Conventional cancer therapies and diagnostic techniques can cause cytotoxicity, genotoxicity and are inefficient. Reportedly nanoparticles-based techniques can effectively carry out detection of type and state of tumor, biomarkers detection, tumor mapping and targeted drug delivery. Organic, inorganic, multifunctional, hybrid and functionalized nanoparticles have revolutionized cancer diagnostics and treatments due to their theragnostic applications, biocompatibility and unique physiochemical properties. Nanoparticles acquired properties for interaction with biological cellular systems, that are not present in their larger dimensions. Nanoparticles gain access to their targets by passive and active drug targeting, gene delivery and stimuli-sensitive drug delivery strategies. Nano-based anti-cancer therapies reduced toxicity, mitigated therapy resistance and improved pharmacokinetics and pharmacodynamics of encapsulated chemotherapeutic drugs and anti-cancer therapies. In this chapter applications of nano-oncology in cancer diagnostics and therapy, future perspectives and challenges in the way of designing and approval of nano-based cancer therapies will be discussed.

**KEYWORDS**
Nano-oncology, Nanoparticles, Theragnostic, Targeted drug delivery, Active drug targeting

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## INTRODUCTION

Cancer with its wide-ranging socioeconomic effects, remains to be a main focus of drug development and delivery studies. However, success rate of developed anti-cancer therapies is still marginal. These therapies can display serious side effects, drug resistance, epigenetic modifications and cytotoxicity. Nanotechnology has navigated in a new age of cancer treatment, presenting unique opportunities to overcome the challenges and limitations of conventional therapies. Extensive research on use of nanoparticles in treatment and diagnosis of various types of cancers, contributed to the development of a new medical purview, nano-oncology, a platform for the development of new aspects of cancer diagnosis and therapy using nanoparticles (Bray et al., 2024; Jiang et al., 2024; Osadchuk et al., 2021). Nanoparticles with their biocompatible nature, smaller size, surface charge, high atomic number and quantum effect acquired exceptional physiochemical properties to interact with biological systems, have gathered significant attention as future of medicine. Reportedly, they can contribute in early cancer diagnosis, enhanced imaging capabilities and targeted drug delivery for more effective and less toxic cancer treatment (Nasir et al., 2021).

### Nanocarriers in Nano-oncology

Ideal anti-tumor-drug delivery nano-carriers remain stable without fluctuating pharmacodynamics of therapeutics, avoid therapeutics' early metabolic degradation during circulation, capable of specific targeted drug release, non-toxic and easy to visualize by imaging techniques.

### Organic Nanocarriers

Liposomes are organic nano-vesicles, first ever nano-drug carriers approved clinically. Liposomes function by adapting lipid layer structure and duplicating biophysical properties of living cells. Polymer-based nanoparticles (1- 1000 nm),

Polylactic-co-glycolic acid (PLGA), dendrimers and polymeric micelles are the studied organic nanocarriers for cancer therapy and diagnosis (Yao et al., 2020).

**Inorganic Nanocarriers**

Inorganic nanoparticles have high surface area to volume ratio. Metallic nanoparticles (1 to 100nm) such as platinum and gold nanoparticles, are widely used for cancer diagnosis and therapy due to their antioxidant properties, prolong circulation time, less toxicity and effective targeting and drug delivery. Other examples of inorganic nanoparticles includes quantum dots, graphene, Fullerene, carbon nanotubes, mesoporous silica nanoparticles and magnetic nanoparticles (10- 50 nm) (Alrushaid et al., 2023).

**Hybrid Nanoparticles**

Organic and inorganic nanoparticles possess different benefits and limitations. Their combined use as a single hybrid system for drug delivery develop multifunctional drug carriers with improved therapeutic efficacy, reduced toxicity and lessen drug resistance. Lipid-polymer hybrid nanoparticles, liposome-silica hybrid nanoparticles, chitosan hybrid nanoparticles, half-shells of metal multilayers, PLGA hybrid nanoparticles and cell membrane coated nanoparticles are some examples of combined targeted drug delivery systems for cancer therapy. Hybrid conjugated nanomaterials have been used for targeted gene or drug delivery, multimodal therapy, reduction of resistance to therapy and noninvasive imaging (Nanda & Yi, 2024; Yao et al., 2020).

**Applications of Nano-Oncology in Tumor Diagnostics**

Early and accurate detection of tumors using imaging techniques, histopathology, cytology and biomarkers detection is significant for effective targeting, therapy and prevention. Conventionally used imaging methods diagnose tumors after visible alterations and metastasis and are unable to differentiate among lesions caused by benign and malignant tumors (Jin et al., 2020). Nanotechnology-based techniques for cancer diagnosis are now being considered as more sensitive, more specific, instant, convenient and worthwhile diagnostic tools.

**Nano-Oncology in Tumor Imaging**

Nano-oncology empowers the development of highly specific and more sensitive nanoparticles-based imaging agents to improve quality of imaging techniques. These imaging agents provide non-invasive imaging of tumors, visualization of metastasis, and processes occurring in cells. Due to large surface to volume ratio of nanoparticles, they largely attract specific surface receptors of tumor cells thus providing effective, sensitive and specific tumor imaging on cellular, molecular and tissue levels (Zhang et al., 2019).

Image-guided strategies for cancer therapy are crucial topic in biomedicine. Accurate diagnosis of multifaceted and soft tumors is challenging, which confines suitable handling options to attain desired therapeutic results. Reportedly, multifunctional nanoparticle-based contrast enhancement agents improved the diagnosis precision of various diseases including cancer. Nanoparticle-based important imaging techniques include positron emission tomography (PET), fluorescence imaging, magnetic resonance imaging (MRI), and photoacoustic imaging (PAI). Nanoparticles as contrast agents for magnetic resonance imaging, computed tomography (CT) and optical imaging are of great interest currently. MRI is based on use of metal nanoparticles as contrast agents being injected in body. Metallic nanoparticles such as gadolinium, manganese, and iron oxide are in frequent use (Akakuru et al., 2019; Baranwal et al., 2023; Cui et al., 2024).

Fluorescence imaging by loading various nanocarrier such as nano-micelle with specific anti-tumor drugs or near infrared dyes aid in adequate detection of tumor boundary. Biocompatible nanomaterials have been reported to provide multimodal imaging for tumor boundary demarcation. B-mode ultrasound by using polymeric nanoparticles and perfluoropentane nanobubbles, provides clear tumor mapping. At normal body temperatures nanoparticles-based nano-droplets convert into nanobubbles forming walls, generating echoes making tumor imaging possible (Jin et al., 2020). Mammography imaging also support use of nanomaterials for ensuing imaging and tumor boundary demarcation. Surface-enhanced Raman spectroscopy (SERS) imaging also involves the use of nanoparticles as substrates for distinguishing tumor cells boundaries (Akakuru et al., 2022).

Superparamagnetic iron oxide nanoparticles (SPIONs) possess high affinity for lymph nodes and can detect nodal metastases when used in conjunction with high- resolution MRI. Photoacoustic imaging (PAI) reportedly also being carried out effectively and without toxic effects using single walled carbon nanotubes as PAI agent. These agents emit near infrared light and detected by their sturdy Raman scattering signal. Positron emission tomography (PET) works using self-assembling amphiphilic dendritic molecules into supramolecular nanoparticles resulting in tremendously sensitive and specific tumor imaging with diminished cytotoxicity and enhanced penetration and retention effect. Magnetic powder imaging using magnetic nanoparticles showed sensitivity and high resolution in lungs cancers (Garrigue et al., 2018). Nanoshells (10-300nm) are made of silicon with thin metal shells. They convert electrical energy mediated by plasma into light energy that can easily be adjusted through UV- infrared emission or absorption arrays. (Jin et al., 2020). Nanoclusters have attained great attention for bio-imaging application because of their brightness, photostability and resistance to photobleaching. Nanoclusters are depicted as such small cluster of metal atoms from

which removal or addition of single atom can alter their photosensitivity and mechanical properties. Nanoclusters support multimodal imaging, such as by CT, MRI, PAI and X-ray because of their tunable incandescence from near-ultraviolet to the IR region (Porret et al., 2020).

**Nano-Oncology in Detection of Tumor Biomarkers**

Oncological biomarkers can be described as naturally occurring biological structures such as proteins and nucleic acids, or characteristics expressions generated by or in response of tumor cells that can be detected in tissues, body fluids or tumors. Variation in their levels or nature such as genomic alterations, represents the incidence, state and prognosis of tumors estimating therapy response. Each type of cell has specific molecular monograms for differentiating healthy cells from tumor cells or benign cells from metastatic cells (Combes et al., 2021; Jin et al., 2020).

Nanoparticles such as magnetic nanocomposites, hydrogels, carbon nanotubes and mesoporous silica nanoparticles gained attention due to their small size, large surface area, adjustable pore size and biocompatibility for proteomics. Fluorescent nanoprobes can detect cell membrane protein-a of tumor associated fibroblasts due to pH-response of tumor environment. Nanoparticles based identification of exosomes that are released by cancerous cells is also under review for early diagnosis. Circulating tumor cells' (CTC) detection in blood is also evidently identified as prognostic biomarker for several tumors, providing understanding on tumor metastases and heterogeneity. Nano-sized immune-magnetic beads and non-magnetic nanorods reportedly used as an alternative for CTCs enrichment. Use of nanoparticles reduced loss of CTCs and cytotoxicity. Polymer dots considered to be ideal for CTCs detection, due to their nontoxic behavior and photostability. Nanotechnology application for manufacturing lab-on-a-chip using quantum dots for multiplexed protein detection for effective immuno-screening of tumor cells (Baranwal et al., 2023; Özyurt et al., 2023; Zhang et al., 2019; Zhang et al., 2022)

**Applications of Nano-oncology in Cancer Treatment**

The ultimate objective of biocompatible nanoparticles in cancer therapy is to efficaciously deliver chemotherapeutics, antigens, adjuvants or other immunotherapeutic agents to the target sites, for tumor detection, tumor regression and immune response activation. Nanotechnology addresses the hydrophobic properties, toxicity, low water solubility, rapid clearance and short half-life of chemotherapeutic drugs by providing improved enzymatic stability and distribution. Nanoparticles or nano-complexes encapsulating therapeutic agents usually administered intravenously.

**Tumor Microenvironment (TME)**

TME comprises of uncharacteristic mass of cells comprising blood vessels, lymphatic vasculature, elements of the extracellular matrix, varied cells populations fibroblasts, cancer stem cells and immune corpuscles In TME hypoxic condition occurs due to excessive cell proliferation leading to activation of angiogenesis. Presence of populations of inhibitory immune cells, tumor-associated macrophages, defective dendritic cells, tumor-associated fibroblasts, tumor-associated neutrophils, adipocytes and vascular endothelial cells along with pericytes play their role in facilitation of angiogenesis, tumor growth and malignancy. The extracellular matrix of TME is dense and contains growth factors for angiogenesis, chemokines and provide elasticity to tissue. To provide sustenance to rapidly dividing cells, the most important metabolic adaptation in tumor cells is Warburg effect (Arneth, 2019; Dong et al., 2020).

**Drug Delivery Systems**

Living cells are normally 10 µm transversely, their parts are in submicron size realm and proteins are even more small, in nanometers. Therefore, the use of nanoparticles as drug delivery system and probes allows to reach out cellular machinery more specifically without causing excessive disturbance. Nanoparticles' accumulation in tumor cells is based on particles size, surface, half-life in cellular environment and level of angiogenesis of tumor site. Optimization of therapeutic efficacy and applications of nanoparticles for cancer therapy is highly dependent on their interaction within tumor cells. Cellular internalization of nanoparticle achieved by receptors mediated entry, endocytosis, and/or direct penetration through cell membrane (passive diffusion) (figure.1). Intracellular transfer of nanoparticles carried through endosomes, lysosomes and cytoplasm of tumor cells to access their targets to release encapsulated anti-cancer therapeutics through diffusion, degradation or stimuli response. Nanoparticles can provoke many biological responses in tumor cells, manipulating cellular signaling pathways, gene expression, and cellular functions. Targeted drug delivery systems based on nanoparticles provide many advantages such as reduction of toxicity of healthy cells and enhanced cellular drug uptake at tumor sites (Gao et al., 2014).

**Passive targeting drug delivery**

The passive targeting strategy described as drug deposition in tumor cells through enhanced vascular permeability and retention effect (EPR). Vasculature of tumor has distorted epithelium, damaged lymphatic drains with a reduced acceptance of interstitial fluid unlike normal vasculature. Passive targeting takes benefit of the intrinsic size of nanoparticles and the exceptional properties of tumor vasculature. Inflammation can increase intercellular gaps in vasculature endothelium by 1 µm aiding drug carrying nanoparticles abundantly. Nanoparticle can also enter cells through fusion merging their layer with tumor cells' membranes and/or by lipid exchange mechanism by exchanging bilayer material with cell membranes. These mechanisms can activate immune system compromising the efficacy of therapeutics. Nanoparticles-based drug delivery systems through enhanced vascular permeability and retention effect involving polymeric micelle-based nanoparticles,

conjugated nanoparticles or other nano-formulation reportedly aid in drug targeted delivery, drop tumor growth and proliferation, reduced cancer resistant protein overexpression, modifies DNA methylation profiles resulting in sensitivity development in resistant cells towards anti-cancer therapies. High heterogeneity of EPR effect due to different in pore sizes distribution can result in dissimilar drug delivery and insufficient accumulation of therapeutics in the tumor cells. There is also a drug outflow induced due to higher osmotic pressure in interstitium leading to redistribution of drug in cancer cells (Yanar et al., 2023).

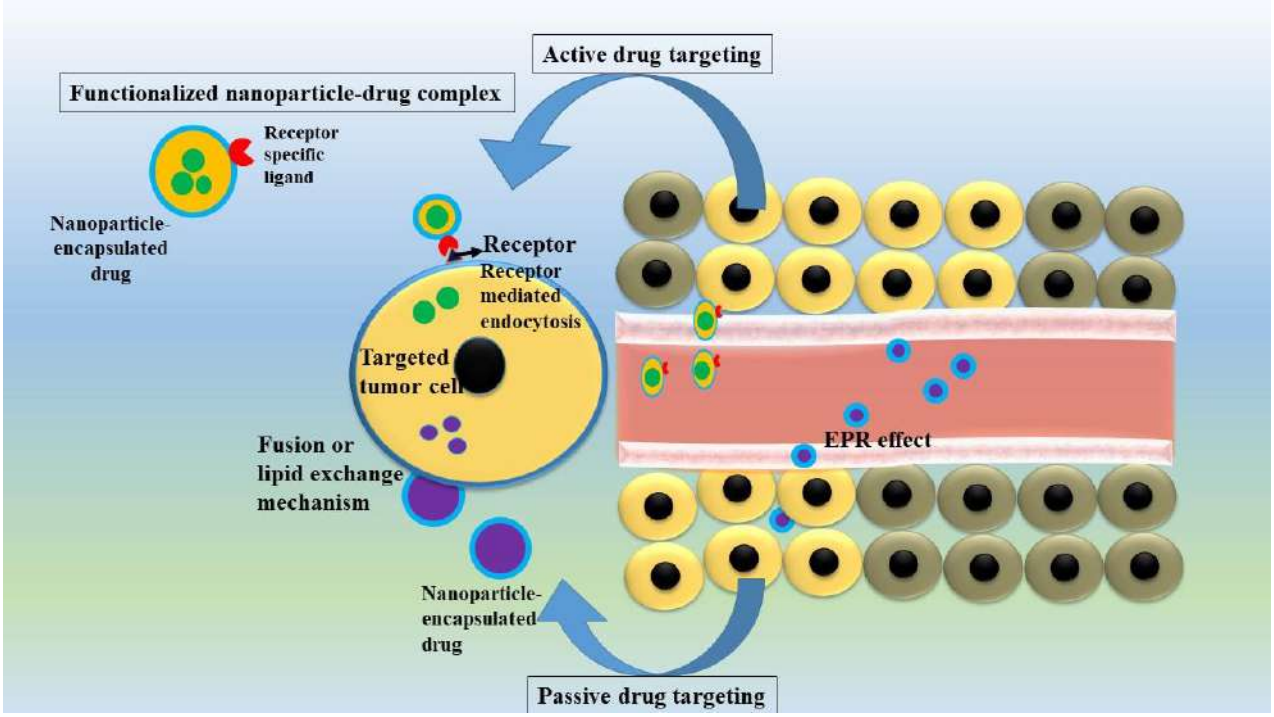


**Fig. 1:** Active and passive drug targeting

**Active Targeting drug delivery**

Active targeting can alternatively surmount limitations of passive drug delivery. Active targeting characteristically includes functionalizing nanoparticle surface that allow their attachment to target cell receptors and to penetrate biological membranes more efficiently. Active targeting decreases unsought side-effects of drugs, while elevating therapeutic efficacy at higher doses (Byrne et al., 2008; Yanar et al., 2023). Functionalized nanoparticles have three layered structure, core nanoparticle covered with a shell embellished with polymers, ions or small molecules and then the outer surface decorated with various ligands such as peptides, antibodies, proteins, aptamers, polymers or micro-molecules. Ligands have reactive groups on both ends, one designed to attach ligand to the surface of nanoparticle and other to bind various units such as antibodies, according to the required function. Functionalization of nanoparticles aids in in-vivo and in-vitro stability of nanoparticles, enhance their targeting specificity, reduce their toxicity, elevating their cellular uptake, improve their bioavailability and avoid drug resistance. Addition of recognition moieties to nano-complex be achieved by two strategies; ligand exchange and conjugation. Ligand exchange is based on ligand replacement with specific biomolecule inducing structural transformation of functionalized nanoparticle while conjugation is based on carboxylic acid activated amide bond formation between ligand and specific biomolecule. Conjugation is considered as method of choice in nanomedicine. Selection of target receptor is crucial, as it should be abundantly expressed on surface of tumor cells while least expressed on normal cells (Combes et al., 2021).

**Gene Delivery Strategies**

Therapeutic nucleic acids such as genes, oligonucleotides, miRNAs or siRNAs carried to tumor cells for silencing tumor promoting genes or reinstating the expression of tumor suppressor genes by gene modulation used for immune therapies and TME targeting. However, these nucleic acids showed lower incorporation in tumor cells due to their high surface charge, large molecular weight, poor stability and toxicity. To overcome this limitation of nano systems are developed, such as designed nanocarriers for shRNA molecules, antisense oligonucleotides-loaded nanoparticles and DNA nanostructure-based codelivery system (Liu et al., 2018).

**Stimuli-sensitive Nanomaterials Strategy**

Stimuli-sensitive nanomaterials employed for targeted drug release due to changes in external or endogenous conditions. Drug delivered and deposited in tumor cells passively through EPR effect. After reaching the tumor site

nanoparticle activated by stimulating factors and release encapsulated therapeutics. According to the physicochemical properties of nanomaterials, they can be categorized into three categories (Li et al., 2019):

a) Endogenic-stimuli (e.g. pH, enzyme and redox reactions) responsive materials
b) Exogenic- stimuli (e.g. temperature, light, ultrasound and magnetic field) responsive materials
c) Multi-stimuli responsive materials

**Nano-Oncology in Strengthening of Standard Anti-Cancer Procedures**

Photodynamic cancer therapy is grounded on the demolition of the tumor cells laser engendered atomic oxygen. Special dyes used for generation of atomic oxygen are absorbed more by the cancer cells but their residues can move into skin and eyes causing daylight sensitivity in patients. Hydrophobic version of photosensitizers packed inside porous nanoparticles to avoid spread of dye to other body parts without altering their oxygen generating property. Photothermal therapy is based on near-infrared light induced heat in tumor cells followed by cell death. Nanoparticles with localized surface plasmon resonance are of greater use in photothermal therapy such as gold nanoparticles or photostable micelles. Nanoparticles used as multifunctional system reportedly showed more specificity towards tumor cells, reduced harm to the normal cells and enhanced efficacy of therapy. Radiosensitization, as an alternative therapy for malignant tumors, is challenging. Nanoparticles with high atomic numbers can act as radiosensitizers when interact with ionizing radiations enhancing the efficacy of therapy without mutilating surrounded healthy tissues. An increased production of reactive oxygen species (ROS) and photoelectric and Compton effect leads to secondary electrons emission, enhancing the therapy effects (Piktel et al., 2016). Hyperthermia, an anti- cancer therapy, displayed difficulty in uniform heating, specifically in tumor region, without harming normal tissue. Magnetic nanoparticles can induce intracellular hyperthermia by generating heat due to their magnetic field and electrostatic effect. Along with the site destruction of tumor cells their induced hyperthermia can initiate immune response and result in acquiring long term immunity (TS et al., 2020).

**Nano-Oncology and Chemotherapeutics Resistance**:

Resistance to chemotherapeutic drugs by tumor cells is a challenging and involves several mechanisms. These may include physiological or non-cellular based resistance and cellular resistance. Non-cellular drug resistance caused due to inadequately vascularized tumor sites or physiological barriers. These mechanisms will reduce cytotoxicity of tumor cells caused by drugs. Cellular drug resistance caused by overexpression of drug efflux pump, activation of detoxification systems followed by enhanced drug outflow, drug- resistance associated proteins, interference in apoptotic mechanism and reduce drug uptake due to activated surface transporters. Drug efflux pump mediated drug resistance is more significant as they have the function to remove drug from tumor cells leading to enhanced capacity of DNA repair and decreased cell death. Reportedly, functionalized nanoparticles can enter cells by endocytosis, bypassing efflux pumps. Nanoparticles can aid in higher accumulation of drug in tumor cells or tissues reducing the drug outflow. Several nano-based drugs induced high level of cytotoxicity in tumor cells inhibiting mitosis, angiogenesis and proliferation than free chemotherapeutic drugs overcoming drug resistance. Polymers, reportedly, can inhibit P-gp pumps leading to improved permeability of resistant tumors cells for drugs by incorporating in cell membranes and influencing intracellular mechanisms such as ATP synthesis. Silencing drug resisting genes, nano formulations can treat resistant tumors by reducing expression of P-gp and increasing mitotic arrest in tumor cells (Piktel et al., 2016).

**Nano-oncology in Immunotherapy**

Immunotherapy eliminates tumor cells by a cancer–immunity cycle. Apoptosis leads to release of tumor cell antigens that are then picked up by antigen presenting cells such as dendritic cells and migrate to lymph nodes where they prime and activate immature T cells into cytotoxic T lymphocytes initiating and modulating adaptive immunity. Activated T cells detect tumor cells and induce apoptosis resulting in release of tumor antigens from apoptotic cells. Nanoparticles can overcome limitations of cancer immunotherapies by assisting at different stages (Debele et al., 2020; Jia et al., 2017; Park et al., 2018; Phan et al., 2003).

**a) Tumor Antigens Delivery**

Tumor antigens' efficient delivery to antigen presenting cells and lymph nodes is of great importance for inducing immune response. Tumor antigens can be categorized as tumor associated antigens (TAA) and tumor specific antigen (TSA). TSA are solely expressed on tumor cells while TAA expressing on normal cells also, leading to autoimmune reaction when used as immunotherapeutic targets. TSA can be utilized as targets of choice but they are prone to enzymatic degradation and low immunogenic.

Consequently, nanoparticles have been widely studied as delivery intermediaries for protected and selective transfer of tumor antigens to lymph nodes and antigen presenting cells. Nanoparticles use for immunotherapy depends upon their size, surface charge, morphology and water solubility. Reportedly, nanoparticles, such as polymers, having hydrophobic domain can easily activate immune cells. Nanoparticles smaller than 5 nm may filter out while circulation and particle greater than 100 nm may get entrapped in extracellular matrix. Functionalized nanoparticles can deliver more selectively to the lymph nodes. Non-spherical nanoparticles, have higher aspect ratios, possess extended margination, high circulation time and higher penetration in tumor tissues. For surface charge, anionic nanoparticles induce higher immune response but have less

tissue permeability due to their immobilization in cationic extracellular matrix, inducing hemolysis and platelets aggregation leading to early antigen release interfering with cellular uptake and antigen delivery. It is more convenient for dendritic cells to pick cationic nanoparticles than anionic or neutral nanoparticles.

**b) Adjuvant delivery**

Adjuvants are molecules that enhance immunogenicity when internalized in antigen presenting cells along with tumor antigens. Adjuvants common for cancer immunotherapy are 3-O-desacyl-4′-monophosphoryl lipid A, lipopolysaccharide, CpG oligodeoxynucleotides, polyinosinic: polycytidylic acid, and stimulator of IFN genes agonists. Nanoparticles based strategies, such as "tumosomes", are designed for adjuvants effective and specific delivery in to the cytoplasm of antigen presenting cells through endocytosis and induce strong immune response.

**c) Tumor microenvironment modulation**

Tumor associated macrophages and myeloid-derived suppressor cells inhibit anti-tumor immune responses by inhibiting immune cells stimulation, maturation, and differentiation. They can overexpress immunoregulatory cytokines, activate Tregs and avoid immune surveillance. Tregs, can inhibit anti-tumor immune cells activity and dwindle immune response in TME.

Engineered nanoparticles can be used to target and eliminate Tregs, kill tumor associated macrophages and/or modulating their polarization, inhibit signaling pathways of immunoregulatory cells using encapsulated inhibitors in lipid-based nanoparticles through active or passive delivery in TME leading to tumor growth suppression and enhanced immune response with least side effects.

Developed nano-systems can specifically deliver nanoparticle-encapsulated angiogenesis inhibiting therapeutics such as epidermal growth factor receptor inhibiting erlotinib and doxorubicin by passive delivery. Nanoparticles loaded with tumor vasculature disrupting agents can cause apoptosis of endothelial cells. Many studies have directed towards role of nanoparticles to mitigate the hypoxic state of TME when used in combination with other anti-tumor therapies by carrying agents that can produce oxygen after penetrating tumor sites. Other than surgical removal, function of tumor draining lymph nodes functions can be modulated using nanoparticles. Fibroblasts overexpressed in extracellular matrix of TME and play a vital role in tumor proliferation and angiogenesis. Nanoparticles, such as nanoceria and iron oxide, for targeting, inhibiting activity and/or destruction of tumor associated fibroblasts have been studied (Poilil Surendran et al., 2018).

**Anticancer Vaccines**

The cancer vaccines which transport antigens to increase immunity against tumor gained high attention recently. Nanoparticles as anticancer vaccines for tumor therapy are extensively researched. Modified nanoparticles with tumor antigens targeting tumor draining lymph nodes exhibited promising results. However, the obtainable tumor antigens are limited for most tumors. Adjuvants are required to attain desirable results of vaccines. A nanoplatform "artificial necroptotic cancer cell" and melittin-lipid nanoparticle are a few examples of utilization of nanoparticles in cancer immunotherapy (Yang et al., 2021).

**Approved Nano-drugs for Cancer Therapy**

Most of nanodrugs are under pre-clinical and clinical trials for their approved clinical use. Only a few nanodrugs have been approved by the *Food and Drug Administration* (FDA) and *European Medicines Agency (EMA)* for clinical use *(*Table.1).

**Table 1:** Approved nano-drugs for cancer therapy (adapted from (Rodríguez et al., 2022; Wicki et al., 2015))

| Nanodrug | Formulation | Therapy |
|---|---|---|
| Doxil (Janssen, Ortho Biotech, Baxter) Caelyx (Schering-Plough) | Doxorubicin HCl PEGylated liposome | Systemic Chemotherapy |
| DepoCyt (Sigma-Tau) | Liposomal cytarabine | Systemic Chemotherapy |
| Marqibo (Spectrum) | Liposomal vincristine | Systemic Chemotherapy |
| Onivyde (Ipsen, Merrimack) | Liposomal irinotecan | Systemic Chemotherapy |
| DaunoXome (Galen) | non-pegylated liposomal Citrate salt of daunorubicin | Systemic Chemotherapy |
| Myocet (Teva UK) | Non-PEGylated Liposomal doxorubicin and anthracycline | Systemic Chemotherapy |
| Mepact (Millenium) | Liposomal mifamurtide | Systemic Chemotherapy |

| | | |
|---|---|---|
| Ameluz (Biofrontera Bioscience GmbH) | 5-aminolevulinic acid (nanoemulsion) | Photodynamic therapy |
| Vyxeos (Jazz Pharmaceuticals) | Liposomal daunorubicin and cytarabine | Systemic Chemotherapy |
| Eligard (Tolmar) | PLGA , Leuprolide acetate | Hormonal Chemotherapy |
| Oncaspar (Les Laboratoires Servier, Baxalta U.S.) | L-asparaginase with polyethylene glycol | Systemic Chemotherapy |
| Abraxane (American Biosciencem, Inc., Celgene) | Albumin-bound paclitaxel | Systemic Chemotherapy |
| Ontak (Eisai, Les laboratoires Servier) | Denileukin diftitox | Immunotherapy based Chemotherapy |
| Kadcyla (Roche Genentech) | Trastuzumab, covalently linked to DM1 (or Emtansine) | Adjuvant Therapy |
| Pazenir (Ratiopharm GmbH) | Albumin bound Paclitaxel | Chemotherapy |
| NanoTherm (Magforce) | Superparamagnetic iron oxide nanoparticles coated with amino silane | Thermal ablation induced by magnetic field |
| Hensify (NBTXR3) (Nanobiotix) | Hafnium oxide nanoparticle | Radiotherapy |
| Provenge (Sipuleucel-T) (Dendreon Pharmaceuticals) | activated autologous $CD54^{+}$ cells with prostate acid phosphatase antigen- granulocyte-macrophage-colony stimulating factor | Immunotherapy (vaccine prostate cancer) |

**Future Perspectives of Nano-oncology**

Nanomedicine helped to settle the toxic effects and efficacy of therapies. Nano-based cancer therapy holds huge potential for evolving precision medicine. Nanoparticles deliver chances for designing and modification of properties that are not likely with other forms of therapeutic drugs. Future nanoparticle-based therapies are anticipated to incorporate manifold functionalities like sensitive tumor imaging, targeting and treatment, into a solitary nanosystem. Advances in development of theranostic nanoparticles is a major future related prospect for nanooncology. These multifunctional nanoparticles can undergo therapy and imaging simultaneously, allowing non-invasive real-time inspection of tumor progression, pharmacodynamics and pharmacokinetics of nanoparticles, and therapy responsiveness at cellular level (Kashyap et al., 2023).

Development of green nanotechnology possess high potential in overcoming toxicity of some nanoparticles. This approach involves incorporation of plants and microorganisms in synthesis of nanoparticles to obtain eco- friendly, less toxic and well optimized nanoparticles (Khoobchandani et al., 2020). Recent trials on intratumoral administration of nano- based therapeutics supported constant drug release and tumor restricted therapy. Along with enhanced penetration of therapeutic in tumor, this strategy can reduce dose rate, systemic toxicity and cost of therapy. Local delivery of anti-tumor therapeutics is accomplished with minimally invasive intravascular catheters or biopsy needles aided by imaging devices (Park et al., 2018; Yun et al., 2023). Advances in development of stimuli- responsive nanoparticles that are able to respond to changes in TME, can significantly influence the anti- tumor therapy response. Nano-oncology proposes innovative expositions for the development of cancer vaccines, proficient enough to induce vigorous antitumor immune responses. Advances in precision medicine will initiate the development of personalized nanotherapeutics according to the genetic and molecular properties of various tumors. Active targeting and functionalization of nanoparticles impacting significantly for tumor-targeted therapy. Advances in development of nano-based highly sensitive and specific diagnostic tools for early tumor detection may allow timely therapy, better prognosis and survival. Nanotechnology based progress in genomic profiling, identification of biomarkers, and sensitive imaging of tumors can aid in improvement of specificity and reduce toxicity of nano-based tumor therapies.

**Challenges in Nano-oncology**

Although advances in nano-oncology undeniably provided many novel therapeutic options for cancer treatment but only a limited number of nanomaterials are clinically approved. There are several considerable challenges in the way of designing and approval of nano-based cancer therapies. Major challenge involves cytotoxicity, genotoxicity, incapacitating biological barriers and difficulty in optimization of internalization, accumulation and pharmacokinetics properties of nano systems at target sites. Successful clinical translation of nano-based anti-cancer therapies are highly dependent on safety evaluation, manufacturing standardization and scalability, designing of clinical trial, identification of reliable biomarkers, comprehensive preclinical studies, physiological variability of recruited patients, cost effectiveness, ethical considerations and meeting requirements for approval from regulatory authorities. This is a complex and challenging process (Rodríguez et al., 2022). Potential toxicity of nanoparticles due to their accumulation in healthy cells or tissues is one of the major concerns for oncologists (Akçan et al., 2020). Physiochemical properties of nanoparticles highly influence their stability, bioavailability, biodistribution, half-life, excretion time and circulation time. Therefore, it is crucial to identify the exact

properties of nanoparticles that allow the maximum acceptance and deposition of therapeutics in the targeted tumor tissues with least toxicity. Acidic pH environments due to high intracellular lactic acid concentration resulted from glycolysis in TME can damage stability of nanoparticle influencing therapeutic efficacy (Bogdanov et al., 2022; Ghaemi et al., 2023). Tumor genome is fundamentally instable; therefore, tumorigenesis majorly occurs in a random manner creating a heterogeneous TME with heterogenous immunological components spatio-temporally. During therapy, tumor cells evolve gradually due to survival pressure leading to intensified complexity of tumor genome and therapy resistance. Although active targeting strategies aided in overcoming these challenges and improved targeting selectivity and specificity but heterogeneity of tumor, abnormal tumor blood vessels with EPR effect, compact extracellular matrix, high pressure of intersitial fluid of TME and interaction of nanoparticles with immunological components such as phagocytosis remain challenging hurdles during cancer therapy (Ge et al., 2022; Paresishvili & Kakabadze, 2023). Therapeutic tactics should address carcinogenic target, characteristic immune checkpoints and tumor heterogeneity for effective and prolong therapeutic effects.

**Conclusion**

Nano-oncology characterizes a paradigm modification in cancer diagnostics, therapy and management. contributing innovative solutions to surmount the limitations of conventional therapies. Recent advances in nanomaterials based targeted drug delivery systems, imaging agents, tumor mapping techniques, immunotherapy, and theranostic usage of nanomaterials have boosted purpose of nano-oncology. Advances in nano-oncology opened the doors for personalized cancer medicine, precise and early tumor diagnosis and effective cancer therapy and prevention. However, nanotoxicity, tumor heterogeneity and clinical translation are major challenges required to be addressed with standardized pre-clinical studies. Nano-oncology evidently holds the hope for imminent effective cancer diagnosis and ultimate cure for cancer.

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Chapter 42

# Nanoparticles in Environmental Epidemiology: Effects on Human Health

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**ABSTRACT**

Nanoparticles (NPs) are becoming more and more significant every day because of their profound effect on human health. The ability of NPs levels to differentiate between natural and artificial causes of air pollution makes them an essential indicator. Due to their ultrafine size, which enables them to stay suspended in the environment for prolonged periods of time, they can cause a range of health issues and move farther. Indoor and outdoor conditions can both cause respiratory and cardiovascular diseases caused by NPs. Exposure to nicotine products at work, home, and through passive smoking has been associated with side effects such as dyspnea, thin septum and elevated levels of interleukin protein and tumor necrosis factor (TNF-α), which can lead to tumor growth in the exposed population. This comprehensive volume compiles information on the origin, exposure and impacts of NPs on numerous organ systems. For researchers exploring air nanoparticles in the fields of epidemiology, this chapter provides background information and scientific data.

**KEYWORDS**
Nanoparticles, Tumor necrosis factor,

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## INTRODUCTION

NPs, which have diameters ranging from 1 to 100 nm, are one of the different sizes of particles that can be found in the environment. Particles in the environment that range in diameter from 0.1 to 10 µm stay there for approximately one week. The only mechanisms that can possibly remove small particles from the mixture are coagulation and diffusion; settling can remove larger particulate debris.Furthermore, the main reasons why NPs are difficult to remove from the atmosphere and represent a risk to human health are their fine size and extended atmospheric retention duration (Sonwani et al., 2021). In addition to being created in the bodies of insects, plants and people, nanoparticles are also released via combustion processes, forest fires, automobile exhaust, and industrial emissions (Jeevanandam et al., 2018). The high rates of urbanization, industrialization, vehicle emissions, extreme events (such as dust storms, volcanic eruptions, forest fires, etc.) and episodic events (like fireworks and crop residue burning) in Asia contribute to the region's high concentration of nanoparticles in the atmosphere (Saxena et al., 2020 and Sonwani et al., 2001).

Titanium dioxide nanoparticles, or TiO2 NPs, are the most commonly produced nanomaterial and are used in personal hygiene products, food additives, pigments and photocatalysis. These nanoparticles represent the cutting edge of a rapidly expanding discipline of nanotechnology. Titanium dioxide (TiO2) is the most often utilized and longest-produced of all these nanomaterials. These TiO2 nanoparticles are extensively utilized in the commercial sector, particularly in the cosmetics industry. High consumption in this method has made the effects of human population toxicity worse. Numerous investigations have demonstrated that the lungs, heart, liver, spleen, kidneys, alimentary canal and heart all gathered TiO2 NPs following oral exposure or inhalation.

Furthermore, they disrupt the equilibrium of glucose and lipids in mice and rats. TiO2 nanoparticles are mostly harmful because they induce oxidative stress, which can lead to immunological reactions, genotoxicity, inflammation, and cell damage. Degradation type and intensity are significantly influenced by the physical and chemical properties of TiO2

nanoparticles, which regulate their reactivity and bioavailability. According to research, TiO2 NPs have the ability to break DNA strands and harm chromosomes. The consequences of genotoxicity are influenced by a number of factors, including exposure route, size and change of the particle surface. The majority of these symptoms can be the consequence of a very high TiO2 NP dosage. Although more TiO2 NPs are being produced and employed, epidemiological data are still scarce (Shabbir et al., 2021). The potential health harm that the increasing amount of nanoparticles (NPs) in our environment may pose to human health must be investigated as soon as possible. Thus, more research is required to evaluate various biological endpoints and use various human cell models as targets.

**Source of Nanoparticles**

Natural and manmade processes are the two potential sources of nanoparticles in the atmosphere.

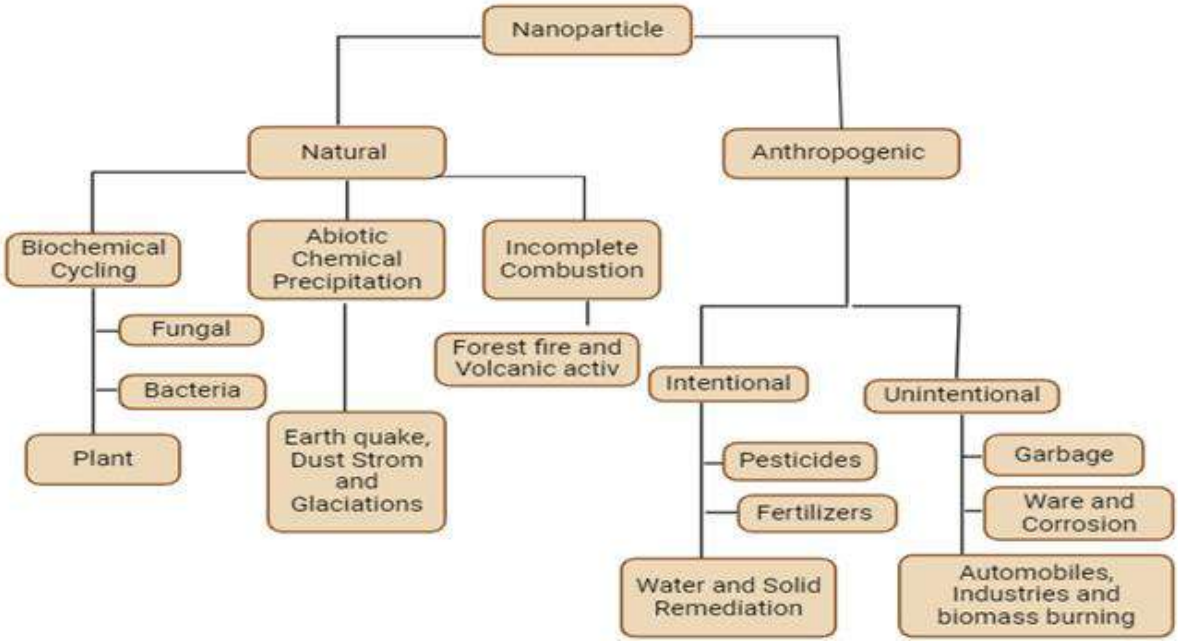


**Fig. 1:** Sources of nanoparticle emissions into the environment, both man-made and natural (adapted from Buzea and Pacheco, 2017).

**Natural Source**

About 10.5% of the aerosols in the atmosphere are produced by human activities; the remaining 90% are created by nature (Pipal et al., 2014; Jeevanandam et al., 2018; Sonwani and Saxena, 2021). In nature, nanoparticles are everywhere and originate from a multitude of sources, including sea spray, forest fires, volcanic eruptions, landslides, dust storms and biogenic emissions. Because of their tendency to react with air particles and clouds, organic chemicals make up a significant fraction of atmospheric nanoparticles.

Nitrogen oxides, volatile organic compounds, and primary organic aerosols are abundant in ambient air due to both natural and anthropogenic sources. These substances eventually combine to form secondary organic aerosols, or SOA (Tiwari and Saxena, 2021). Numerous studies (Sonwani et al., 2016) estimate that 90% of SOA generated globally is produced by biogenic volatile organic compounds (bVOCs).

Location-specific factors, such as the kinds of local sources and their relative contributions, greatly influence the chemical composition of nanoparticles (NPs). The roles of NPs in varying ambient atmospheres have not been extensively researched. According to a Pittsburgh study, organic carbon (OC) and ammonium and sulfate salts, which make up 45–55% and 35–40% of NPs, respectively, are the main components of NPs. Furthermore, incomplete burning and geological origins make up the other main atmospheric causes of nanoparticle production.The main combustion-generated sources are forest fires and volcanic eruptions, while the main geological sources are earthquakes, glaciers, dust storms and volcanic eruptions (Strambeanu et al., 2015).

**Human-based Source**

In comparison to natural sources, anthropogenic sources of nanoparticles are found primarily in urban areas. Intentional and inadvertent anthropogenic sources are distinguished. Burning biomass, incinerating non-biodegradable garbage, and incomplete combustion from autos and factories are examples of unintentional sources. One deliberate source of NPs is the application of pesticides and fertilizers, which generates large amounts of them. Non-primary and secondary sources are another division of NPs based on origin. Transportation-related activities, industrial emissions,

resource mining, and energy generation are examples of primary sources. Both stationary and mobile primary sources are possible. Stationary sources of emissions include mining operations, thermal power plants, and the chemical and metallurgical sectors. The largest source of NPs is thermal power plants, especially in metropolises and semi-urban/rural areas where there are more of them than anywhere else (Sonwani et al., 2021). The bulk of the sources of mobility, according to (Strambeanu et al., 2015), include engines, extra-atmospheric rocket launches, automobiles, ships, submarines, airplanes, and engines.

The sharp increase in the number of cars on the road is one of the main causes of air pollution. In 2002, there were more than one billion cars on the road worldwide, and the number has only increased since then. Carbon monoxide (CO), hydrocarbons (HCs), nitrogen oxides (NOx), sulfur oxides (SOx), particulate matter (PM), volatile organic compounds (VOCs) and their secondary consequences are the main components of exhaust, according to Banerjee and Christian (2018). The second-largest sources of anthropogenic NPs emissions into the atmosphere are the mining and construction sectors. Indirect processes including decantation, sedimentation, and flotation can also produce nanoparticles (NPs); direct production of NPs can occur via surface mining and excavation through mine shafts (Strambeanu et al., 2015). Meteorology and demolition methods both have an impact on the amount of NPs in the atmosphere. In addition to dust, one can find lead, glass, wood and other dangerous particles at demolition sites, as well as respirable asbestos fibers. These particles can travel quite far into the atmosphere and sometimes they form dust clouds that can reach several kilometers, affecting nearby areas (Kumar et al., 2013).

**Movement of NPs Across Borders**

An additional significant factor in enhancing the pollution load and atmospheric concentration of particulates in a given area is the transboundary migration of air pollutants. Since air mass migration from desert and ocean areas includes a range of minerals and salts in addition to tiny particles, it has a considerable impact on the quality of the air in remote areas (Sonwani and Saxena, 2021). For instance, seasonal disturbances are brought to the Caribbean basin by the transportation of mineral dust from Africa (Buzea and Pacheco, 2017). The forest fires in 1997 and the volcanic eruptions in Iceland in 2011 caused comparable environmental destruction in Asia, especially in Singapore and Malaysia.Numerous writers from all over the world, especially in South Asia, have reported on the transportation of fine particle emissions from anthropogenic sources (Saxena et al-2020). Roughly 70–80 million tons of crop residue are burned each year, contributing significantly to air pollution and aggravating respiratory disorders. In October and November, daily newspapers in Northern India carried stories on the frequent occurrences of dense clouds of smog caused by farmers burning agricultural waste, which reduced visibility and raised the Air Quality Index (AQI) to an extreme level. In pre-monsoon Asia (March to early June), temperatures and wind speeds are higher than in other seasons, which makes dust storms more frequent (Badarinath et al., 2009).

**Table 1:** Indicates Effects of nano-particles on various organ of body

| S. No | Biological System | Target Organ | Effect of Nanoparticles | Reference |
|---|---|---|---|---|
| 1 | Neural System | Epithelial lining and Brain | DNA damage, apoptosis And hormonal imbalance | Valdiglesias et al., 2013; Pujalté et al., 2011 |
| 2 | Endocrine System | Epithelial lining, Thyroid and Hormone Receptors | Oxidative Stress, Apoptosis, Overproduction of T3 hormone and blocking of signal cascades | Jaing et al., 2019 Leso et al., 2018 |
| 3 | Respiratory System | Lungs | Inflammation, Oxidative Stress and Genotoxicity | Clif et al., 2014 Sharma et al., 2012 |
| 4 | Cardiovascular System | Heart | Increase blood pressure and Decrease Heart Rate | Yu et al., 2016 |
| 5 | Excretory System | Kidney Epithelial lining | Nephrotoxicity, DNA damage, Shrinkage of Kidney Cell and Vasoconstriction | Sramkova et al., 2019 |
| 6 | Digestive System | Stomac Intestine | Increase mucus production, Inflammation and Accumulation in lamina propria | Georgantzopoulou et al., 2015 |

**Impact of Nanoparticles on the Respiratory System and Inhalation Exposure**

When humans inhale nanoparticles, they can cause a variety of respiratory disorders. According to Esztati et al. (2004), indoor air pollution from burning home fuel is the eighth-largest risk factor for the worldwide burden of disease. The primary cause of indoor nanoparticle pollution in homes is smoke from inefficient stoves and biomass fuels, which are most frequently used in rural regions. Ignorance and customs are the main barriers preventing rural populations from switching to contemporary chulas and eco-friendly fuels. Unprocessed biomass solid fuel, which is 50 times more polluting than gas stoves, is the most popular fuel used for residential cooking in rural areas (Ravindra et al., 2019). Another frequent cause of NPs exposure is cigarette smoking. It has been observed that a single cigarette releases nearly 8.8 × 109 nanoparticles. There are few research on distinguishing the various types of particles found in cigarette smoke, perhaps due to the large concentrations of particles and their quick dilution in air. An investigation conducted in the Netherlands provided experimental evidence of the direct correlation between a greater number of NPs and a longer puff length and

higher tar concentration in a single cigarette.

NP exposure at work poses health risks that are more prevalent in poor and underdeveloped countries. Traffic officers have been found to have higher incidences of respiratory and cardiovascular conditions as a result of their prolonged exposure to vehicle emissions. In a joint study conducted in 2017 by the United Kingdom and India, various medical tests of 532 traffic cops exposed to air nanoparticles were compared with 150 office workers working interior environments. According to the findings, there were 50% higher incidences of thick sputum, joint discomfort, and dyspnea in the traffic cops (Bajaj et al., 2017).

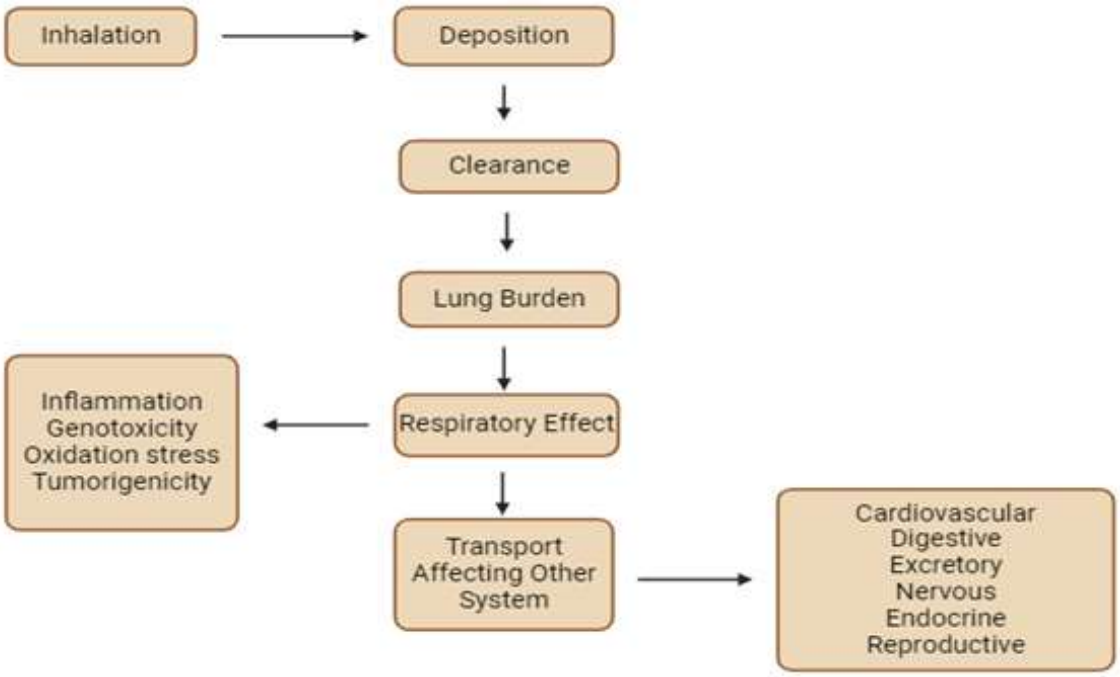


**Fig. 2:** Overview of pathway of nanoparticles from inhalation to disease site.

**Inhalation**

The human lung has an internal surface area of approximately 75–140 m2 and approximately 3,00,106 alveoli. The lungs are the main site of entry for nanoparticles into the body because of their direct contact with the external environment. According to Pacurari et al. (2016), they are also thought to be important and the primary focus of study on the effects of nanoparticles. Air enters the body through the mouth, nose, and throat, travels through the tracheobronchial tree, and then arrives in the alveoli. The Weibel bifurcating tubes model can be used to depict the conducting zone and respiratory zone, which were previously covered in depth and establish the contour of the respiratory airway. Particle transit is influenced by this structure. Furthermore, some of them can enter the cardiovascular system and other internal organs by overcoming alveolar epithelium and capillary endothelial cells (Qiao et al., 2015). Furthermore, deep into the cytoplasm and karyoplasm of the pulmonary epithelium and mesothelial cells of the lungs, electron imaging demonstrates that nanoparticle penetration can occur in both the outer and inner cellular compartments (Bakand et al., 2012).

**Deposition**

The aerodynamic dimension of the particle determines where NP will deposit in the respiratory tract (Ferreira et al., 2013). Larger diameter fibers are mainly deposited in the respiratory airways' "saddle points" where they branch off. As a result, they are unable to enter the respiratory system very far. For smaller particles, Brownian motions dominate the deposition process. Aerosol nanoparticles (NPs) measuring 20 nm or less have been found to elude most clearance systems and end up in the alveolar region of the lungs after 24 hours of inhalation, according to data gathered by energy filtering transmission electron microscopy (EFTEM).

**Lung Burden**

It is demonstrated that there are notable differences in the lung behavior of soluble and insoluble particulate matter (NPs). Once dissolved in aqueous solution, soluble nanoparticles enter the circulatory systems. By the way of macrophage phagocytosis or mucociliary escalator, on the other hand, the insoluble NPs (black carbon) are eliminated (Buzea et al., 2007). According to reports, insoluble particulate matter is responsible for increased inflammation, lung tumors, and tissue damage (Ferreira et al., 2013). Lung damage results from the insoluble particulates accumulating more quickly than the ability of macrophages to clean them. As a result, the lungs' defense mechanisms are unable to function. Additionally, it was found that the bronchoalveolar lavage fluid's shortened half-life of IL-1β and TGF-β1 induces acute lung inflammation.

Increased exposure durations, however, lead to the production of collagen, which irritates the lungs and can induce pulmonary fibrosis (Lin et al., 2014).

**Nano-Toxicity**

The pseudostratified epithelium that makes up the lung-blood stream barrier is present in human lungs. The mucous layer covers the thin columnar epithelium, bronchial epithelium (3-5 mm) and bronchiolar epithelium (0.5-1 mm), which make up the airways (Prapathawatvet et al., 2020). Since there are more than forty different cell types that make up lung tissue, a number of cell models were developed and assessed to look into the overall effects of nanoparticles on the lungs. The main focus of NP exposure is the epithelium of the respiratory system. A549, which is derived from human lung adenocarcinomas, is the most often used cell line for toxicity testing; Calu-3, 16HBE14o- and BEAS-2B cell lines are utilized as models for the bronchial barrier system.

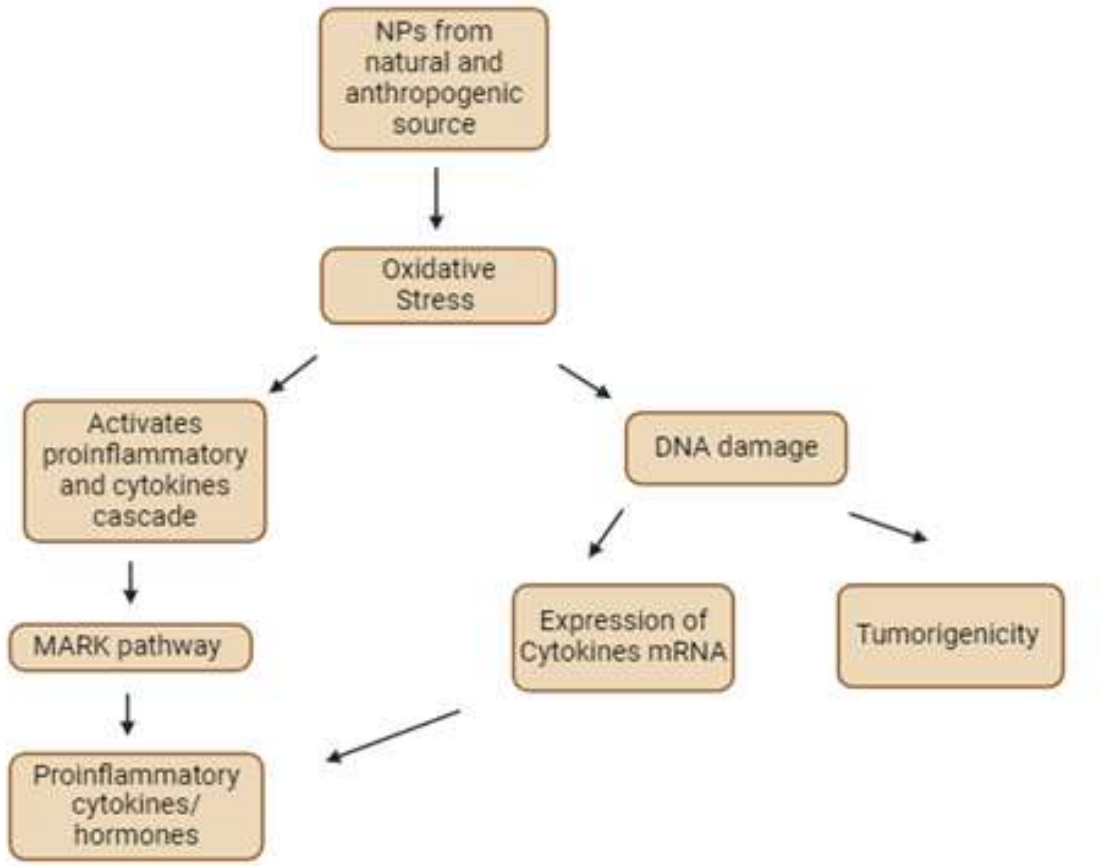


**Fig. 3:** Effects of toxicity caused by nanoparticles on human body (adapted from Li et al., 2010; Chakraborty et al., 2018).

According to Donaldson et al. (2005), the damage that nanoparticles (NPs) inflict to the respiratory system is inversely proportionate to their size, since smaller particles are more easily deposited in the distal portions of the throat. Herein, four primary categories of nanoparticle toxicological impacts are examined.

- Stress Caused by Oxidation
- Inflammation
- Genotoxicity
- The ability to grow

**Oxidative Stress**

The main effect of nanotoxicity is oxidative stress, which is brought on by an imbalance between antioxidants and free radicals in the body. According to Sharma et al. (2012), this leads to an imbalance in the respiratory system when excess free radicals with an unbalanced number of electrons accidentally react with other molecules. Reactive oxygen species (ROS) production. The production of reactive oxygen species (ROS) is a major oxidative mechanism that damages human lungs. According to Donaldson et al. (2010), NPs' involvement in the electron transport chain of the cell's mitochondria causes an excess of reactive oxygen species (ROS). According to Martin and Sarkar (2017), metallic NPs can trigger Fenton-type reactions that lead to free-radical-mediated toxicity, whereas carbon NPs are known to affect mitochondrial functions. According to Li et al. (2008), Fenton's reaction is the process that produces exceptionally reactive hydroxyl radicals (OH·)

when hydrogen peroxide (H2O2) has an enhanced potential due to specific metals' unique oxygen transfer characteristics.

The surface groups' catalytic activity determines how much ROS is created by a specific NP. About 10% of the molecules in a particle with a size of 30 nm are expressed, compared to only about 20% and 50% of the molecules in particles with a size of 10 and 3 nm, respectively (Hao and Chen, 2012). As nanoparticles are deposited, molecular oxygen-dependent superoxide anion radicals (O2−2), H2O2 and hydroxyl radicals (OH·) are produced. It has been demonstrated that BEAS-2B bronchial epithelial cells respond cytotoxically to several chemical species. Additionally, it was noted that the respiratory system releases neutrophils and macrophages, which are inflammatory phagocytes, in reaction to particulates.

**Inflammation**

There are two types of immune systems in humans: innate and adaptive. The innate immune system is the body's initial line of defense against any foreign particle that gets inside. If the foreign particle (antigen) cannot be neutralized by the innate immune system, the much more powerful and complex adaptive immune system is activated. The body's dendritic cells carry out this activation. Because they are foreign particles, nanoparticles also trigger the activation of dendritic cells, which release ROS, chemokines and cytokines and stimulate naïve T-cells and different inflammasomes. Factors that Determine Inflammation. NPs trigger an inflammatory response in the respiratory system, just like they do in other body systems (Padmanabhan and Kyriakides, 2015). The zeta potential (ξP) of a particle is a well-known indicator of its capacity to induce inflammation. The electric potential generated by the interaction of charged groups (found on a particle's surface) with the suspension medium is known as ξP. A particle with more positively charged groups on its surface will be more soluble in the acidic medium of the human body. This will enhance the particle's interaction with macrophages, leading to an inflammatory response (Schins, 2013).

The amount of white blood cells (WBCs) in the blood is another sign of inflammation in the body. An elevated WBC count above normal indicates inflammation and consequently, a reduction in immunity. Inhaled nanoparticles trigger the release of pro-inflammatory hormones when they settle in the lungs and interact with the main immune system, the alveolar macrophages. Thus, the dormant macrophages are aroused to facilitate the transport of several pro-inflammatory cytokines (IL-1 family, IL-6, IL-8, and i-CAM pro-inflammatory protein synthesis) to the affected location (Clift et al., 2011; Foldbjerg et al., 2011).

**Genotoxicity**

Inflammation or oxidative stress can produce genotoxicity indirectly, or NPs can directly interact with DNA to cause it. Genotoxicity can be separated into major and secondary categories based on various studies and the ensuing effects. Primary genotoxicity is the state in which minuscule particles penetrate the nucleus and modify DNA. There is another significant indirect process that is linked to the DNA repair cascade. Secondary genotoxicity is caused by NP-induced oxidative stress and inflammation (Zhu et al., 2013; Magdolenova et al., 2014).

DNA oxidation causes carcinogenic changes, such as adenine and guanine hydroxylation, which results in the creation of DNA adducts (Berube et al., 2007). When specific carcinogens are present in DNA, covalent changes take place that lead to DNA adducts. Particulate carcinogens can penetrate cellular membranes and the nucleus directly. Examples of such substances include asbestos and crystalline silica. According to Magdolenova et al. (2014), they then interact with and disturb the many components of the mitotic spindle's process and functionality, leading to dysfunctionalities. All biological functions, including mitosis, DNA replication and DNA transcription into mRNA, can be interfered with by these foreign particles. NPs unintentionally promote mistakes in nuclear processes indirectly by binding to the nuclear proteins responsible for DNA repair mechanisms and interfering with their function. Topoisomerases are the enzymes responsible for carrying out this repair cascade. Human topoisomerase-II alpha has been shown to bind to Carbon-60 fullerenes in the ATP-binding domain, which can interfere with the enzyme's ability to function as an enzyme (Magdolenova et al., 2014).

**Tumourigenecity**

A network of proteins in a cell called the MAPK/ERK pathway, also known as the Ras-Raf-MEK-ERK pathway, is responsible for transmitting signals from surface receptors to DNA found in the nucleus. When a signaling molecule attaches to the epidermal growth factor receptor (EGFR) receptor on the cell surface, the signal is initiated. When this receptor is in its normal state, external ligands like epidermal growth factors bind to it, phosphorylate it, activate it and trigger a series of docking proteins that eventually produce mRNA, which is then translated to build other proteins. Here, these receptors are interacting with ROS and occasionally direct NPs, and because of the altered DNA code, aberrant proteins may or may not arise. The formation of many cancers begins at a fundamental stage when one of the pathway's proteins mutates and becomes stuck in the "on" or "off" position. The body produces higher amounts of 8-hydroxy-2′-deoxyguanosine (8-OHdG) as a result of the entrance of NPs. Tumors can arise from 8-OHdG-induced G-to-T transversion mutations in important genes implicated in the development of cancer (Guo et al., 2017). As a result, the incidence of carcinogenicity, which reduces immunity, is directly correlated with an elevated level of 8-OHdG.

**Effect of NPs on Digestive System**

The digestive system, sometimes referred to as the gastrointestinal tract, is primarily composed of the oesophagus, stomach, and intestines. Food and drink are the main routes via which NP reaches the intestines. Interleukin-8 is elevated

by silver nanoparticles, and this is directly associated with inflammation and increased mucus formation (Georgantzopoulou et al., 2015). Certain nanoparticles (NPs) can disturb the layers of mucous and epithelial cells by penetrating blood vessels and avoiding the junctions between intestinal epithelial cells. That depends on how big they are.

Depending on their size, certain nanoparticles (NPs) have the ability to break through the intestinal epithelial cell junctions and cause damage to the mucus and epithelial cell layers in blood vessels. Furthermore, they eventually congregate in the intestinal lamina propria, where they impede the function of goblet cells. The rate of NP formation rises if an individual already has underlying medical disorders that cause inflammation in the intestinal regions, such as Crohn's disease or ulcerative colitis (Jones et al., 2015). As a result, toxicity levels rise, increasing the incidence of carcinomas and colon cancer.

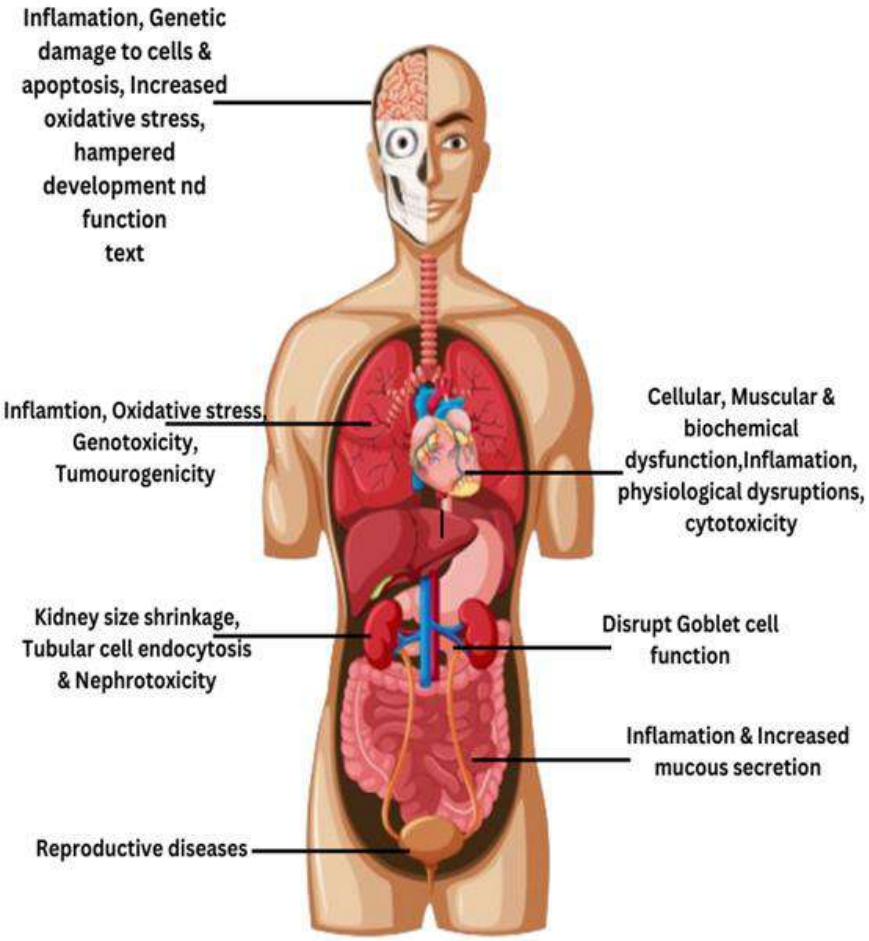


**Fig. 4:** Impact of Nanoparticles on various systems of the human body.

**Impact of Nanoparticles on the Cardiovascular System**

NPs can enter our bodies through the mouth or respiratory system and travel through the digestive tract before entering the circulatory system. These can significantly affect the way the system usually operates. According to Yu et al. (2016), some of the early effects of NPs on the cardiovascular system include changed vascular tone and dysfunction, a lowered heart rate, and elevated blood pressure. Nanoparticles (NPs) can affect our bodies in a variety of ways, depending on their concentration, physical characteristics, and duration of retention. These effects can include angiogenic or antiangiogenic, vasodilation or constriction, pro-oxidant or antioxidant, cytotoxic, apoptotic, or phagocytic (Gonzalez et al., 2016).

The two effects of silver nanoparticles (Silver NPs) on blood composition are antagonistic: angiogenesis (the formation of new blood vessels) and membrane permeability. These days, pacemakers, medications and related antibodies all contain silver nanoparticles. Garcia and associates (2016). TiO2 NPs can accumulate in the heart after prolonged exposure and result in inflammation, cellular necrosis, sparse cardiac muscle fibers and cardiac biochemical malfunction. In addition to cardiac ischemia damage and atrioventricular occlusion, a study on SiO2 NPs in aged rats showed elevated blood viscosity and Fbg levels (Yu et al., 2016).

**Impact of Nano-particles on the Excretory Organ System**

The toxicity of a particle rises with its size, according to Wang et al. (2009). Indicators of apoptosis, such as shrinkage and nuclear condensation, were observed in kidney cells treated to SiO2 nanoparticles at dosage levels of 20–100 µg/ml in this investigation on HEK293 cells, or cultured human embryonic kidney cells. Furthermore, oxidative stress activation and

ROS formation in HEK293 cells imply the possibility of nephrotoxicity for nanoparticles. Impaired nephrotoxic potential can cause cerebral epilepsy by disrupting nephron elasticity. To evaluate the cytotoxicity of NPs, Pujalté et al. (2011) additionally employed the glomerular mesangial (IP15) and epithelial proximal (HK-2) cell lines.

**Impact of Nanoparticles on the Endocrine System**

The kidney is highly vulnerable to xenobiotics and the bioaccumulation of toxins because each kidney nephron includes a network of blood capillaries that filter toxins from blood (Pujalté et al in 2011). After glomerular filtration, NPs typically gather in the proximal convoluted tubules (PCT), where tubular cells may endocytose the particles. TH1 cells treated to inorganic NPs were demonstrated to display DNA damage in addition to an acceleration of NP-induced nephrotoxicity (Sramkova et al., 2019). According to Pujalté et al. (2011), there was a similar result observed in another investigation when copper nanoparticles were introduced to the tubular cells of mice. This will lead to chromosomal anomalies as well as genetic diseases.

**Impact of Nanoparticles on the Reproductive System**

It has been established that the buildup of NPs is one of the primary causes of the present increase in cases of infertility. According to Zhang et al. (2020), sperm concentration and motility rates were found to be decreased and sperm abnormalities rates were elevated when silica NPs, which are frequently found in workplaces, were administered to rats in an experimental investigation that involved a high-fat diet. Significant sperm DNA integrity loss was seen as a result of the genotoxic effects of TiO2 NPs, which are demonstrated when they breach the blood–testis barrier and produce inflammation and cytotoxicity (Santonastaso et al., 2019).

According to one study, a subject administered MoO3 had a marked reduction in the weight of both the uterus and the right ovary, which eventually had a negative impact on reproduction (Asadi et al., 2019). Consequently, NPs play a major role in lowering birth rates and birth defects in their progeny. These conditions are frequently observed in females living in urban areas who are regularly exposed to air pollutants such particle matter (NPs).

**Conclusion**

Nanoparticle exposure can result in major respiratory and cardiovascular disorders, as is widely known from their health impacts. Epidemiological and toxicological studies have shown that nanoparticles are significantly more harmful than coarser particles because of their ultrafine size, chemical reactivity, and prolonged residence time in the environment. Furthermore, a correlation has been noted between the size of the particles and their capacity to go deep into the lungs. Nanoparticles can originate from man-made sources such as industrial emissions, forest fires, dust storms, volcanic eruptions and welding fumes, as well as natural sources like cigarette smoke and vehicle emissions. Nanoparticles (NPs) damage and delete segments of single- and double-stranded DNA and cause genetic material mutations that result in lung cancer and neoplasms. In addition to damaging the lungs, nanoparticles (NPs) also impair the cardiovascular and gastrointestinal systems, which can result in ulcers and cardiac arrest, respectively. Because of the buildup of NP, comorbid patients are constantly more vulnerable to many health issues.

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Chapter 43

# Role of Nanoparticles for Treatment of *Staphylococcus aureus*

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**ABSTRACT**

Antimicrobial resistance (AMR) is an emerging problem worldwide and is a major problem to the public health and results in increasing the health hazards, increase the treatment costs in hospitals and causes higher mortality. The effectiveness of treatment has been decreased because of the increase in the severity of the AMR. Nanoparticles (NPs) have different mechanisms of action and high penetrating power into the microbes, therefore, proved to be effective in many cases of drug resistance treatment. Such properties are also utilized in technology, biotechnology and many other branches that result in overall improving human life. Currently, NPs have been added to glass, fibers, computers, and many other technologies. There is a need for a lot of research on the mechanism of the action of NPs for treating different infections in humans and animals. Future research should also focus on the toxicity and overuse effects on health and environment. This chapter will focus on the emerging AMR, NPs size, classification, synthesis methods, mechanism of action, their antimicrobial effects and their effective treatment results against resistant microbes, especially *Staphylococcus aureus*.

**KEYWORDS**
AMR, *Staphylococcus aureus,* Nanoparticles

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## INTRODUCTION

*Staphylococcus aureus* is a significant pathogen that infects humans and leads to various clinical condition (Lowy, 1998). Infections caused by *Staphylococcus aureus* occur frequently in both community and hospital environments, posing challenges for treatment expected to the rise of drug-resistant strains like MRSA (Buck et al., 2005). Transmission usually occurs through direct contact, although certain infections may involve alternative modes of spread (Rasigade and Vandenesch, 2014). This is a Gram-positive pathogen that seems purple when it is stained with the Gram stain. It is spherical in shape (cocci) and prefers to form clusters that seem like grapes. These bacteria can develop in surroundings containing up to 10-12@ salt and generally result in colonies that are golden or yellow in color. They are capable of both aerobic and as well as anaerobic growth (facultative) and can bear heats ranging from 18-40°C. Standard tests of biochemical used for identification contain the positive results for catalase, coagulase (to differentiate (*Staphylococcus aureus* from other *Staphylococcus* species), The fermentation of mannitol and susceptibility to novobiocin (to separate from *Staphylococcus saprophyticus*) (Weiss, 2017). It's estimated that approximately half of grown people are populated by *Staphylococcus aureus*, with approximately 14-15% of people consistently carrying these pathogens in their nasal ways. Some individual, like healthcare workers and those individuals who often use the needles, sick patients, and those who have already weak immune systems, have a greater number of colonization, sometimes reaching up to 75-80%. Transmission of this pathogen can occur through direct from one person to another through contaminated objects (Tong et al., 2015). This is a concerning development because bloodstream infections caused by *Staphylococcus aureus* often lead to unfavorable outcomes, including a high risk of secondary infections such as infective endocarditis, septic arthritis, and osteomyelitis (Frimodt-Møller et al., 1997). Alongside the increasing prevalence of MRSA, there have been clinical observations of resistance to vancomycin, underscoring the necessity for the development of novel and more

effective antimicrobial agents for the treatment of *Staphylococcus aureus* bacteremia (SAB) (Howden et al., 2010). While it's common for individuals to carry *Staphylococcus aureus* on their skin and mucous membranes without experiencing harm, the opportunistic nature of this bacterium means that carriage can progress to various infections, spanning from minor soft tissue and as well as skin issues to rigid interfering conditions like bloodstream infections (SAB), infective endocarditis (IE), and meningitis (Von Eiff et al., 2001). The process by which carriage of *Staphylococcus aureus* progresses to infection is not fully understood, but it is believed to involve the breach of the skin or mucosal barrier, which can occur through factors like abrasions, surgical procedures, or the use of intravascular devices. Additionally, various host factors, including both general and local immunosuppression, play a role in this transition (Laupland et al., 2008). The intricate architecture of biofilms offers bacteria a distinct advantage for survival and virulence within the human body, contributing to the development of rigid pathological environments (Parsek and Singh, 2003). Nanotechnology has become increasingly essential across various research domains, involving the fabrication and control of nanoparticles, thereby inducing significant alterations in metal properties. This technology holds potential as a viable solution to combat the rising challenge of multidrug resistance in *S. aureus* (Wang et al., 2017). Various resources, including polymer-based and liposomal and also carriers of the nanodrugs, have been investigate, with the vectors of metallic like gold nanoparticles (NPs) being particularly attractive as core materials because of their inert and non-toxic properties (Burygin et al., 2009). Previous research has detailed the process by which metal nanoparticles, or NPs, suppress the growth of harmful microorganisms. This is how metal nanoparticles work to kill bacteria through various mechanisms, including wall of the cell damages, generation of reactive oxygen species (ROS), and damages of the cytoskeleton disrupting pathways of signaling, and inhibition of enzymes involved in membrane formation. That's why nanoparticles can serve as antibacterial medicines are effective against widespread antibiotic resistance. Specifically, in the case of urease inhibition, greater effectiveness was observed when ciprofloxacin-capped silver nanoparticles (AgNPs) or gold nanoparticles (AuNPs) were utilized (Burygin et al., 2009). Small zinc oxide nanoparticles were found to exhibit β-galactosidase inhibitory properties in a biomimetic manner, demonstrating Superior antimicrobial effect towards Methicillin-resistant *S. aureus* (MRSA) (Nisar et al., 2016).

***Staphylococcus aureus***

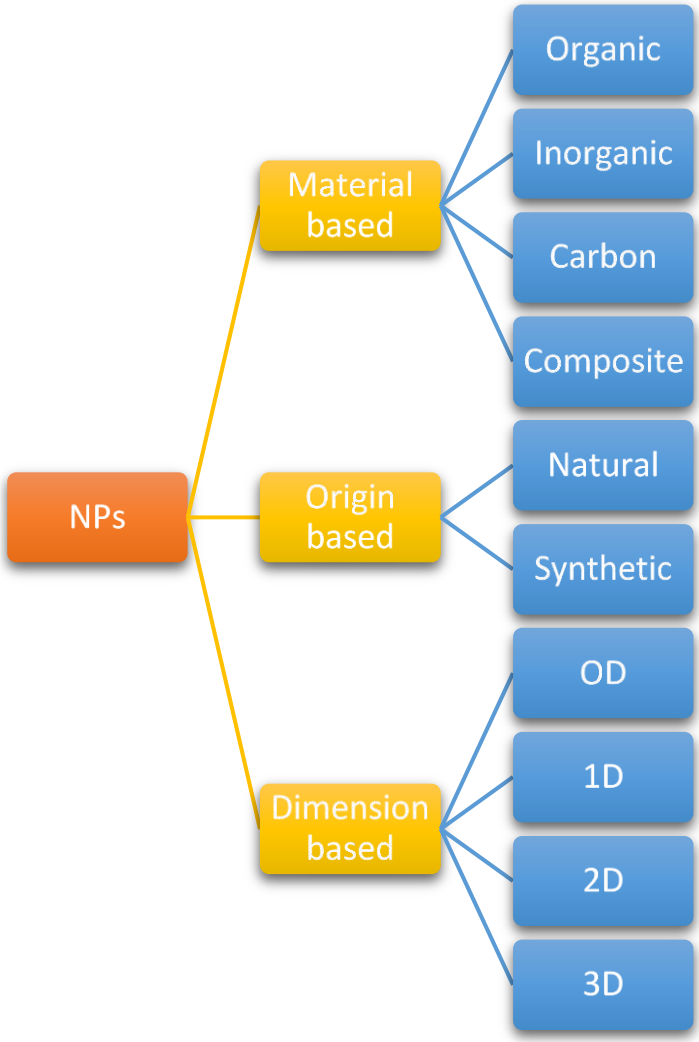
*S. aureus* is notable for its high tenor to develop resistance to many drugs, such as antibiotics. This tenor can occur through several mechanisms, depending on the specific drugs. These processes include the use of efflux pumps, which actively expel some drugs, such as antibiotics, from pathogen cells; moderation of the drugs such as antibiotic's target the protein, reducing its binding attraction; and degradation of the enzymes of the antibiotic, deliver it inefficient. The rise of resistance from instinctive mutations of genetics within bacterial populations, the resistant cells of the positive selections, and horizontal gene transfer where bacteria obtain genes that resist from other species, such as bacterial pathogens (Colon 2024). One particular strain that poses significant risks to humans is methicillin-resistant *S.aureus* (MRSA). This variant is characterized by a genetic mutation that confers resistance to methicillin, a synthetic form of penicillin used to treat staphylococcal infections that are resistant to natural penicillin derived from molds. MRSA was initially identified in the early 1960s, soon after the introduction of methicillin as a widely used antibiotic. Although methicillin is no longer commonly prescribed today, MRSA strains persistently colonize human skin, nasal passages, and can be found in blood and urine samples (Ali et al., 2024).

In recent studies involving consecutive cases with both methicillin-resistant *S. aureus* (MSSA) and methicillin-sensitive *S. aureus* (MRSA) bloodstream infections (SAB), various primary clinical foci or manifestations have been observed, showing consistent patterns across different cohorts (Laupland et al., 2008). As the epidemiology of *S. aureus* infections evolves, it is expected that the distribution of primary clinical foci in cases of *S. aureus* bloodstream infections (SAB) will also change. For instance, improvements in infection control practices and the use of central line bundles has led to a reduction in catheter-related infections, resulting in catheter-related SAB accounting for a lesser proportion of all SAB victims (Burton et al., 2009). Likewise, societies with high incidence of infections of the skin often experience higher rates of bloodstream infections (SAB) associated with prevalence of epidermal infections, including epidermis and tissue illnesses. For instance, the incidence of Methicillin-resistant *S. aureus* linked to people around them bacteremia caused by USA300 MRSA has risen with the extensive rise of USA300 MRSA SSTIs (Tattevin et al., 2012). In a single-center study involving 722-724 reports of *Staphylococcus aureus* bloodstream infections (SAB), infections were classified as either "complicated" or "uncomplicated." A complicated infection was defined as one which led to death that may be caused by it, such as CNS involvement, embolic consequences, infection at the sites of tumors, or infection that returns within a year(Fowler et al., 2003). These classifications are crucial as they influence the depth and diagnostic assessments type, period of antibiotic therapy, and complete diagnosis for patients (Fowler et al., 2003). Recent reviews have independently confirmed that prolonged fever and positive monitoring cultures of blood are associated with complicated *Staphylococcus aureus* bacteremia (SAB) and subsequently lead to lesser results (Van Hal et al., 2012). The source of illness is a significant predictor of 30-day mortality in *Staphylococcus aureus* bacteremia. Mortality rates are notably higher for cases without a specific focus of infection (21 to 48%), infectious endocarditis (25 to 59%), and lungs disorders (38 to 66%). In contrast, poorer mortality amounts are observed for catheter-related bacteremia (6 to 20%), soft tissue and skin infections (14 to 16%), and urinary tract illness (10%) (Van Hal et al., 2012).

**Nanoparticles**

The development of resistance and emerging diseases is a serious problem worldwide. In spite of increasing knowledge of pathogenesis of microbial agents and mode of action of drugs there is still morbidity and mortality are high due to microbial infection (Kolář et al., 2001). Before the use of modern chemotherapeutics in the healthcare system, inorganic chemical like silver and copper were being used since ancient times to treat the infections (Moghimi, 2005). In recent years, due to advances in the field of nanotechnology, these organic and inorganic nanosized particles showed great results in fields of industrialization, medicine, and food packaging (Gajjar et al., 2009). Nanoparticles range in size from 1-100nm in diameter (Kandeel et al., 2022). These NPs are classified into different categories. Firstly, NPs were classified on the basis of crystalline form and chemical composition, but that was unsatisfactory. Then classification on the basis of dimension was added later on (Gleiter, 2000; Hamidi et al., 2017). On the basis of material, NPs are classified into following four categories: i) Organic based NPs; ii) Inorganic based NPs; iii) Carbon based NPs; iv) Composite based NPs (Hochella et al., 2015). On the basis of origin, they are classified into the following two categories: i) Natural ones are those that are derived from air, water, soil and other living organisms; ii) Synthetic ones are those that are synthesized from physical, chemical or biological methods or combination of any two or three of these methods (DemİRcİ et al., 2018; Sharma et al., 2015). While on the basis of dimensions, they are classified into 0 dimension (D),1D,2D and 3D (Pokropivny and Skorokhod, 2007). Fig. 1 showing classification of NPs.

**Fig. 1:** Classification of NPs




NPs have many antimicrobial specific applications that were not reported until 19$^{th}$ century due to lack of cauterization techniques that colloidal silver (Ag) NPs have been used as an antimicrobial agent for more than 100 years (Nowack et al., 2011) Lea, M.C in 1889 claimed to synthesize first colloidal Ag NP of 7-9nm size and stabilized them in citrate medium (Lea, 1889). For some reasons of lack of appropriate techniques, the physiochemical properties of AgNPs had not been studied until 1969 (Frens and Overbeek, 1969) and synthesis methods were not fully known until 2009 (Dong et al., 2009). Due to discovery of antibiotics like penicillin in 1940 antimicrobial NPs (AMNPs) was not an area of interest of researchers until the resistance of antibiotics was observed (Clement and Jarrett, 1994; Chopra, 2007).

There are various methods of NPs synthesis but there are two major methods: 1) Bottom-up method. 2) Top-down method. Bottom-up method is further classified into various categories like:

**1) Bottom-up Method**

A) Gas phase synthesis: In this method, NPs are produced by the combination of gas particles on the catalyst surface. For example, Chemical vapor deposition (CVD) is a method when a thin layer of gas is deposited over the substrate surface and then product is obtained as a thin gas layer when it is heated at ambient temperature (Bhaviripudi et al., 2007).

B) Liquid phase synthesis: There are two processes which produce NPs through liquid phase that are 'Sol-gel' and 'Microwave assist'. This is limited to produce metallic oxides, but it is better than gas phase synthesis due to higher rate of production of NPs at lower temperature (Charitidis et al., 2014).

C) Biosynthesis method: This method is further classified into two classes mycosynthesis (utilization of fungi) and Phyto nanotechnology (utilization of plants) (Singh et al., 2016; Hernández-Díaz et al., 2021). These are ecofriendly methods and produce NPs in their active form and are also very cost effective (Hernández-Díaz et al., 2021).

**2) Top up Method**

This method mostly involves the mechanical and physical techniques that decreases the particle size from micro to nanoscale with the help of strong mechanical shearing forces like grinding, milling, evaporation, heating and radiations etc. (Mukhopadhyay et al., 2002; Mueller et al., 2003; Yadav et al., 2012 D'Amato et al., 2013).

**Mechanism of Action of Nanoparticles**

NPs may or may not have the same mechanism of action as that of antibiotics. But they are effective against microbes and showed marvelous results for treatment of microbial infections. Mechanisms of action of nanoparticles can be categorized into several primary mechanisms:

**1-Disruption of Cell Membrane Integrity**

Nanoparticles cause physical damage to the bacterial cell membranes through direct contact with them. Nanoparticles are positively charged and bacterial cell membranes are negatively charged, the interaction disrupts bacterial cell membranes, resulting in leakage of cell content and ultimately cell death (Le Ouay and Stellacci, 2015). Oxidative stress: Many nanoparticles produce reactive oxygen species (ROS) such as zinc oxide nanoparticles (ZnO) and silver nanoparticles (Ag). Within bacterial cells, these reactive oxygen species (ROS) can cause oxidative damage to lipids, proteins, and DNA. These ROS are included hydroxyl radicals, superoxide radicals, and hydrogen peroxide (Xiu et al., 2012).

**2-Protein Dysfunction**

Nanoparticles disrupt essential cellular processes such as metabolic pathways, DNA replication, and protein synthesis by binding with bacterial proteins, especially enzymes, and inhibit their function (Rai et al., 2009; Lara et al., 2010).

**3-DNA Damage**

Nanoparticles can lead to mutations or the inhibition of DNA transcription and replication by gaining entry to the bacterial cells and interaction with DNA, causing fragmentation or structural changes ( Morones et al., 2005; Cui et al., 2012).

**4-Biofilm Inhibition and Disruption**

Biofilms are protective covering on the surface of the bacteria. They protect the bacteria against antibacterial agents. Nanoparticles also penetrate existing biofilms, making the bacteria more susceptible to antibacterial agents by inhibiting the formation of biofilms over the bacteria (Martinez-Gutierrez et al., 2013; Goh et al., 2015).

**5-Metal Ion Release**

In an aqueous environment, some nanoparticles, particularly metal-based ones like silver. These metal-based nanoparticles release their metal ions in an aqueous environment. These metal ions disrupt the function of proteins and enzymes by interacting with thiol groups and causing the death of bacterial cells (Li et al., 2010; Marambio-Jones and Hoek, 2010).

**6-Immune System Modulation**

Some nanoparticles can boost the host immune response by activation of macrophages and other immune cells against bacterial infections, leading to increased phagocytic activity and bacterial clearance ( Pal et al., 2007; Ivask et al., 2014).

These are the main mechanisms by which NPs showed their antimicrobial activity. Different NPs may have the same mechanism of action or are different from each other.

**Types of Nanoparticles used against *Staphylococcus aureus***

There are different kinds of NPs that can be used against *Staphylococcus aureus*

**1-Silver Nanoparticles (Ag)**

They have strong antibacterial properties. They release silver ions which react with the bacterial cell membrane, DNA, and proteins, causing cellular damage and death. They can inhibit the growth and biofilm formation of *Staphylococcus aureus* (Rai et al., 2009).

**2-Gold Nanoparticles (Au)**

They can enhance the efficacy of antibiotics or other antimicrobial agents. Their work is to damage the cell membrane integrity and interfere with the metabolic processes of the bacterial cells. They have strong antibacterial activity against *S. aureus* (Saha et al., 2012).

**3-Copper Nanoparticles (Cu)**

They have strong antibacterial activity by producing reactive oxygen species (ROS) and damaging the bacterial cell membranes. They also prevent biofilm formation and effectively kill *S. aureus* (Ren et al., 2009).

**4-Zinc Oxide Nanoparticles (ZnO)**

The properties of zinc oxide nanoparticles are antibacterial and photocatalytic. Under UV light, they produce reactive oxygen species (ROS), leading to oxidative stress in bacterial cells. They are effective against both biofilm-forming and planktonic *S. aureus* (Zhang et al., 2010).

**5-Chitosan Nanoparticles**

They can damage the bacterial cell wall and cell membranes, causing the leakage of bacterial cell contents as a result of cell death. They also have mucoadhesive properties. Due to this property, they increase their activity and effectiveness. They also reduce the biofilm over the bacterial cells (Qi et al., 2004).

**6-Silica Nanoparticles**

Their function is to increase the effectiveness of antimicrobial agents. They damage the bacterial cell walls and interfere with metabolic processes (Slomberg et al., 2013). Those NPs which are tested for their antimicrobial activity and especially against *S. aureus* have been mentioned in Table 1.

**Table 1:** Summary of those nanoparticles which are used against *S. aureus* because of antimicrobial activity

| Nanoparticles | Mean size | shape | Tested bacteria | References |
|---|---|---|---|---|
| Copper (Cu) | 9nm | Quasi-sphere | *S. aureus, E. coli* | (Ruparelia et al., 2008) |
| Silver (Ag) | 13.5nm | Spherical | *S. aureus, E. coli* | (Kim et al., 2007) |
| Copper oxide | 20-95nm; 20-28.9nm | Rectangle and rod | *EMRSA MRSA S. aureus S. sepidermidis E. coli P. aeruginosa; P. aeruginosa B. subtilis S. aureus* | (Azam, Ahmed, Oves, Khan, and Memic, 2012; Ren et al., 2009) |
| Zinc oxide | 20nm | | *E. coli P. aeruginosa S. aureus B. subtilis* | (Azam, Ahmed, Oves, Khan, Habib, et al., 2012) |
| Silver-gold alloy | <200nm | Spherical | *S. aureus* | (Bahrami et al., 2014) |
| Titanium-Nickel-Copper alloy | N/A | N/A | *S. aureus E. coli* | (Li et al., 2016) |
| Platinum-Silver alloy | N/A(Azam, Ahmed, Oves, Khan, and Memic, 2012) | N/A | *S. aureus E. coli* | (Cai et al., 2017) |

**Application of Nanoparticles in the Treatment of *Staphylococcus aureus***

Materials used for nanoparticles are gold, silver, organic and inorganic, etc. They have a variety of uses, e.g. transportation of drugs to the target sites x-ray, ultrasound, treatment by photons and antiseptics. For example, hollow and perforated nanoparticles are made from silica and Ca3Po4 (Wang et al., 2006; Son et al., 2007; Tang et al., 2009). The mode of action of nanoparticles is still unknown. But one study has shown that these particles combined with lipid bilayers of microorganisms and release drugs within the cell wall or at cell membrane.NO combined with cytokines and change them to produce an antimicrobial effect by inhibiting the function of Zn metalloproteins during replication of DNA and respiration by the cell. NO also hastens the wound closure and reduces the bacterial load on the wound. Chitosan nanoparticles have anti-MRSA properties, and they have a quaternary ammonium group on the surface which is obtained by gelation of ions. Chitosan is a polysaccharides of β linked D -2-glucosamines which is a strong antibacterial agent (Crossley et al., 2009; Tang et al., 2009). Colistin is biocompatible and can easily be degraded by living organisms. Silver ions act as bacteriostatic and bactericidal, therefore, they are considered as broad spectrum antibacterial agent (Berger et al., 1976). Silver ions released potassium ions from plasma and cytoplasmic membrane of bacteria. These sites have important enzymes and DNA. Silver ions target these sites ( Miller and McCallan, 1957; Fuhrmann and Rothstein, 1968; Rayman et al., 1972; Schreurs and Rosenberg, 1982). In this way, they inhibit bacterial growth. When the growth of bacteria was stopped, Silver ions retain as a granules in the vacuole and cell wall (Brown et al., 2012). They stopped the cell division and destroyed the cell envelope and cellular content (Richards et al., 1984). These ions also cause abnormality in the structure of cytoplasmic membrane, cytoplasmic contents and outer cell layers by increasing the size of bacteria cells. They also combine with bases of nucleic acid (Yakabe et al., 1980). These also form free radical and act as antibiotic agents (Kim et al., 2007). Zinc oxide and copper oxide have antibacterial activity and are used in medical and skin coating. Zinc oxide is

used as a paint in the walls of hospitals and acts as an antibacterial agent. It also uses in the cosmetic products such as cream, lotion etc. due to its antibacterial property (Martınez-Flores et al., 2003). Gold nanoparticles combine with a phosphorous portion of DNA and inhibit the replication of DNA of bacteria. These also react with proteins having Sulphur and stop the function of enzyme. These are used against MRSA.

**Conclusion**

In this chapter firstly reviewed some introduction of *S. aureus* and nanoparticles. There are discussed some details of *S. aureus* and diseases related to this bacterium, affected organs and organs systems and increasing mortality and morbidity related to this bacterium. This chapter also highlighted the development of resistance of this bacterium to different classes of antibiotics, focusing on the emerging global problem of antimicrobial resistance (AMR). It is also mentioned NPs as an alternative strategy to control this bacterial infection, MOA of NPs, methods of synthesis of NPs and list of NPs that are tested against *S. aureus* as antimicrobial therapy. Different classes of NPs and applications of NPs against *S. aureus* and some other bacteria have been elaborated in the chapter. This chapter also includes the future of NPs like their increases uses in the medical field have shown marvelous results and proved to be a miracle in many fields. The information related to toxicity of NPs when they are used in excess has also been given.

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Chapter 44

# Interplay of different Nanoparticles in Semenology of Equine and Camelus

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**ABSTRACT**

Managing reproduction is important for animal production in the camel, horse, and cow industries. The significance of optimizing reproductive efficiency for financial and environmental reasons is emphasized. The application of nanotechnology to assisted reproductive technologies (ART) is being studied, with a particular emphasis on sperm quality improvement and cryopreservation methods. The effects on sperm quality and cryopreservation outcomes of zinc oxide nanoparticles (ZnONPs), cashew gum (CG) nanoparticles, copper nanoparticles (CuNPs), iron oxide or magnetic nanoparticles (MNPs), and selenium nanoparticles (SeNPs) are investigated. The problems and advancements in the reproduction of equine and camels are also covered in this chapter. All things considered, increasing animal reproductive efficiency through the use of nanotechnology offers workable solutions, which is crucial for long-term animal production.

**KEYWORDS**
Equine, Camelus, ART, ZnONPs, CuNPs, MNPs

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## INTRODUCTION

The regulation of reproduction holds true for animal production as well. It is important to maximize the times when animals are productive while breeding them, for both environmental and financial reasons. Animals with protracted durations between weaning and conception or delayed puberty have a disrupted economic equilibrium between productive outputs and inputs such as food and management; this is significant for both the financial success of large corporations and the long-term viability of small-scale farming. (Hashem and Gonzalez-Bulnes, 2020). In terms of the environment, these animals receive no advantage from exploiting natural resources other than the production of greenhouse gases and environmental debris. Therefore, it is imperative to optimize reproductive control. The field of reproductive management, particularly the application of assisted reproductive technologies (ART), is rapidly expanding and is critical to the control of reproduction in the cattle industry. The primary goal is to create and apply appropriate instruments for raising farm animal fertility using methods that are simple, inexpensive, and efficient. At the moment, this philosophy must take into consideration the idea of smart production, which includes considerations for human health, animal welfare, and environmental safety in addition to ideas like technology and cost efficiency (De Graaff and Grimard, 2018). In order to achieve the objectives of smart production, scientists therefore put a great deal of efforts in controlling reproductive activity, multiple ovulation and artificial insemination (AI), embryo transfer (MOET), and pregnancy management, particularly in the field of animal reproduction. These fields need the handling of living cells, such as spermatozoa, oocytes, and embryos, as well as the growth factors, administration of hormones, and other chemicals. Nanotechnology has the potential to enhance all of these aspects technological, economic, environmental, and health that limit the efficiency of these processes. Combining the fields of biology, chemistry, physics, engineering, mathematics, and computer science, nanotechnology is a cutting-edge field (Feugang et al., 2019). Although nanotechnology has been employed to generate therapeutic and diagnostic agents for human medicine, there is currently limited usage of nanotechnology in animal medicine and production. This technique works on the principle of converting chemical

molecules into particles that are tiny (between 1 and 200 nm). Enhance cellular absorption, charge, surface area and reactivity, and binding qualities are among the new chemical and physical properties that are attained as a result, and these attributes may have created a new avenue for biosciences innovation (N. Hashem and Sallam, 2020). The use of polymeric and metal nanoparticles' germicidal properties as antibacterial has led to the development of medication delivery systems at the nanoscale, both as therapeutic substances and as means of administering therapy. Compounds for delivery can be entrapped and protected by natural and nanostructured materials (Hill and Li, 2017). Numerous nanomaterials might be helpful in removing some of the barriers preventing ART from being as effective in animal reproduction.

Certain nanomaterials are being created specifically for males to optimize the processes of sperm preservation (freezing or cooling) and sperm purification (removing defective sperm cells) during post-collection sperm handling for AI. This will ensure the provision of high-quality sperm doses. For instance, fresh semen ejaculates and frozen-thawed semen that were intended to be used in in vitro fertilization (IVF) or field-scale artificial insemination (AI) have both been purified using nano-magnetic iron oxide ($Fe_3O_4$ NPs) pods, which are distinguished by their bio-compatibility and bio-function (Huang and Juang, 2011). According to the first research, boars may produce large amounts of highly recovered, pure sperm cells for semen (Feugang et al., 2015), and AI in cattle can lead to higher conception rates (Odhiambo et al., 2014). Molecules with strong antioxidant properties as nanoparticles, such cerium oxide (Falchi et al., 2016), selenium (Khalil, El-Harairy et al., 2019) and zinc may also be added to frozen semen to lengthen its shelf life and guard against reactive oxygen species during the cryopreservation procedure (Jahanbin et al., 2015).

Horses are a significant component of animal husbandry and have been for ages, serving a variety of purposes, particularly in developing nations. They are an important source of transportation, leisure, and draught power. In the developing world, there are around 93.6 M horses, with central Asia and east Africa having the largest number of these animals (Burn et al., 2010). There are 0.4 M horses, 4.5 M asses, and 0.2 M mules in Pakistan, in that order (GOP, 2009-2010). Even with their acknowledged importance, draught animals are raised in substandard circumstances. Animals are made to labor longer than they should and are malnourished. In addition to stressing out animals, improper handling, overcrowding, overloading, and lengthy transit durations without adequate food and watering can occasionally result in fatal injuries or severe injuries (Ragnarsson and Lindberg, 2010). As a result, these problems cause these animals' working efficiency to decline, which then lowers the revenue of the extremely poor society that depends on them. These animals' productivity may be greatly increased by carefully planning their tasks, taking use of the cooler hours of the day, utilizing better tools and equipment etc. (Burn et al., 2010).

Based on the latest statistics available from the Food and Agriculture Organization (FAO), around 38.6 M camels exist worldwide, with dromedary camels accounting for an approximately 95% of that number (Faye, 2024). There are two accepted species of camels: the two-humped Bactrian camel, *Camelus bactrianus*, is commonly called the Bactrian camel. The semi-arid, rocky hilly regions and the flat areas of Kazakhstan, Iran, Russia, Mongolia, and China comprise the majority (90%) of the Bactrian camel habitat (Alhaj et al., 2024). Instead, *Camelus dromedarius*, sometimes referred to as an Arabian camel or a one-humped Dromedary camel, is a domesticated camel that is found in the Middle East, northern and eastern Africa, and southwest Asia. People living in desert areas depend on camel milk because it minimizes the risk of certain illnesses and supplies vital nutrients (Alshuniaber et al., 2021).

**Table 1:** Overview of various nanoparticles and their roles in improving sperm quality

| Nanoparticles | Species | Roles | References |
|---|---|---|---|
| Zinc Oxide | Equine and Camel | Boosts antioxidant enzymes, which are important for spermatogenesis, stabilizes sperm membranes, counteracts the effects of ROS, raises sperm quality, enhances ATP pathways, lowers lipid peroxidation, and decreases anti-sperm antibodies. | (Ghallab et al., 2017; Shahin et al., 2020) |
| Selenium | Equine and Camel | Functions as a ROS scavenger, guards against oxidative damage to sperm cells, increases sperm motility, improves sperm quality, and prevents spermatogenesis from oxidative damage. | (Abdelnour et al., 2021; Ghallab et al., 2017) |
| Copper | Equine | Participates in oxygenation, hydroxylation, and dismutation processes; binds to nucleic acids; oxidises proteins and lipids; produces free radicals; affects pituitary receptors and sperm motility; | (Gao et al., 2007) |
| Iron Oxide or Magnetic | Equine | Improves sperm morphology and membrane integrity, reduces sperm DNA fragmentation, selects high-quality spermatozoa, and is used for sperm sexing. It also detects spermatozoa carrying certain chromosomes. | (Assumpção et al., 2023; Domínguez et al., 2018) |
| Cashew Gum | Equine | Improves the quality of semen, maintains sperm motility in cold storage, and may lessen the negative effects of seminal plasma on sperm motility. | (Loureiro et al., 2020) |

The camel, or *Camelus dromedarius*, is a useful animal that has evolved to withstand the harsh desert climate. It is utilized for its meat and milk (Skidmore, 2018), as well as a sports animal (Spencer et al., 2010). There isn't as many research on camel reproductive physiology as there are on other farm animals since camel fertility declines are caused by a

complicated combination of environmental and genetic variables. Scientific and scientific works on the camelid family have multiplied significantly in recent years (Swelum et al., 2014). There exist several approaches to enhance the production and reproductive capabilities of Arabian camels (Swelum et al., 2019), such as artificial insemination (AI). AI is a helpful technique for improving the genetic makeup of animals, but because camel cryopreservation procedures are not followed correctly, it has not yet been optimized for usage in camels (Abdel-Aziz Swelum et al., 2019). The challenges associated with this approach are as follows: (1) semen collection from vigorous males during rut; (2) poor sperm concentration; (3) lack of appropriate extenders for storing it; (4) viscous semen; and (5) low sperm motility (Skidmore et al., 2018). This Table 1 provides an overview of various nanoparticles used in the studies mentioned and their roles in improving sperm quality or aiding in specific processes related to reproductive health.

**ZNO NP**

Sperm quality is enhanced and antioxidant enzymes are increased by zinc oxide nanoparticles (ZnONPs) (Afifi et al., 2015), and is consequently thought to be a necessary component of spermatogenesis. Rats lacking zinc (Zn) have decreased amounts of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and protein, along with increased ribonuclease activity. Zinc is essential for cell development because it is a cofactor for RNA-dependent DNA polymerase, DNA, and RNA polymerase activities (Cheah YunSang and Yang WanXi, 2011). Zinc is largely contained in the mature sperm tail and has a role in phospholipid regulation and adenosine triphosphate activities in sperm motility. Zn affects stabilization of the sperm membrane, nucleic acid synthesis, and the protein metabolism, all of which are crucial for the vitality and motility of sperm (Dissanayake et al., 2010). Zn can also counteract ROS effects, which boosts the effectiveness of ATP pathways (Baumber et al., 2000). Zinc has anti-oxidant qualities; it lowers ROS produced by faulty leucocytes and spermatozoa, lowers the quantity of antisperm antibodies in circulation, and decreases lipid peroxidation by phospholipase inhibition (Roy et al., 2013).

In exploration of enhancing Arabian stallion sperm quality, we delved into the realm of non-enzymatic antioxidants. The study, conducted on semen from four distinguished purebred Arabians, sought to understand how supplementing semen extender with varying concentrations of BSA, trehalose, or zinc sulfate would affect the sperm's resilience during cooling or cryopreservation.

After chilling at 5 °C for two hours, the addition of nano-sized zinc to the extender significantly increased sperm progressive motility, vitality, and sperm membrane integrity. Supplementing sperm with nano-sized zinc greatly enhanced their post-thawing characteristics. In comparison to the conventional salts, vitamins, and control groups, there was a considerable increase in sperm progressive motility, vitality, and membrane integrity in the nano-sized zinc treatments (Shahin et al., 2020)

**Se NP**

Selenium (Se) is another vital trace element that is necessary for animal health. Se has been shown in several studies to possess protective antioxidant properties and is involved in signaling pathways, apoptosis, and cell growth (Balázs and Rácz, 2013). Se is a potent antioxidant that modifies the production of selenoproteins. Selenoenzymes (GPxs) and seleno-amino acids (L-selenocysteine, L-selenomethionine) are used to replace sulphur in proteins. Glutathione peroxidases 4 (GPXs4) is an essential factor that influences male fertility and sperm quality. Therefore, if the selenium level of the selenoproteins is low, spermatozoa may be susceptible to oxidative stress (Tareq et al., 2010). In several studies, nano-selenium (SeNPs) has been used as a ROS scavenger to shield sperm cells from oxidative damage. SeNPs added to the semen extender improved the oxidizing rooster semen variables and post-thawing quality (Khalil et al., 2019). The spermatogenesis and spermatozoa quality (DNA integrity, motility) were additionally shielded from oxidative damage by the oral supplementation of SeNPs from cisplatin, a male reproductive toxin (Rezvanfar et al., 2013)

Comparing the nano-Se group to the nano-zinc, control groups, sodium selenite, zinc sulphate, vitamin E, and vitamin C, there was a substantial increase in PI-negative sperm (Table 1). When compared to the other groups, the control group had a considerable increase in early apoptotic and apoptotic sperm; the group treated with nanoselenium had the lowest value. Supplementary Material Figure S3 displays sample images from the flow cytometry study.

Comparing the nano-Se group to the zinc sulfate-supplemented and control groups, there was a notable rise in the proportion of intact plasma membranes. There was a notable rise in the swollen membrane, although the frequency of atypical ultra-structures (slightly swollen plasma membrane and swollen) varied across the groups (Shahin et al., 2020)

**Cu NP**

Reactions involving oxygenation, hydroxylation, and dismutation include copper. On the other hand, large concentrations of copper can bind to nucleic acids, oxidize proteins and lipids, and create free radicals (Murray et al., 2006). While the residues of plasma copper are associated to many transporters, including transcuprein, copper-amino acid complexes, and albumin, the bulk of the copper is transported bound to ceruloplasmin (95%) (Cunninghamn et al., 1995; Evans et al., 2002). Due to its strong redox activity, free copper is poisonous and can lead to redox imbalances (Tabassomi and Alavi-Shoushtari, 2013).

The motility of spermatozoa and pituitary receptors, which control the release of LH, seem to be impacted by copper.

Examined the benefits of supplementing with iron and copper for the maturation media (Gao et al., 2007).

**Iron Oxide or Magnetic Nanoparticles (MNP)**

Research found that when the sperm sample is first incubated with MNPs and then subjected to a magnetic field, it is simple to identify those spermatozoa bearing the X chromosome. This was the first study to use MNPs to achieve sperm sexing using flow cytometry. In fact, 90% of the spermatozoa had the X chromosome, according to flow cytometry. Consequently, the proportion of X spermatozoa produced by this straightforward method was comparable to that obtained using cell sorting (Johnson and Welch, 1999). We are presently doing further studies using quantitative polymerase chain reaction (qPCR) to corroborate our findings. Nonetheless, the cell sorter has a major impact on sperm physiology, including sperm membrane and DNA damage (Garner, 2006) Alternatively, a sperm population enriched in X-containing spermatozoa as well as live, motile, uncapacitated spermatozoa with intact DNA comparable to the control samples were obtained using the MNP approach. Despite the relatively modest levels of motility and survivability in both treatments, this might be because the frozen-thawed technique is not yet standardized for donkeys. The results are comparable to those found for frozen-thawed equine spermatozoa, despite the fact that the sperm capacitation technique for donkeys' is yet poorly described (unpublished data). The sperm DNA fragmentation level in sexed samples is significantly lower than in samples sorted by the cell sorter, but it is still the same as in control samples (Domínguez et al., 2018).

The subpopulation of non-apoptotic spermatozoa have a greater morphological quality and membrane integrity, and sperm selection by magnetic activation effectively selected high-quality spermatozoa in equines, expressively lowering defects. This study's use of nanoparticles in spermatozoa selection turned out to be effective in producing high-quality morphological semen samples for assisted reproductive techniques. To improve our understanding of the unique characteristics of horse sperm cells and increase the effectiveness of this procedure, additional research is necessary (Assumpção et al., 2023).

**Cashew Gum (CG)**

For commercial horse AI, chilled semen samples are frequently used. To enhance the process, additional options are required, as current one still damages the spermatozoa during cold storage. This is the first study that aware of that uses of cashew gum and NP produced from cashew gum to enhance the quality of semen from stallions. During the whole storage time (up to 48 hours) in the current investigation, we found that only the NP1 group was able to sustain both progressive motilities and totals (Loureiro et al., 2020).

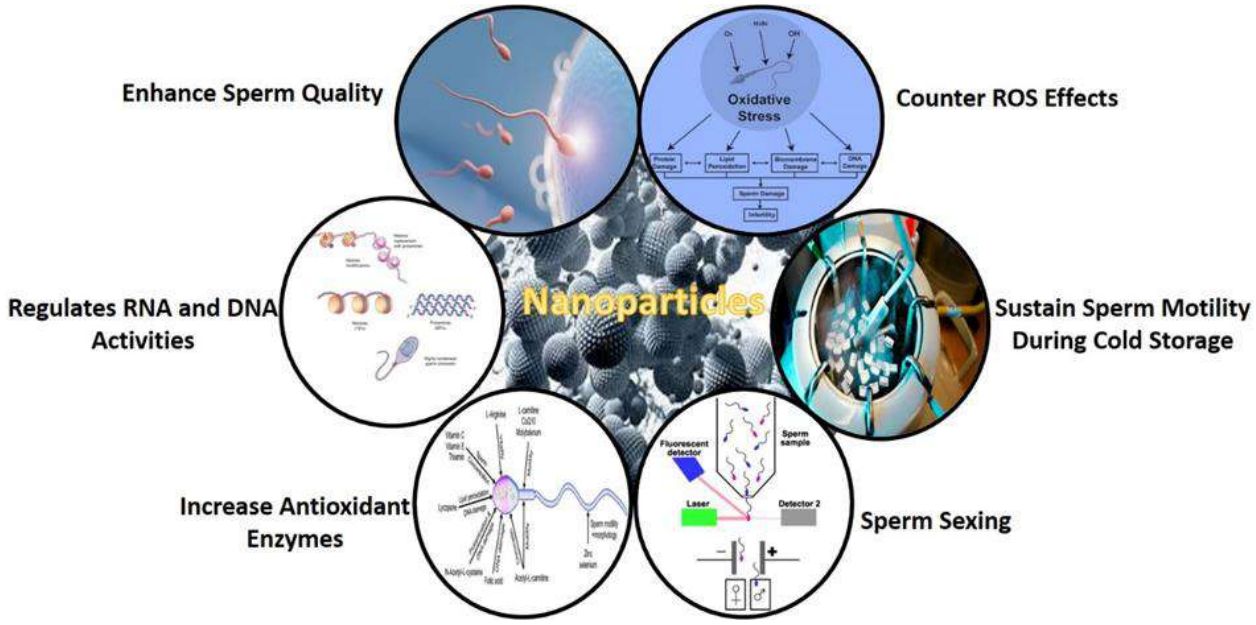


**Fig. 1:** An illustration of nanoparticles in different aspects of male reproduction

Unlike what we discovered, Pugliesi et al. (2012) demonstrated that applying Arabic gum at varying concentrations to horse semen that had been refrigerated for up to 96 hours reduced sperm motility. These latter writers suggest that the reason for this outcome is the normal rise in viscosity that happens when an aqueous solution is combined with a hydrocolloid. The fact that there were no viscosity changes in the tested media in our investigation might have led to the two research differing conclusions Furthermore, Kenney's extender's Cashew gum + NP + α-tocopherol combination would have been perfect for preserving motility during the cold storage time (Loureiro et al., 2020). Another thing to keep in mind is that, because our experiment followed the commercial station's regular husbandry procedures for sample

collection, when an aqueous solution is combined with a hydrocolloid. The benefits of removing seminal plasma are up for dispute since different research found different outcomes depending on whether seminal plasma was present or absent during cooling or cryopreservation (Al-Essawe et al., 2018). Additionally, it has been noted that using extenders significantly lessens the seminal plasma's deleterious impact on sperm motility when they are being stored at a low temperature (Love et al., 2005). It has been demonstrated physiologically that environment influences sperm, the features of seminal plasma, and motion both stimulate sperm activation and enhance sperm motility (Heise et al., 2011) At every storage time, all of the treatments showed comparable outcomes when mitochondrial activity was examined (Fig. 1). Given that sperm defects are directly linked to lower mitochondrial activity, this assessment is an important way to measure sperm quality (Barroso et al., 2006). Our results imply that cashew gum and NP did not have any harmful effects on sperm cells, given that all experimental groups were comparable to the control group, which merely used Kenney's as an extender (Gallon et al., 2006).

**Conclusion**

In conclusion, the discipline has a promising future when it comes to the use of nanotechnology to the control of animal reproduction. Additional research into the possible use of various nanoparticles, including cashew gum, zinc oxide, copper, iron oxide, and selenium, may result in significant improvements in sperm quality, cryopreservation techniques, and overall reproductive efficiency in many species.

Future studies should focus on developing nanotechnology-based strategies to address specific challenges in animal reproduction, such as enhancing the quality of semen, increasing the pace of conception, and lowering oxidative stress. Additionally, efforts must be directed towards developing practical and economical methods of incorporating nanotechnology into animal production systems, particularly in resource-constrained settings.

Collaboration between researchers, veterinarians, and industry partners will be necessary to translate these advancements into practical solutions that support both small and large-scale farmers. To preserve sustainability and minimize any unanticipated consequences, more research is required to ascertain the potential environmental implications of employing nanoparticles in animal husbandry. Future improvements in animal welfare, enhanced productivity, and breeding plan optimization may come from the expanding use of nanotechnology in animal reproductive management.

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Chapter 47

# Nanoparticles: Reshaping the Future of Breast Cancer Therapeutics

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**ABSTRACT**

Breast cancer is the most prevalent cancer globally, particularly in women, and has significant side effects from therapy. A potential substitute for the treatment of breast cancer is nanomedicine. Good clinical results have previously been obtained from the widespread usage of nanomedicine products like Doxil® and Abraxane® as additional treatments for breast cancer. Potential therapeutic nanocarriers having the capacity for targeting, imaging, and tracking include both organic and inorganic nanomaterials. Nanoparticles have the potential to revolutionize breast cancer gene therapy by decreasing the likelihood of immune system-based identification, boosting bioavailability, increasing the circulation period, and supplying the gene regulator precisely. Herein, we discuss the potential of nanotechnology, current obstacles in breast cancer therapy, and the targeted medication delivery strategy of nanoparticles, nanoparticles-based imaging, biosensing, two types of gene therapy for breast cancer: one based on nanoparticles and the other using nanoparticles and targeted death.

**KEYWORDS**
Nanoparticles, Breast cancer, Drug delivery, Gene therapy, imaging, Biosensing

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## INTRODUCTION

A group of disorders known as cancer are brought on by unchecked cell proliferation, which gives these aberrant cells the ability to proliferate and invade other bodily regions. Due to inadequate techniques for early cancer detection, cancer claimed the lives of approximately 9.6 million people worldwide in 2018 (Bray et al., 2018). According to American Cancer Society statistics, the most prevalent invasive cancer found in women worldwide is breast cancer (BC). In 2018, there were 266,120 newly identified cases of invasive BC with a possible death toll of 40,920. However, if discovered early enough, especially before metastasis, it is believed to be treatable (Liyanage et al., 2019). It is necessary to use novel and creative perspectives in order to handle breast cancer efficiently.

### Current Obstacles in Breast Cancer Treatment

The disease's great biological diversity (tumor heterogeneity) in how it presents amongst cases, each case's rapid evolution (clonal evolution of cancer stem cells), the multiple canonical pathways driving the disease's progression, the serious side effects of the traditional treatment cocktail (chemo and adjuvant therapies), and the onset of therapy resistance make breast cancer a difficult disease to control (Tanaka et al., 2009). Managing recurrence and treatment resistance are two difficulties in the treatment of BC. In fact, metastases account for 30% of recurrent illness in early-stage BC (Pisani, 2002).

Therefore, in order to properly treat each BC subtype, new strategic medicines must be developed. Since different habitats coexist at the metastatic location, controlling BC in metastatic cases is a far more difficult task. The standard therapeutic approach was excising the tumor by surgery, followed by radiotherapy and chemotherapy, which frequently had a great deal of negative side effects on healthy tissues. Not driven by the HER2 protein or the hormones progesterone or estrogen, ten to twenty percent of cases of breast cancer are triple negative (TNBC) and is an aggressive form that is not

well responsive to chemotherapy or hormone treatments. TNBC is also associated with BRCA1 mutations. Patients with TNBC often have a worse prognosis due to medication resistance, unfavorable side effects, and possibly cancer recurrence (Foulkes et al., 2010).

**Potential of Nanotechnology**

The multidisciplinary field of nanotechnology, which has applications in medicine including targeted thermal ablation, gene therapy, nanoparticle-based drug delivery, imaging, diagnostics, and bio sensing, is founded on ideas from physics, biochemistry, chemistry, and materials science. Nanotechnology is the manipulation of cellular and molecular components of matter. The use of nanotechnology has expanded over the past few decades, and drug-loaded nanoparticles have a high loading capacity and are a promising tool for cancer treatments since they are less toxic, stable, efficacious, selective, and tolerable than conventional chemotherapy medicines. During BC therapies, anticancer drugs can be delivered to tumors either actively or passively using nanoparticles laden with the drugs. (Singh et al., 2017). Some of the most significant cancer therapy approaches have been made possible by the quick development of nanotechnology. (Tran et al., 2020). In cancer and reconstructive surgery, nanotechnology has great promise. Underlying conditions related to breast cancer recurrence through implants may benefit greatly from modified Nano medicine (Alshareeda et al., 2023).

**Nanoparticles**

Nanoparticles are extremely small crystals with a size of less than 100 nm, meaning they are limited to the Nano scale in all three dimensions. An integrated interfacial layer surrounds the nanoparticles, which is usually made up of ions or other inorganic or organic molecules. Since nanoparticles are used in Nano medicine to improve drug delivery and activity, they are of great scientific interest (Mirza and Karim, 2021). Because of their unique coating and typical tiny size, hydrophobic anticancer medicines are easier to administer to specific body locations with less immune system opsonization (Tang et al., 2017). Many nanoparticles that selectively target metastasized breast tumors have been developed, including those made of Carbon nanotubes, quantum dots, fullerene, gold, silica, and magnetic materials. Due to the conjugation of semiconductor fluorescent Nano crystals, like quantum dots, to antibodies, these target proteins can be accurately quantified and simultaneously labeled in a single section of breast tumor (Yezhelyev et al., 2005). The simultaneous detection and quantification of multiple proteins on small tumor samples will be made possible by the use of nanoparticles, including gold-containing nanoparticles (i.e., Raman probes) and quantum dots of various sizes and emission spectra. This will ultimately enable the customization of a particular anticancer treatment to a patient's unique tumor protein profile. Carbon nanotubes, fullerenes, grapheme, and Nano diamond-based nanostructured materials are the main carbon-based Nano systems being developed for cancer theranostics (Augustine et al., 2017).

**Nanocarriers**

The medical and/or therapeutic goals guide the multi-component architecture of nano-carriers. (Bolhassani and Saleh, 2013). The size, shape, and surface properties of the nanomaterials used in cancer research can be altered to treat particular tumor types. Size plays a crucial role in how well the nano carriers enter the bloodstream and reach the tumor tissue (Bregoli et al., 2016). Optimizing the size of nanoparticles could enhance their selective uptake into tumor tissue. The fluid dynamics and subsequent uptake of the Nano carriers can be affected by their form. Due to difficulties in manufacturing and testing, it seems that spherical Nano carriers are utilized more often than nonspherical ones (Truong et al., 2015).

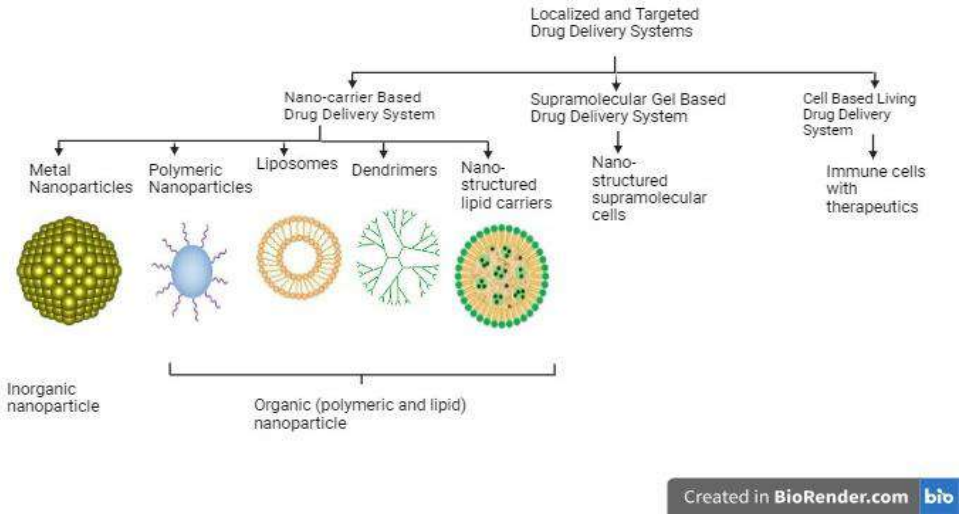



**Fig. 1:** Structure and classification of a system of nanocarriers.

By delivering the active ingredients to the intended tissue, the idea of a Nano carrier promises to lessen the harmful effects that would otherwise affect healthy tissues. Utilizing nanoparticles could enable tumor cells to receive tailored

medicine delivery. Using active or passive targeting raises the drug's concentration in the cancer cells. Given these benefits, patients with metastatic disease may have better results if nanoparticle medicines continue to go in the correct direction. However, the main obstacle to treating cancer is treatment resistance (Shapira et al., 2011). The composition of Nano carriers can be made sensitive to stimuli (such as pH or temperature changes) so that they release their cargo appropriately. Additionally, ligands that are recognized by receptors overexpressed in cancer cell tissues can be used to functionalize nano carriers. Finally, Polymers (such polyethylene glycol, or PEG) can be coated on nano carriers to increase their circulation time and remain invisible to the immune system (Viseu et al., 2018). The versatility of Nano carrier systems has been further investigated in relation to the development of theranostic (therapeutic + diagnostic) cancer treatment tools, which integrate many therapies with imaging techniques to track the body's distribution of therapeutic substances (Tabish et al., 2018).

**Breast Cancer Treatment using Nanotechnology**

The fast-moving clinical translation of effective breakthroughs based on nanotechnology has completely transformed the field of cancer treatment (Ferrari, 2005). Pharmacological uses of rapidly developing nanotechnology are available to identify and manage a range of illnesses, particularly cancer. The primary goals of Nano technological breakthroughs in cancer treatment include improved diagnostics, more effective medication delivery to tumor cells, and the development of targeted therapies (fig.2). The physio-biochemical characteristics of nanoparticles for therapeutic application are the focus of innovative Nano therapy, which is followed by targeted nanoparticle drug delivery aimed at reducing antitumor drug side effects with a promising fall in traditional treatment costs, a major barrier to cancer treatment (Mirza and Karim, 2021).

**Nanoparticles-based Drug Delivery**

Clinical trials are currently underway for a number of Nano particulate-based chemotherapeutic delivery systems for the treatment of BC, including liposome-, polymeric-, and nanoparticle albumin-bound paclitaxel formulations that have already received US FDA approval. With a unique coating that prevents immune system attack, Doxil® and Abraxane®, Two US FDA-approved drugs based on nanotechnology currently on the market, represent significant advances in cancer treatment. This allows for the precise medication administration to cancerous cells with a reduced risk of side effects compared to current chemotherapies.

Global experts in cancer treatment have become interested in Nano particulate drug delivery systems (NDDS) and, in particular, lipid nanoparticles (which have a increased level of biocompatibility and adaptability) because of their size-dependent features and prospective benefits. It is possible to create lipid Nano particulate formulations with a variety of characteristics that are pertinent to different disease states and administration routes. Additional specialized formulation criteria, such as affordability, stability in terms of chemicals and biology, decreased poisoning, and increased effectiveness, can also be met by customizing the product. Furthermore, lipid-based Nano carriers are seen to be attractive options for the formulation of both immunotherapeutic medicines and conventional chemotherapeutic drugs due to their safety and efficacy profiles (Mizrahy et al., 2017). Trans membrane glycoprotein HER2 is encoded by the human ErbB2 gene and is a target for immunotherapy against breast cancer (Lee and Muller, 2010). To target breast cancer that is HER2-positive, vaccines and other Nano medicines may be developed using overexpression of HER2, which induces an immunological response (Savas et al., 2016).

The FDA approved albumin Nano particle chaperones of paclitaxel for treatment in recurrent and metastatic BC ten years after approving liposomally encapsulated (pegylated) doxorubicin (anthracycline) for use in metastatic cancer in 1995. The latest generation of liposomal dox is safer and less cardio toxic than conventional dox, while yet having efficacies comparable to those of the former (Hortobagyi, 1997). Specifically, the proteins expressed on cancer cells are not targeted by first-generation classified vectors (Ferrari, 2008). Active targeting, or the ability to identify and focus on certain biological substances found on the surface of cancer cells, is what has allowed the "second generation" of therapeutic nanovectors to develop. Applying this method has the potential to decrease potentially fatal systemic cytotoxicity and increase the therapeutic window for delivering larger concentrations to sick lesions (Tanaka et al., 2009). High affinity ligands, including folate (Gabizon et al. 2004), prostate-specific membrane antigen (Farokhzad et al. 2006a), and Arg–Gly–Asp (RGD) (Pasqualini et al. 1997), can be chemically coupled to the surface of nanoparticles to achieve this. This enhances the interaction between the cancer cells and the nanoparticles, leading to a significant advancement in the bio distribution of nanoparticles in comparison to non-targeted first-generation Nano vectors.

Using a multi-stage approach, According to Tasciotti et al. (2008), the "third generation" of nano vectors is characterized by a greater degree of multi-functional integration and as a carrier for nanoparticles. First stage biodegradable mesoporous silicon micro particles can be loaded with one or more second stage nanoparticle types carrying various payloads for imaging and therapy. Since the first stage of the third-generation vectors' delivery method directs the particles towards the vascular endothelium and the second stage particles pass via the fenestrations, it does not depend on the EPR effect. Third-generation vectors' modularity offers a potent tool for addressing a variety of unmet medical needs, with an emphasis on the creation of multifunctional and multimodal medicines (Tasciotti et al., 2008).

DNA nanostructures are carefully built and developed to have controllable size, shape, function, surface, and chemistry. DNA nanostructures have been effectively loaded with two powerful anticancer drugs, doxorubicin (Dox) and CpG oligonucleotides, to increase their cellular absorption effectiveness (drug payload) (Hu et al., 2018).

**Nanoparticles-based imaging**

Using common imaging modalities including computer topography, positron emission tomography, magnetic resonance imaging, and near-infrared fluorescence imaging, both active and passive targeting NPs can be utilized to target

and scan breast cancer and metastases (Mu et al., 2017). Tracking the course of cancer and the effectiveness of used therapy requires both qualitative and quantitative imaging. Receptors, anchoring proteins, transporters, enzymes, and other cell membrane protein indicators are often targeted for imaging because of their 2- to 100-fold higher amounts on tumor cells. Because of its extreme overexpression in tumor cells (HER2 is one of the most expressed membrane proteins) and its low expression in healthy cells, HER2 is a perfect biomarker for cancer. For the treatment of metastatic BC with HER2 overexpression, the FDA-approved anti-HER2 monoclonal antibodies trastuzumab and pertuzumab have previously been produced (Cai et al., 2008).

**Table 1:** Chemotherapeutic agents including nanomaterials in cancer clinical trialss (Jin et al., 2020)

| | Year | Drugs | Disease | Findings |
|---|---|---|---|---|
| Liposome | 2015 | Doxorubicin | Platinum-Sensitive Ovarian Cancer | beneficial risk-benefit ratio |
| | | Paclitaxel | Non-Small Cell Lung Cancer | significant resection rate and disease response, with manageable toxicity |
| | | Ursolic acid | Advanced Solid Tumors | reasonable, controllable toxicity, increasing the rate of patient remission |
| | | Mitomycin C | advanced cancer | lengthy half-life, bearable, and efficient |
| | 2016 | miR-34a Mimic | Advanced Solid Tumors | efficient |
| | | Vincristine Sulfate | Refractory Solid Tumors or Leukemias | lacking neurotoxicity with a dosage limit |
| | | 5-fluorouracil and Leucovorin | Advanced Solid Tumors | extended half-life, reduced peak plasma concentration, and greater area |
| | | Cytarabine | Childhood Acute Lymphoblastic Leukemia | no long-term detrimental neurological effects |
| | 2017 | Amphotericin | Acute Lymphoblastic Leukaemia | efficient |
| | | Irinotecan | Recurrent High-Grade Glioma | nothing atypically poisonous |
| | 2018 | Cytarabine and Daunorubicin | Newly Diagnosed Secondary Acute Myeloid Leukemia | notably higher survival rate |
| | | Curcumin | Locally Advanced or Metastatic Cancer | resilient |
| | | Daunorubicin | Pediatric Relapsed/Refractory Acute Myeloid Leukemia | well-accepted and exhibited high rates of reaction |
| | | Lipovaxin-MM | Malignant Melanoma | safe and devoid of harmful side effects that are scientifically noticeable. |
| | | Vincristine Sulfate | Acute Lymphoblastic Leukemia | offered a significant clinical advantage and safety |
| | | Oligodeoxynucleotide | Refractory or Relapsed Haematological Malignancies | tolerant, efficient |
| | 2019 | Eribulin | Solid Tumours | well-accepted with a pleasant pharmacokinetic profile |
| polymeric Micelles | 2017 | Epirubicin | Solid tumors | demonstrated less toxicity than conventional epirubicin formulations and was well tolerated in patients with a variety of solid malignancies |
| | 2018 | Genexol-PM plus carboplatin | Ovarian Cancer | Well-tolerated toxicity and superior efficacy |
| | 2019 | Paclitaxel (PTX) | Breast cancer | NK105 had a better PSN toxicity profile than PTX |

The poly(d,l-lactide-co-glycolide) (PLGA) polymer, which has FDA approval for therapeutic use in humans, served as the basis for the development of the majority of polymeric nanoparticles. For preclinical research in TNBC tumor models, nontargeted polymeric nanoparticle drug carriers have been developed. One such example is an active metabolite of irinotecan (SN38) encapsulated in polymeric nanoparticle that demonstrated anticancer effectiveness in the 4T1 mouse mammary tumor model (Sepehri et al., 2014). For the purpose of tracking medication administration, Certain polymeric nanoparticles have had a variety of imaging agents added to them. Because NIR imaging is easy to use, quick to detect when drugs are delivered via nanoparticles, and can be used to optically image tumor cells that are resistant to treatment before excision, it has been fully studied (Miller-Kleinhenz et al., 2015).

One non-invasive method of medical diagnosis is magnetic resonance imaging (MRI). Certain exogenous contrast agents are used to boost contrast and provide improved resolution and sensitivity in magnetic resonance imaging (MR) since it can be challenging to distinguish between normal and diseased tissues in these images. A measurable magnetic

resonance (MR) signal is produced by the metal nanoparticles' enormous surface area and size, which exhibit super paramagnetic phenomena. Specifically, a great deal of research has been done on super paramagnetic iron oxide nanoparticles (SPIONs) as T2 contrast agents in magnetic resonance imaging (MRI). This is due to the fact that phantom pictures' negative contrast (darkness) can be enhanced by the T2 relaxivity of water protons (Núñez et al., 2018). There is a development underway to create "smart" Nano probes that can sense changes at the molecular level and interact in vivo with natural systems. Systematically administered, ligand-biomarker-accumulated Nano probes provide deep diagnostic imaging by relaying signals from malignancies (Ferrari , 2005).

Using imaging probes specific to biomarkers for image-based diagnosis and therapy monitoring is a promising way to improve cancer imaging's sensitivity and specificity (Weissler, 2006). Despite having a high sensitivity, nuclear imaging methods need complex and costly radiochemistry and have poor resolution and anatomic localization of the tumor lesion. Injectable nanoparticles have proven to be useful imaging probes for cancer detection in animal experiments because they attach to specific proteins on cancer cells, such BC cell mammaglobin. In addition to enabling non-invasive therapies such employing infrared light to kill cancer cells, an imaging contrast ant with nanoparticles can reach a tumor site and help identify cancerous cells. It is anticipated that widespread adoption will happen during the next five to 10 years. Though confusing, adaptable Nano medicine will play a significant role in providing superior alternatives to conventional cancer treatments.

**Nanoparticles-based gene therapy**

When it comes to breast cancer gene therapy, nanoparticles have the potential to be a game-changer since they can be an efficient carrier of a particular medicine or gene by increasing bioavailability, decreasing the likelihood of recognition based on the immune system, and precisely supplying the gene regulator (Mirza and Karim, 2021). Gene therapy aims to substitute a malfunctioning gene with a functional one, healthy variation. Prosperous gene delivery requires the crossing of both extracellular and intracellular barriers. Immune reaction is deliberately dodged or reduced, and immunologically inert minicircles are also employed for effective systemic delivery of gene. Usually, lipid bilayer membranes are disrupted to allow for the methodical delivery of naked pDNA using laser irradiation, sonoporation, or electroporation (McCrudden and McCarthy, 2013). A magnetic field can be used to drive gene editing in vivo in Zhu et al.'s prospective translation approach for CRISPR-Cas9 therapeutic systems. In tumor-bearing mice models, they used recombinant baculovirus vectors multiplexed with Nano magnets to maximize efficiency, reduce genotoxicity, and improve transduction in target cells and targeted tissue distribution (Mirza and Karim, 2019).

In triple negative breast cancer, TP53 is the most often altered or deleted gene. One neighboring gene that is a potential collateral susceptible target of TP53 is POLR2A (RNA polymerase II subunit A). Cancer cells are extremely vulnerable to additional TP53 suppression or inhibition since nearly Every TNBC with a decrease in TP53 copy number also has hemizygous POLR2A deletion. It's interesting to note that TNBC has partial deletion of this specific crucial gene, which may provide a targeted treatment alternative. POLR2A is the target of a recently developed and patented low pH-triggered Nano bomb (Xu et al., 2019). Upon delivery to a TNBC cell, with a controlled release, this POLR2A inhibitor-containing Nano-bomb multiplied 100 times its initial size and killed cancer cells specifically.

In addition to offering great promise for the creation of multimodality agents with imaging and therapeutic properties, nanoparticles are also highly promising as diagnostic imaging agents. However, some of the most promising nanoparticles have heavy metals and show extended tissue preservation. There are significant toxicity concerns with this. By minimizing the time of exposure to these compounds, the production of nanoparticles with appropriate clearance properties will reduce the risks associated with toxicity (Longmire et al., 2008). Therefore, creating nanoparticles with the best possible clearance or biodegradability is a necessary requirement before using them in a clinical setting.

**Nanoparticles-based Diagnosis**

By using fluorescent in-situ hybridization, fluorescent nanoparticles can be used in cancer diagnostics to identify a variety of genes and matrix RNA, as well as to multiplex simultaneous profile of tumor indicators (Yezhelyev et al., 2006). The quality of life and survival rates of BC patients are severely impacted by late diagnosis; therefore, earlier cancer detection and more accurate diagnosis are desperately needed. Recently, a number of Nano-based techniques, such as aptamers, QDs, and organic and inorganic NPs, have shown promise as methods for detecting and screening HER-2+ BC (White et al., 2020). The majority of targeted agents take advantage of mAbs, Fabs, scFv, and anti-HER-2 humanized monoclonal antibodies (Trastuzumab and Pertuzumab). The dual benefit of labeling NPs with these targeting moieties is that it stabilizes the targeting agent and loads many contrast agents onto a single vector, greatly enhancing the signal's specificity and intensity. Additionally, the ability to alter the constructs' physico-chemical characteristics offers far more control over the bio distribution and clearance kinetics, which enhances tumor retention and permits longer imaging times for the tumor tissue (Sitia et al., 2022).

In the past few years, molecular diagnostic techniques based on nanotechnology have advanced very quickly, and in the future, they will become a vital possibility for the diagnosis of cancer. Aptamer-guided silver–gold bimetallic nanostructures have a notable cytotoxic effect on the BC cell line MCF-7. The highly active surfaces of these nanostructures enhanced Raman scattering, allowing for the potential treatment of BC cells using near-infrared photothermal therapy (Wu et al., 2012). Concurrently doing clinical breast examination, breast imaging, and biopsy is known as a triple test, which increases diagnosis accuracy and lowers the amount of false negative results. As a cutting-edge method for breast cancer early detection, iron oxide nanoparticles offer a lot of promise. The latest goal of Google Inc. is to create wearable detectors

and iron oxide NP diagnostics, which will be included in the upcoming Google Wear operating system and device versions (Nounou et al., 2015).

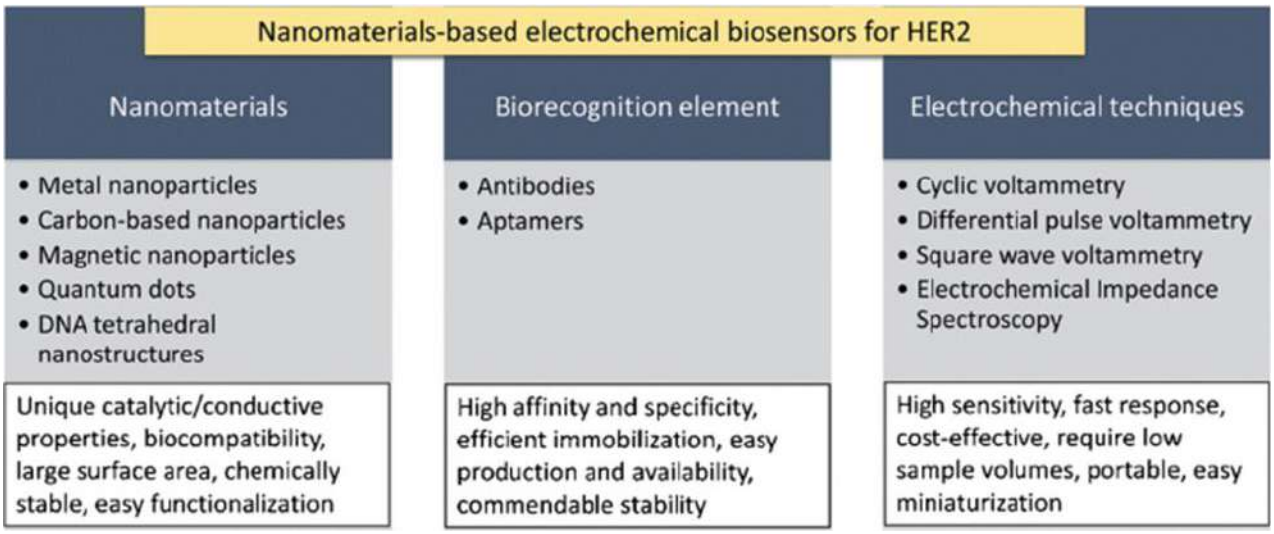
Since miRNA can either restore or repress miRNA expression, they appear to be a promising diagnostic biomarker with the potential to be employed for early identification of BC. Enhanced clinical relationship between the illness type and stage can be achieved by mining the regulation mediated by miRNA. As an alternative, nanotechnology offers a strict and sensitive miRNA detection method with significant implications for cancer theranostics. Strongly targeted therapeutic miRNA for tumor targets has also been demonstrated to be delivered noninvasively using nanoparticles and Nano pores (Cai et al., 2015).

**Nanoparticles-based Thermoablation**

Though it is thought to be the greatest option for treating confined tumors, surgical resection is ineffective for treating heterogeneous cancer, metastases, and small tumors (less than 3 cm in size). Tumor ablation therapy is a minimally invasive local treatment approach that has unique benefits for treating tumors that are difficult to surgically remove. Nevertheless, ablation therapy is unable to entirely eradicate all tumor tissues and cells at once due to its physical and chemical makeup as well as the limitations of equipment technology. Not only that, but the ablation process invariably causes some normal tissues nearby to be damaged. According to recent research, certain types of ablations can kill tumor tissues by physically inducing an immune response. This can then produce a large number of immune cells that kill tumor tissues and cells again. Metal nanoparticles coated with immunological medicines can enhance the effectiveness of this anti-tumor immunotherapy. Consequently, there is a lot of room for research on the use of metal nanoparticles in conjunction with ablative treatment (Xie et al., 2023).

**Nanoparticles-based Biosensing (immunosensing)**

Electrochemical biosensors that utilize nanomaterial, antibodies, and aptamers have gained significant traction as effective and efficient methods for detecting HER2-ECD. These biosensors take advantage of the distinct conductive characteristics, large surface area, chemical stability, and high affinity and specificity of aptamers and antibodies, as well as the unique properties of nanomaterial to assess HER2 in a selective and sensitive manner (Ahirwar , 2021). As bio recognition elements or detection probes, (Peng et al., 2014).




According to reports, aptasensors and electrochemical immunosensors for HER2 have been developed utilizing label-free and label-based detection methods. The label-free methods make use of HER2's inherent properties, which impose electrode impedance fluctuations and permit label-free detection. On the other hand, the label-based approaches increase test sensitivity by producing an enhanced signal by attaching labels to biomolecules or electrode surfaces, such as fluorescent tags, enzymes, and nanoparticles (Bogomolova et al., 2009). Improved bio recognition element immobilization increased electrochemical signal output, and decreased background noise (i.e., a rise in the signal-to-noise ratio) have all been made possible by the use of nanomaterial in bio sensing applications (Shan et al., 2020).

**Breast cancer gene therapy via nanoparticle-mediated targeted death**

This type of treatment aims to cause malignant cells to die by expressing genes whose protein products trigger several death pathways. Typically, pDNA is used to administer these genes. When compared to the protein itself, pDNA has a number of benefits. For example, even when purified on a large scale, pDNA does not cause an immune response, and tumor cells do not become resistant to it. Three main methods are used in gene therapy to cause cell death: a) Gene-directed enzyme prodrug treatment (GDEPT); b) Toxin-induced; and c) Induced apoptosis (Vago et al., 2016). A series of genetic mutations and aberrations discovered in BC are excellent for gene therapy, which involves giving cancer cells a nucleic acid-based drug to either correct the genetic defects or kill the malignancies. The goal of death-induced gene therapy is to completely

eradicate malignant cells, so its difficulty is to cause death only in diseased tissues while sparing healthy ones. One of the main challenges in gene therapy is creating the ideal transfection vector, and for breast cancer gene therapy, nanoparticles seem to be a promising fit(Roacho-Perez et al., 2018).

**Conclusion**

Nanomaterials are being carefully chosen to change the objectives of breast cancer medication and examination by reducing the discomfort associated with conventional chemotherapy, overcoming present clinical challenges and influencing the therapy landscape going forward. A summary of the latest advancements Systems for delivering drugs or genes against cancer, particularly breast cancer, using nanoparticles have been developed, focusing on the drug delivery method and strategy design utilized in nanomedicine. Nanoparticles-based drug delivery systems provide superior stability, pharmacokinetics, biocompatibility, and tumor targeting in contrast to traditional drug. Moreover, they reverse medication resistance and greatly reduce systemic toxicity. Notwithstanding moral and security issues, nanotechnology offers a useful arsenal in the fight against cancer.

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Chapter 48

# Role of Nanoparticles in Livestock Management

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**ABSTRACT**

This paper explores the potential of nanoparticles in transforming livestock management practices. At nano scale, Nano particles have shown number of characteristics which prove to be the solution of number of problems in livestock industry related to nutrition animal health and production. Nano particles have shown promising effects on semen quality enhancement, improving fertility and reproductive efficiency, environmental implications and sustainability, production efficiency, growth promotion, vaccination, bacterial infections, viral infections, parasitic infections, antimicrobial properties of nanoparticles, disease prevention and control, and feed supplementation. To ensure the ethical and sustainable use of nanoparticle in livestock management, it is important to address the ethical concerns and establishing a strong regulatory framework. Continued research and innovation in nanoparticle technology are needed to take its advantage and improve the sustainability and resilience of livestock industry.

**KEYWORDS**
Nano particles, Livestock management, Sustainability, Disease prevention, Nutrition, Fertility

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## INTRODUCTION

Livestock management is a challenging essential to the health, production, and sustainability of animal agriculture. The expanding global population contributes to up demand for products derived from animals, including meat, milk, and eggs. So that in order meet needs that developing by avoiding dangerous impacts and maintaining animal welfare, it is very necessary to meet developing needs. To improve the efficiency and sustainability, modern technology has been used in agricultural sector these days. One of these modern technologies is use of nanoparticles in livestock management. Due to their unique characteristics at Nano scale, Nanoparticles give the solution to different problems including boosting animal nutrition and health faced by livestock producers. This chapter explores the potential of nanoparticles in transforming livestock management practices (Sargison, 2020).

### Definition and Characteristics of Nanoparticles

Nanoparticles are defined as particles having a size between one and one hundred nanometers. At this level, materials demonstrate unique characteristics that differentiate them from bulk materials. These characteristics, which include increased solubility, enhanced reactivity, and larger surface area, make nanoparticles extremely versatile for a variety of uses (Mody et al., 2010). Nanoparticles provide enormous potential for enhancing animal welfare, food intake, and general efficiency in livestock management. These tiny structures have altered electromagnetic characteristics and an increased surface area to volume ratio, in addition to size-dependent qualities that make them desirable for a range of applications (Talapin andShevchenko, 2016). Materials that can be employed to create nanoparticles with distinctive characteristics and features include metals, metal oxides, polymers, and ceramics (Astruc, 2020). In addition to their tiny size, which allows for efficient transport and individual action inside biological systems, they are also good candidates for application in illness prevention, growth promotion, animal feed vitamin supplementation, and reproductive control (Mohanraj and Chen, 2006).

### Livestock Management

Livestock management contains number of different techniques which maintain the production and health of livestock in agriculture system. These techniques include reproducing and breeding, giving housing and shelter, treating and

preventing diseases, controlling nutrition, and basics husbandry procedures. Livestock manager take decisions on a variety of factors including resource availability, market demand, animal genetics, and environmental considerations to ensure the best productivity for their animals.

Different sustainable techniques emphasize a balance between the objectives of environmental protection and needs of livestock for managing grassland and cattle (Nienaber et al., 2007). Putting grazing management plans into actions is essential to preserve the health of grassland and prevent overgrazing, to support the sustainable livestock managing system (Teague et al., 2020).

Implementing different livestock management approaches like land degradation, resources efficiency, and greenhouse gas emissions enhances the productivity, animal welfare, and environmental stewardship in livestock operations (Kleppel, 2020; Hassan et al., 2023).

**Importance of Nanoparticles in Livestock Management**

**Nanoparticles in Feed Supplementation**

**A. Types of Nanoparticles Used in Feed**

Depending on their composition and properties, different types of nanoparticles are used as feed additives to improve livestock digestion and absorption (Fig. 1).

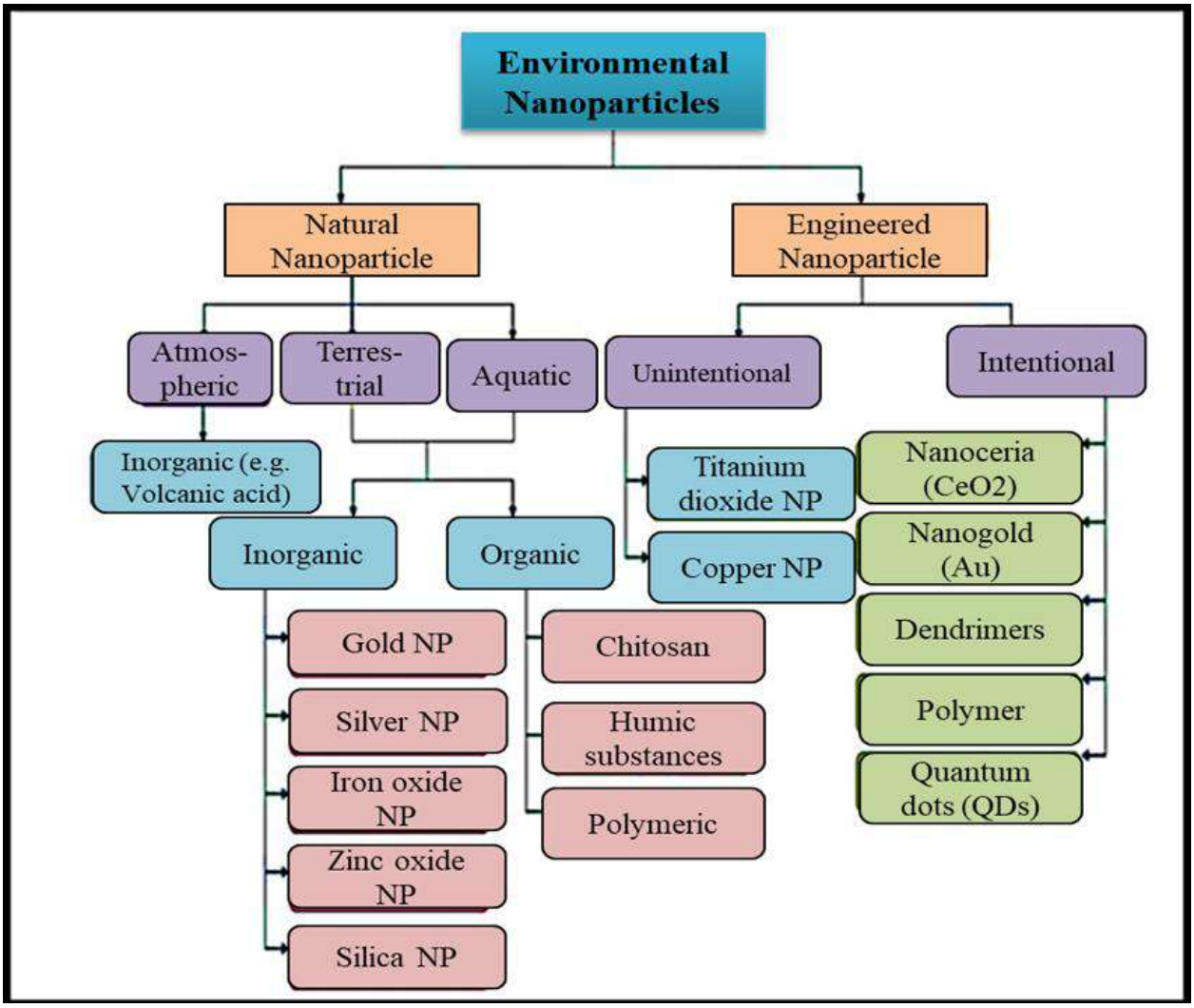


**Fig. 1:** Types of Environmental nanoparticles used in feed.

**a. Nanocarriers:** Nutrients like as vitamins, amino acids, and fatty acids can be condensed and delivered via Nano carriers (nanoparticles). These carriers enable the targeted transport of nutrients to specific areas for absorption while protecting them from breakdown in the digestive tract. Nutrient and bioactive material delivery solutions for animal feed are being demonstrated by Nano carriers such as ABA type tri-block copolymers (Mehrazar et al., 2015).

**b. Nanominerals:** These include nanoparticles of essential minerals such as zinc, copper, selenium, and iron. By increasing their bioavailability and absorption and nanoscale versions of these minerals an animal's development, immune system, and overall health can all be enhanced (Abdelnour et al., 2021).

**c. Mettalic nanoparticles:** Metallic nanoparticles like silver, copper, and gold, which have antibacterial qualities and can boost animal growth (Bunglavan et al., 2014; Scott et al., 2018).

**d. Metal oxide nanoparticles:** Magnesium oxide and zinc oxide frequently consumed in food formulations can enhance feed digestibility and nutrient utilization (Adegbeye et al., 2019; Mohd Yusof et al., 2019).

**e. Nanoemulsions:** Colloidal distributions of oil droplets in water, or vice versa, stabilized at the nanoscale by surfactants, are known as Nano emulsions. Encapsulating lipophilic materials in Nano emulsions can improve their solubility and absorption in the digestive tract. Examples of these materials include fat-soluble vitamins and bioactive compounds (Wilson et al., 2022).

**f. Nanoclays**: Several types of Nano clays, such as montmorillonite, bind mycotoxins and other contaminants in feed to lessen the animal's digestive tract's absorption of them (Nadziakiewicza et al., 2019).

**g. Nanoparticles of plant extracts:** Enzymes are encapsulated in nanoparticles to prevent degradation in the digestive tract and to increase the efficiency of the breakdown of feed components for improved nutritional absorption (Sorbiun et al., 2018).

**h. Nanoparticles for enzyme delivery:** Nanoparticles are used to encapsulate enzymes, protecting them from degradation in the digestive system and improving their efficacy in breaking down feed components for better nutrient absorption (Galliani et al., 2018)

**B. Benefits of Nanoparticle Supplementation**

Nanoparticles supplement provide number of advantages such as animal performance, production, and overall health. Nanoparticles given in the form of vitamin and minerals provide the better absorption and utilization by the animal's body and increase growth rates and feed efficiency (Alkhtib et al., 2020). Furthermore, the phytochemicals present on nanoparticles help in distribution of nutrients to specific tissues or organs, hence enhancing their effectiveness (Abdelnour et al., 2021). Additionally, the antibacterial property of nanoparticles maintains the gut health of cattle and help in treatment of microbial diseases (Baldissera et al., 2020). In animals nanoparticles stimulating the general health by reduce the oxidative stress and progresses their antioxidant state, and also increase the resistant to different stressor (Del Vento et al., 2019). There are number of different benefits of using nanoparticles as feed additives that livestock welfare and performance.

**Nanoparticles in Disease Prevention and Control**

**A. Antimicrobial Properties of Nanoparticles**

Due to their exceptional antibacterial abilities, nanoparticles have attracted a lot of attention from a variety of industries, such as agriculture and healthcare. The distinct physicochemical features of nanoparticles, counting their size, shape, and surface area to volume ratio, are the source of these attributes. Due to their strong antibacterial action against a variety of pathogens, including as bacteria, fungus, parasites, and viruses, metal nanoparticles—in particular, silver nanoparticles—have been the subject of substantial research (Hajipour et al., 2012). Nanoparticles combat bacteria by producing reactive oxygen species, break down microbial cell membranes, and changing microbial enzyme function and by altering their surface and addition functionality (Usman et al., 2013; Gharpure et al., 2020). Furthermore, green synthesis techniques conserving natural resources and lessening their negative effects on the environment by creating antimicrobial metal nanoparticles (Maťátková et al., 2022).

Nanoparticles, due to their unique physicochemical properties, offer numerous advantages in combatting infectious agents.

Functionalization of nanoparticles can improve their interactions with pathogens and increase the effectiveness of antimicrobial treatments (Look et al., 2010).

Nanoparticles are used in drug delivery vehicles, diagnostic instruments and immunotherapies; lets the medicine to be delivered to the infection zone, identification of infectious diseases which makes it easier to contain epidemics and intervene in a timely manner, and to strengthen immune responses against infectious diseases respectively (Torres-Sangiao et al., 2016; Singh et al., 2017; Kirtane et al., 2021).

**B. Potential Applications in Vaccination**

The major benefit of nanoparticle is to improve the efficacy and security of vaccination, offers a potentially new avenue in immunization.

The use of nanoparticles for transcutaneous vaccine delivery, in which the particles penetrate the skin to deliver antigens and possibly do away with the requirement for conventional injections, is one possible use (Kohli and Alpar, 2004). Due to their adaptable characteristics, polymeric micro/nanoparticles exhibit potential for vaccine delivery applications, providing regulated release and augmented immune responses (Yue and Ma, 2015). Furthermore,

nanoparticles can be created to function as artificial vaccinations, eliciting strong defenses against particular infections (Smith et al., 2015). Significantly, studies have looked into how well nanoparticles might penetrate antigen-presenting cells to increase vaccination effectiveness and uptake (Gregory et al., 2013).

**Nanoparticles for Growth Promotion and Performance Enhancement**

In plant development and agricultural production, nanopesticides, nanofertilizers, nanosensors, and nanobiotechnology are the uses of nanotechnology, which uses nanomaterials as carriers. The efficacy and durability of agrochemicals are facilitated by the distinctive architectures of nanomaterials, which include high specific surface area, centralized distribution size, and great biocompatibility (Chhipa and Joshi, 2016). Additionally, applying the right nanomaterials at the right times of plant development or under stressful circumstances helps plants thrive and become more resilient to adversity (Table 1). In order to improve crop yields and quality, nanotechnology has been applied to a different agricultural production processes, including seed germination and plant growth (Figure 2).

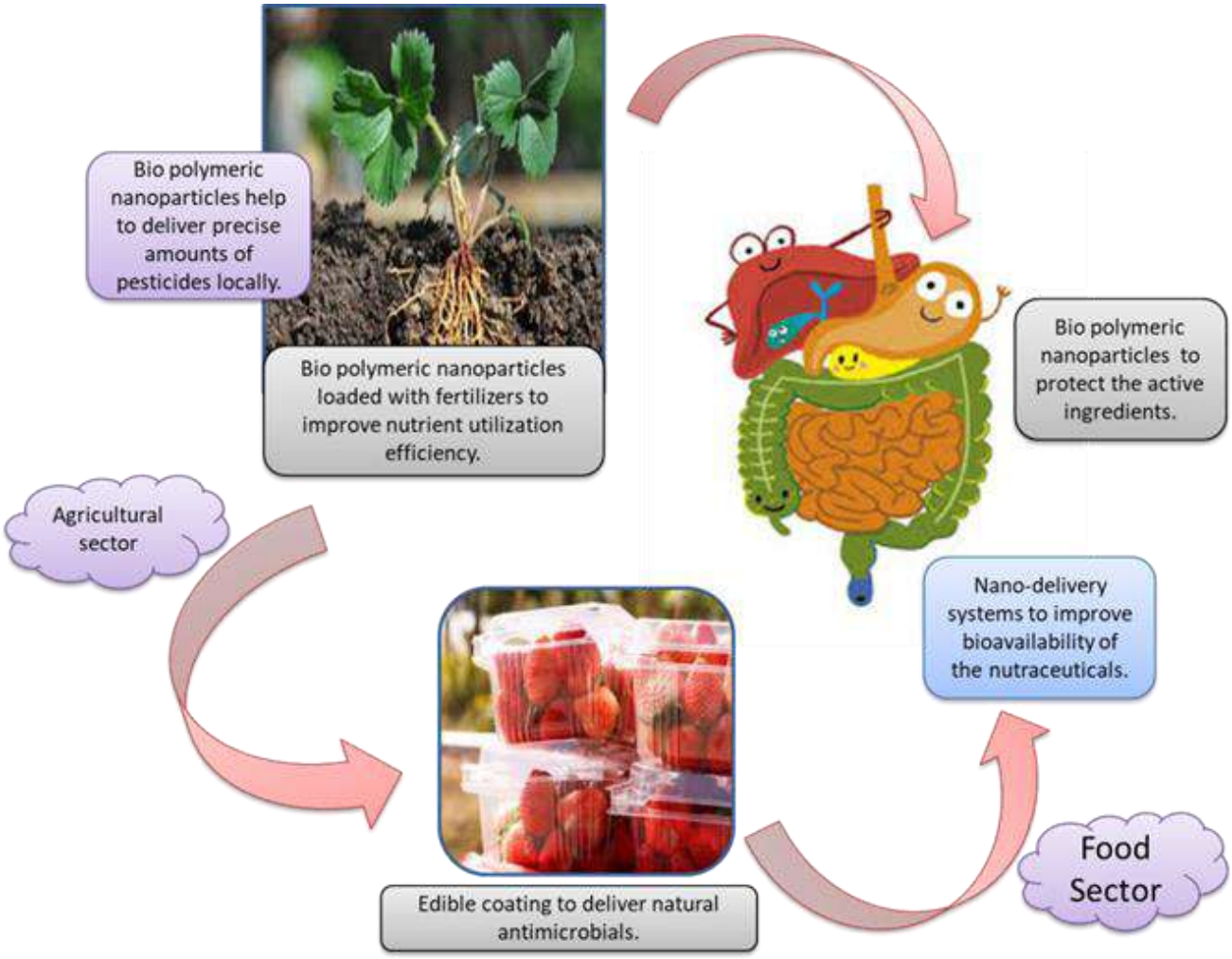


**Fig. 2:** Nanoparticles for Growth Promotion and Performance Enhancement.

Studies conducted recently have showed the beneficial impacts of nanoparticles (NPs) on seed germination in agricultural plants. Exogenous TiO2 NP treatment improves the seeds' absorption of water and oxygen, which shortens the germination period. TiO2 NPs are useful for boosting seed germination. For example, compared to the untreated control, tomato seedlings immersed in TiO2 NPs showed a germination percentage that was about 8% higher (Wang, 2014). TiO2 NPs have been shown in another work to promote seed germination and significantly shorten the mean germination time in wheatgrass. Furthermore, by improving the capacity of the seed to absorb water, nonmetallic NPs like multi-walled carbon nanotubes (MWCNTs) can promote seed germination in a variety of crops. As MWCNTs were sprayed by air over soybean, barley, and maize seeds, the germination rate of the seeds was successfully raised by at least 25% as compared to the untreated control. Subsequent tests demonstrated that MWCNTs were able to pierce the seed's surface. Furthermore, in soybean, barley, and corn seeds sprayed with MWCNTs, the relative gene expression of many genes associated to water channels increased dramatically (Lahiani et al., 2013).

**Table 1:** Types of nanoparticles and their role in Growth promotion.

| Type | Nanoparticle | Experiment | Animal production application | References |
|---|---|---|---|---|
| METALS | Silver | Evaluation of antimicrobial activity of Alkali lignin bound silver nanoparticles to cellulose fibers. | Biocide and Veterinary Medicine | (Hu et al., 2015) |
| | Copper | copper's growth-promoting properties are enhanced with nanoscale copper. | Nutrient delivery and Biocide | (Gonzales-Eguia, et al., 2009) |
| | Gold | Use amoxicillin to functionalize in order to overcome the resistance of bacteria. | Biocide | (Kalita et al., 2016) |
| | Iron oxide | Applications of scanning in in vivo functional research. | Veterinary Medicine | (Soenen et al., 2010) |
| | Calcium carbonate and calcium citrate | Disparities in microparticle and nanoparticle bioavailability | Nutrient delivery | (Huang et al., 2009) |
| POLYMER | Polyacrylate | Assessing resistance to loaded penicillin and enhancing its antimicrobial efficacy. | Biocide | (Turos et al., 2007) |
| | Chitosan | Analyzing the effectiveness of drug dispensing and transferring. | Biocide | (Ghosh et al., 2010) |
| | qPDMAEMA-CNC | Examining the capacity of viruses to bind in order to collect and concentrate infections and particles resembling viruses. | Biocide | (Rosilo et al., 2014) |
| | Triclosan | Increasing organic compounds' antibacterial activity with aqueous nanodisperive methods | Biocide | (Zhang et al., 2008) |
| NANOSTRUCTURED | Carbon (glucose- and sucrose-derived) | Evaluating the activity of drugs against cancer. | Veterinary Medicine | (Ajmal et al., 2015) |
| | Mesoporous silica | Structural visualization of drug discharge in the body | Veterinary Medicine | (Croissant et al., 2014) |
| | Poly(L-lactide)- and Poly(D-lactide)-b-poly(acrylic acid) | Exploration of new number of therapeutic properties | Veterinary Medicine | (Sun et al., 2014) |
| | Albumin-dextran | Create aqueous solutions by Binding with hydrophobic drugs | Veterinary Medicine | (Li et al., 2009) |
| | Biocellulose | Wound dressing by stimulating of collagen | Biocide and Veterinary Medicine | (Napavichayanun et al., 2015) |

**C. Environmental Implications and Sustainability**

When nanoparticles are added to a host polymer, two environmentally beneficial outcomes can occur: first, sustainable nanocomposites made of recycled or bioplastic materials could take the place of common petroleum-based polymers; second, significant plastic savings could be realized by taking advantage of the superior specific properties of the nanocomposites. However, the anticipated advantages may be jeopardized by nanoparticles' inherent environmental load. "Green" polymer nanocomposites made of recycled and bioplastics are environmentally sustainable. A scathing analysis of life-cycle assessment research on nanocomposites and their component parts is provided. Despite their often low concentration, nanoparticles have an amazing effect on the environment. The manufacture of typical nanofillers (nanocellulose, titanium dioxide, silver, and, most importantly, carbon nanotubes) generates significant amounts of greenhouse gases and consumes a lot of energy, negating the benefits of employing green polymer matrices, with the exception of organo-clays and graphene. Achieving optimal performance is therefore essential to genuinely sustainable polymer nanocomposites through material conservation. For this reason, adding more nanoparticles or functionalizing them to improve their dispersion in the host polymer may have unforeseen positive effects on the environment (Carroccio et al., 2022).

In agriculture, crop protection products such as insecticides, herbicides, and fertilizers are essential for maintaining crop growth. The development of sustainable nano fertilizers, nano insecticides, and nano herbicides is imperative due to the substantial losses in food and money caused by plant diseases and insect pests. Maintaining soil fertility is aided with nano fertilizers.

On the other hand, nano pesticides and herbicides improve solubility, prolong the duration of pest control, and shield plants against early deterioration. Plant diseases can be inhibited by directly applying pesticides to seeds and grains (Arora et al., 2024).

Nanoparticle stabilizers (NPS) find application in several domains such as food packaging, nanocoating (which

prevents scratches on surfaces), nanofiltration, nanodelivery systems for agriculture, and numerous agro-industries. In food sciences and food microbiology, NPs are essential because they help identify foodborne infections and prolong food shelf life. For root elongation and seed germination, NPS also garners a lot of attention. They are used in cosmetics like skin creams that fight skin aging by using proteins produced from stem cells (Figure 3).

NPs are used in wastewater treatment, a method that is used all around the world. To supply clean water for drinking and irrigation, they lessen pesticides, fertilizers, and heavy metals. This method is economical and ecologically friendly (Arora et al., 2024).

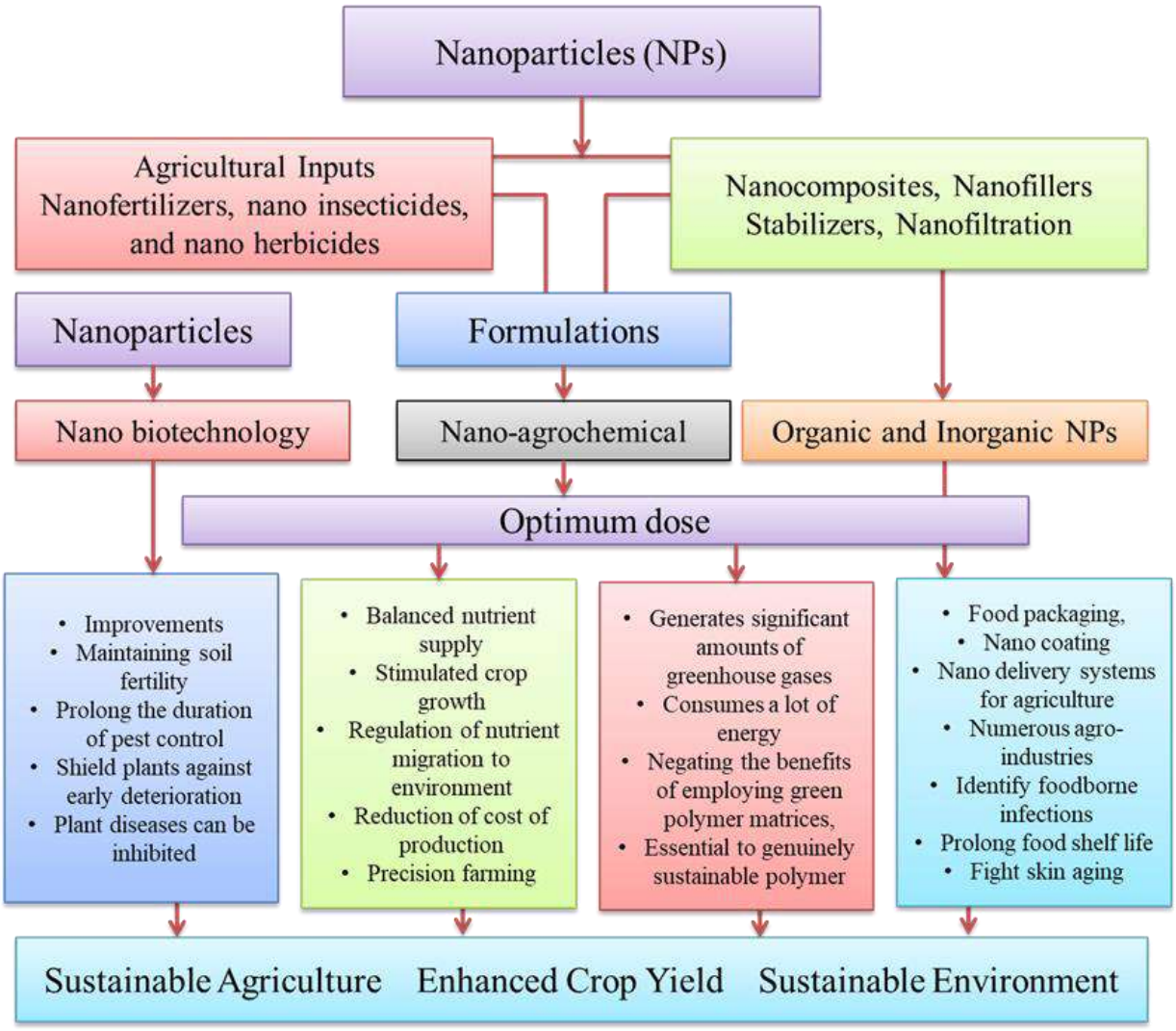


**Fig. 3:** Environmental Implications and Sustainability due to nanoparticles

**Nanoparticles in Reproductive Management**

**A. Improving Fertility and Reproductive Efficiency**

Nanotechnology used different techniques to improve fertility and reproductive efficacy. The use of nanotechnology has the potential to enhance both semen purification and sire fertility testing. The use of nanotechnology applications in cattle farming systems could offer novel and creative ways to address problems with reproductive control (Hill et al., 2017). Nanotechnology can give several pharmaceuticals (including hormones and antibiotics), biological molecules, and nutrients new physicochemical qualities. These include enhanced bioavailability, increased cellular absorption, regulated sustained release, and decreased toxicity as compared to conventional versions.

Nanoparticles improve the hormone based therapies and assisted reproductive techniques, ultimately enhancing fertility of farm animal (Hashem and Gonzalez-Bulnes, 2020).

**B. Applications in Semen Quality Enhancement**

Nanoparticles in semen extender improve the quality of semen and reproductive management. soybean lecithin nanoparticles and selenium nanoparticles in semen extender improved the quality of rooster frozen-thawed semen and bull sperm during cryopreservation leading to overall rise in progressive motility and viability (Khalil et al., 2019; Sun et al., 2021).

While adding thymoquinone nanoparticles GnRH-loaded chitosan nanoparticles to semen extenders boost the ability of buffalo bull spermatozoa to fertilize and cryotolerate and stimulatimg the LH secretion and viable ovulations in rabbits during artificial insemination protocols (Hassanein et al., 2021; Khalil et al., 2023).

Hence, the use of nanoparticle-based techniques proposing novel avenues for the regulation of reproduction and enhancing semen quality and fertility.

**Advancement in Nanotechnology for Agricultural Innovation**

**a. Applications of Nanotechnology in Animal Science**

Nanotechnology offers solutions for veterinary care, food production, and disease treatment in animal science. Nano-capsules protect enzymes and proteins in livestock feed, enhancing yield and effectiveness. Nanoparticles improve the efficiency of medications like antibiotics, vaccines, and probiotics, addressing infections and metabolic disorders. Silver nanoparticles disinfect livestock environments, while nanotubes track hormone levels for better breeding management (Chakravarthi and Balaji, 2010).

**b. Applications of Nanotechnology in Pests and Plant Diseases Management**

In order to manage pests and plant diseases, nanotechnology offers targeted and controlled delivery, revolutionizing chemical application procedures. Nanoparticles reduce environmental contamination while maximizing the effectiveness of fertilizers and insecticides. More precise diagnosis of illnesses like viral infections is made possible by nano-based diagnostic kits, which allow for prompt treatment (Sharon et al., 2010).

**c. Applications of Nanotechnology in the Food Industry**

In the food sector, nanotechnology improves safety, preservation, and packaging. While nanobarcodes track the safety and quality of food, nanocoatings stop oxygen from penetrating food packaging. Foodborne infections and pollutants are identified using biosensors, protecting consumers. Chemicals such as pest are absorbed by nanofibers, which also enable targeted delivery and lessen environmental pollution (Kuswandi, 2016).

**d. Nanofiltration for Water Purification**

In order to alleviate the shortage of freshwater, nanofiltration technologies enhance the desalination and water purification processes. Microbiological pollutants in water are identified by nano-based sensors, guaranteeing its safe ingestion (Khan et al., 2021).

**e. Applications of Nanotechnology in Agronomy**

Nanosensors are useful for precision agriculture because they maximize resource utilization and identify crop diseases and pests. Nanomaterials enhance crop resilience, soil fertility, and machinery durability, all of which support sustainable farming techniques. Food security, environmental sustainability, and agricultural output can all be improved with the help of nanotechnology (Yadav et al., 2023).

Hence, nanoparticles play important role in livestock management in many ways (Fig. 4).

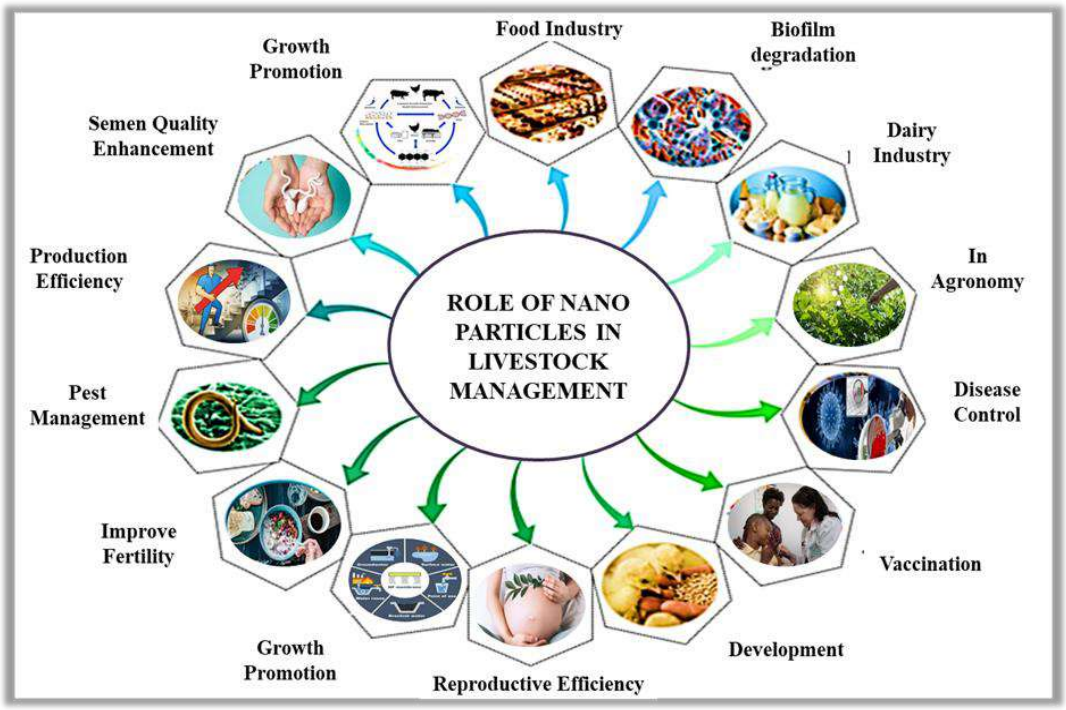


**Fig. 4:** Roles of nanoparticles in livestock management.

**Nanoparticle Safety and Regulatory Considerations**

An important part of nanotechnology is the safeness solicitude associated with the use of nanoparticles. And organizing structure and guidelines are essential to negiciated these issues. Laws are necessary to build protocols for handling nanoparticles and to ensure conformity to safety requirements (Isibor, 2024). A organized approach to criticizing possible risks and controlling the associated risks susceptibility of nanoparticles is presented through a risk assessment and risk management structure (Oomen et al., 2018). Assessing the executive structure to address nanoparticle-related issues and decide their relevancy and effectiveness is an essential step in nanomaterial resource assessment (Linkov and Satterstrom, 2008). Organize the risk control measures and telling decision-making processes is promoted by comparing appraisement of safety assessment factors and definitions of nanomaterials (Boverhof et al., 2015). Additionly, specific criteria for settling the presence of nanoparticles and assessing the risks related to the nanospecificity are provided by the instructions on Technical Requirements for controlled Food and Feed Products applications (EFSA, 2021). Overall, evaluation of risks and risk management techniques are integral and important in the normative framework to assure the safe execution of nanoparticles in various zone and applications.

**Future Perspectives and Emerging Trends**

The oncoming books opinions and arising Trends in Nanoparticles Research for farm animals' administration has great potential to improve farming practices. A notable progress is the combining of nanotechnology with perfection livestock farming that refine sustainability in smart farms by allowing precision sensing and biological heterogeneity (Zhang et al., 2021). These developments are predicted to enhance observing of livestock health and certainly reduce the industry's environmental effects. Real-time data collection is accelerating by advances in wearable sensors and biosensoing technologies that monitor animal health, and provides perceptive information about farm durability management (Neethirajan, 2017). Nanomaterials, nanominerals and nanofertilizers have the implicit to improve crop nutrition and productiveness, which will ultimately improve livestock nutrition and health (Ditta et al., 2015; Raliya et al., 2017; Bhagat and Singh, 2022). Moreover, the use of nanotechnology in stock raising also offers chhances to refine tissue-targeted therapies and mechanobiology that could transform animal care and livestock welfare (Wang et al., 2022). The livestock industry as a whole can greatly increase animal welfare and productivity by using nanotechnology in livestock management.

**Conclusion**

Finally, incorporating nanoparticles into livestock management techniques is a fanatical arrangement which extend many privileges in a field of agricultural setting. Livestock producers can promote animal health, production and welfare while reducing the environmental impact by exploiting the special properties of nanoparticles such as improved bioavailability targeted administration and antimicrobial action. Elevated opportunities to enhance agricultural practices originate from theoretic requisition of nanoparticles in model development, disease prevention, food supplementation, reproductive management, and environmental sustainability. Addressing safety concerns and establishing a strong regulatory framework are essential to ensure the ethical and sustainable use of nanoparticles in agriculture. Additional research and concoction are necessary to fulfill the promise of nanoparticles technology and to talk about new difficulties. This will eventually help create a more springy and viable livestock sector.

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# Chapter 49

# Nanomedicine Marvel

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**ABSTRACT**

Nanomedicine, which combines nanotechnology and medicine, is a new healthcare frontier. Nanomedicine has transformed diagnostics, treatments, and drug delivery methods, as seen in this chapter. Nanotechnology allows early cancer and infectious disease diagnosis with unparalleled precision and sensitivity. Nano-scale biological sensors and imaging agents help clinicians to diagnose and treat patients by revealing cellular and molecular processes. Nanomedicine also solved medication delivery problems, revolutionizing therapeutic treatments. Nano-formulations improve drug solubility, stability, bioavailability and reduce off-target and systemic toxicity. Site-specific medication delivery technologies like nanoparticles and liposomes maximize the effectiveness of therapy while minimizing tissue damage. Nano medicine also has potential for tissue engineering, regenerative medicine, and customized medicine. Nano-materials scaffold regeneration of tissues and maintenance, while nano-scale devices precisely control cellular activity and function. Nanotechnology is used to personalize nanomedicine treatments, improving outcomes and reducing risks. Nanomedicine offers novel solutions to longstanding diagnosis, treatment, and customized medicine problems, transforming healthcare. Nano-medicine has endless potential to transform healthcare and enhance patient outcomes as nanotechnology advances. So, after reading this chapter one will be able to know deeply about the potential and applications of nano medicines marvels.

**KEYWORDS**
Nanotechnology, Nanomedicine, History, Formation, Cure and Applications

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## INTRODUCTION

The Greek word "nano" denotes diminutiveness or extreme smallness and corresponds to a measurement of a one millionth of a meter (10-9 m). Nanotechnology and nanoscience are distinct terms. Nano-science is exercise of nanotechnology to develop functional products such as electronics and other objects. On the other hand, nanotechnology focuses on the examination of structures and molecules within the size range of 1 to 100 nm (Mansoori and Soelaiman, 2005).

Nanotechnology and bio-nanotechnology are completely new concepts from the late twentieth century, and biotechnology has only been around for a few generations; its scope is still being defined. Biotechnology uses scientific techniques and skills to modify cellular, molecular, and genetic phenomena to create items or commodities that are used in a wide range of industries, from medicine to agriculture (Rani, 2017). Bio-nanotechnology is a subfield of nanotechnology that includes atomic-level engineering and manufacturing that is affected by biological antecedents. It is also directly related to biotechnology, but it includes the ability to design and modify the atomic-level complexities of the organisms produced. Bio Nano technology is built to the atomic size and accomplishes 3D molecular tasks clearly, or in broad terms, including (Tolochko, 2009).

By limiting the applications of digital technology, nanotechnology can be defined as an innovation that allows for the controlled initiation of nanomaterials and their implementation, which is, impacting or simply viewing them for their intended use. As a result, it has the potential to taper many biological sciences pathways, and nanotechnology is already deeply ingrained in common biological matters (Tolochko, 2009) (Boulaiz et al., 2011). It can also change our preconceived conceptions and cognitive processes and clearly distinguish between biology, physics, and chemistry. The loading capacity of biopolymer nanoparticles opens up a different amazing possibility in consumption of those nanoparticles as nano-containers used for important targeted approaches (Yousaf et al., 2024). The development of nanotechnology throughout

all fields is based on recently generated nanomaterials that, because of their unique characteristics, rule all fields. Within domains such as medication delivery, diagnostics, aesthetic agents, tissue engineering, and agriculture, the merging of biological topics with nanoparticles is crucial.

It is urged that the biotechnology and pharmaceutical industries use nanobiotechnology more frequently. Throughout the whole pharmaceutical design process, from creating formulations for effective absorption to identifying applications in clinical trials, nanotechnology will be employed The field of nanotechnology is incredibly diverse, ranging from the addition of new techniques based on molecular self-build to the development of novel products using nanoscale real estate and the direct manipulation of atomic barriers. This idea involves applying a wide range of scientific fields, including surface science, organic chemistry, semiconductor physics, cell genomics, and microfabrication (Nasrollahzadeha et al., 2021).

Opponents voiced worries that nanoparticles, such as asbestos, might harm people. A few years back, a study by American experts at the University of Massachusetts revealed that nanoparticles could harm DNA and cause cancer. They caution against the release of nanoparticles into the environment and advocate for strict safety regulations for the manufacturing procedures. Consequently, insurance providers set a maximum coverage amount for nanotechnology-related insurance contracts. International safety standards are what they demand (Nasrollahzadeha et al., 2021).

The term "the future technology" that can solve a wide range of issues is frequently used to describe nanotechnology. Some even predict a revolution in nanotechnology. While there are undoubtedly many advantages and possibilities associated with nanotechnology, it is important for everyone to be aware that even this relatively new technology has risks and is still very much understudied. Environmental issues like pollution and climate change are said to be solvable as well. However, there are drawbacks to nanotechnology as well. For instance, creating useable nanoparticles necessitates the use of hazardous chemicals and solvents, as well as significant energy and water usage. Moreover, food is kept fresher for longer thanks to the application of nano packaging, allowing for longer food transportation (Nasrollahzadeha et al., 2021).

**What is Nanomedicine?**

Nanomedicine is the practice of diagnosing, treating, building, and controlling molecular-level human biological systems through the use of manmade nano-devices and nanostructures. Nanomedicine is exercise of nanotechnology in the field of medicine. It is utilization of nano-materials, nano biosensors andbiological devices (Sahoo, 2021).

Nanomedicine implies to use of nanotechnology in medicinal applications. Nanomaterials, nanobiotech, nano electronic biosensors, and the potential future uses of nanotechnology at the molecular level as biological machinery all contribute to nanomedicine (Chee, 2022).

**History of Nanomedicine**

The field of nanomedicine is relatively new; it all started in 1959 with presentation titled "There's Plenty of Room at Bottom" delivered by Nobel laureate physicist Richard Feynman at the American Physical Society convention. Nanotechnology has been investigated in the fields of medicine, medical technology, and pharmacology since the 1990s. Nanotechnology has only been in existence for a few decades (Freitas, 2004).

The creation of HD microscopy led to simultaneous advancements in the fields of physics, biologyand chemistry throughout the 20$^{th}$ century. This development gave rise tonew disciplines like biochemistry, microelectronics and molecular biology (Wagner et al., 2006). This research study became feasible in the early 20th century with the advent of groundbreaking microscopes, which were essential in exploring the nano universe across all disciplines.

**Classification of Nanostructures**

Currently, nanostructures are classified into two categories: those that exist naturally in nature and those that are artificially created by humans. Natural nanoparticles are formed through the decomposition of plant or animal remnants, erosion of geological materials, volcanic emissions, or through the burning of mineral fuels (Griffin et al., 2017). Nanostructures can also be classified based on their chemical makeup.

The criterion allows for the distinction of the following structures: 3-dimensional, 2-dimensional, single-dimensional, and zero-dimensional (Boverhof et al., 2015).

**Table 1:** Classification of nanostructures according to different criterion (Boverhof et al., 2015)

| Source | Formation | Size |
|---|---|---|
| Natural | Organic | 3D |
| Man-made | Inorganic | 2D |
| | | 1D |
| | | Zero dimensional |

**Formation of Nanostructures: (Top down and Top up method)**

The two most widely used approaches for obtaining nanostructures are the "top down" and "bottom up" procedures (Mabrouk et al., 2011). When structures are obtained via top-down processes, it indicates that macroscopic materials are ground, divided, or disintegrated. This technology comprises grinding, normal material processing and

lithographic process. High-Energy Ball-Milling is a widely used technique for producing nano materials. The initial substance is finely powdered mixture (<100µm) with distinct chemical composition and crystalline structure, as opposed to mechanical synthesis method which utilizes highly pure metal powders. Throughout the process, the material experiences induced stresses. After a prolonged period of time, an indeterminate substance is acquired. The application of heat treatment induces the reversion to the structure of crystals, known as recrystallization. The lithographic method is widely employed in the electronics industry. Its primary application is in the manufacturing of circuits with integrated circuits and transistors that utilize a substrate made of silicon (Salah et al., 2011). This approach comprises a series of sequential phases.

1. One approach is to coat the outermost part of the substrate with a layer that provides protection and is sensitive to light.
2. During the second stage, a negative or positive pattern of desired structure is applied to the insulating layer.
3. Next, the entire object is exposed to radiation and then treated with an etching process. The etching agent selectively reacts with exposed areas that are not protected by a layer of protection. By following this approach, the intended layer arrangement is achieved.

The "bottom up" approach involves the synthesis of nanoparticles by assembling individual atoms, essentially building structures from the ground up. This approach encompasses molecular beam epitaxy, plasma aided deposition, and vapor deposition (Salah et al., 2011).

Physical Vapor Deposition (PVD) is a technique that involves the deposition of solid materials onto a surface by spraying them, using an electron beam, from a target material. A well-prepared surface, known as the substrate, is positioned in close proximity to the material being sprayed, while maintaining a carefully regulated temperature. The sprayed substance gradually descends onto the underlying surface. The thickness and structure of the resulting layer are influenced by the duration of deposition, the velocity of spraying, the temperature of the substrate, and composition of the diluted gas environment (Salah et al., 2011).

Plasma Assisted Chemical Vapor Deposition (Plasma Assisted Deposition) is a technique that utilizes low frequency and radio frequency current discharges to deposit thin layers on both electrically conducting and non-conductive materials. Its goal is to produce layers with unique surface and volume characteristics as well as hard surface layers. Typically, chemical reactions occur when the gaseous atmosphere is electrically activated in this process. (Salah et al., 2011).

Molecular Beam Epitaxy is a procedure that uses molecular beams to deposit thin semiconductor layers in a high vacuum setting with pressures less than 10-7. By employing a diverse range of methodologies, it is feasible to achieve the desired nanostructures based on their intended purpose and application orientation (Salah et al., 2011).

**What are the Reasons for Selecting the Nanomedicine Approach?**

Nano materials can acquire additional functionalities by integrating them with biological molecules or structures. Nano materials possess a size comparable to that of typical biological molecules and structures. Consequently, they hold potential for biomedical research and applications in both in vivo and in vitro settings. In the realm of physical therapy applications, nano-materials have facilitated the creation of diagnosing devices that employ contrast agents, instruments, and drug delivery passage (Sahoo et al., 2020).

Nano-medicine aims to provide a valuable collection of research tools and clinically beneficial gadgets in future. Advanced drug delivery, innovative therapeutics, and in vivo imaging are possible pharmaceutical applications of the Nanotechnology Initiative. Nanotechnology enables targeted drug delivery to specific cells within the body through the use of nanoparticles (Sahoo et al., 2020).

The advantage of utilizing nanoscale materials in medical technology lies in their reduced invasiveness and potential for implantation within the body. Additionally, the use of such materials results in significantly quicker biochemical reaction times. These devices exhibit superior speed and heightened sensitivity compared to conventional drug testing methods (Lavin et al., 2003).

**Applications of Nano-Medicine**

**Cancer cell-imaging**

Cadmium selenide nano-particles, often known as quantum dots, emit light when they are subjected to UV radiation. When injected, these substances permeate cancerous tumors. The surgeon is able to visually perceive the luminescent tumor, which serves as a reference point for doing more precise procedures to remove the tumor (Rani, 2006).

**For Cancer**

Gold nanoparticle shells specifically can directed to adhere to the malignant cells. By locating tumor area using infrared lasers, flesh is not burned while the alloy of gold is heated enough to kill the cancer cells.

**Utilization in Medicine**

This innovation has the potential to address the challenges and hemorrhaging that arise when surgeons attempt to suture blood vessels that have been severed during kidney or cardiac transplantation procedures (Lavin et al., 2003).

**As Nano Electronic Biosensors**

Nanotechnology refers to the progress made in joints swelling recognition equipped with lights and recording devices, enabling surgeons to do surgery through smaller incisions.

**For Physical Therapy**

Photodynamic therapy utilizes a tiny particle that is inserted into the body and then detected using external light. When a particle absorbs light, it can heat up if it is made of metal. This heat can then affect both the particle and the tissues around it (Cavalcantiet al., 2008).

**As Neuro-electronic Interfaces**

The Neuro-electronic interfacing aims to develop nano technologies that enable the integration and connection of computers with the neural system.

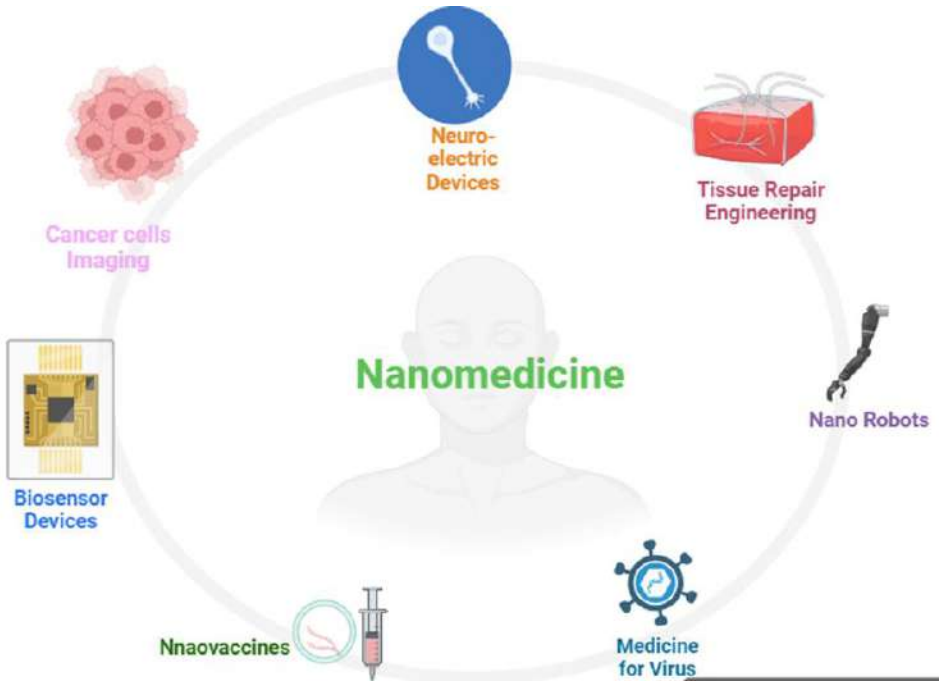


**Fig. 1:** Illustrates the various applications of Nanomedicine across several areas.

**The Application of Tissue Repair**

Nanotechnology has the potential to facilitate the replication or restoration of impaired tissue. Tissue engineering utilizes nano materials, such as scaffolds and growth factors, to stimulate cell proliferation. For instance, carbon nano tube scaffolds can be used to regenerate bones. Tissue engineering has the potential to replace traditional treatments like transplants of organs or artificial implants (Bissau and Baton, 2011).

**Molecular Nanotechnology**

Nanomedicine employs nanorobots that are introduced into body to detect and repair infections and damages. Carbon, specifically diamond/fullerene composites, is the preferred material for constructing these nanorobots due to its inherent strength and other desirable properties. Dedicated desktop nano factories are used exclusively for fabricating these nanorobots. Nanomedicine utilizes nanorobots as miniature surgeons capable of repairing damaged cells and intervening in intracellular structures. These nanomachines have the potential to self-replicate and address genetic deficiencies by modifying or substituting DNA molecules (Raoet al., 2014).

**Nanotechnology for Cancer Treatment**

In recent years, there has been a significant increase in the use of nanomedicines for antitumor therapy, with a particular focus on developing efficient and low-toxic nanoscale drug delivery systems (NDDSs). Among these, liposomes have gained considerable attention and several antitumor liposomes are currently available in the market. DOX liposomes are among the anticancer liposomes that have been investigated the most. By preventing tumor cells from synthesizing DNA and RNA, DOX has strong anticancer effects (Nirmala et al., 2022). On the other hand, DOX also has negative consequences such heart disease and stem cell suppression, in addition to its mutagenic and carcinogenic qualities. Through the enhanced permeation as well as retention (EPR) effect in solid tumors, liposomes provide the benefit of passive medication targeting to tumor tissues, hence lowering drug toxicity. Doxil® is the first commercially marketed DOX liposome product, created in the US by Sequus. It is primarily used for the treatment of recurrent ovarian cancer and Kaposi's sarcoma induced by human immunodeficiency virus (HIV) (Boulaziet al., 2011).

**Utilizing Nanomedicine to Combat Infectious Disorders**

Three primary categories can be used to categorise pathogenic microorganisms: bacteria, fungus, and viruses. The different types of anti-infective medications are classified according to the particular infections they aim to eradicate. They include antiviral medications like albendazole; antibacterial medications like macrolides, penicillin, and metronidazole; antifungal medications like itraconazole, fluconazole, and voriconazole; and antiparasitic medications like albendazole. Based on the various illnesses that these bacteria can cause, this classification was created (Clercq, 2002).

**Nanomedicine for HIV**

HIV/AIDS is now the most common infectious cause of death in adults across world. Despite a lot of research, a cure for HIV/AIDS has not been found yet. There have been 25 approved medications which, for use in environments with limited resources, are available in generic and fixed-dose combinations. However, the 1990s saw a revolution in the treatment of AIDS with the development of protease inhibitors and triple-drug therapy. Because of this, highly aggressive anti-retroviral therapy (HAART) was created, which entails taking three or more medications at once (Clercq, 2002).

**Antiviral Activity against Influenza Virus**

According to Tavakoli et al. (2017), influenza viruses are major causes of respiratory tract illnesses in humans and can lead to seasonal epidemics and even worldwide pandemics. There are just two drugs that have been authorized by the FDA at the moment (Toledo et al., 2018), but the rise of drug-resistant strains in the past few years is really concerning. Consequently, nanotechnology is essential in the search for alternative treatments to combat drug-resistant bacteria. When treating influenza, it is common practice to provide antiviral drugs using metal-based NPs (Levina et al., 2016). In order to create an influenza medication that targets NA inhibitors, the nicotinic antagonist amantadine and the not competitive N-methyl-D-aspartate antagonist have been associated with silver-nanoparticles (AgNPs). Influenza viruses can have their reproduction slowed by these substances. Nanoparticles coupled with antiviral drugs can halt influenza invasion and protect cells by preventing intracellular ROS generation and caspase 3-controlled apoptosis (Li et al., 2016).

**Combatting Hepatitis C Virus**

Hepatitis C, the most common viral infection transmitted through the bloodstream, primarily stems from the hepatitis C virus (HCV) and primarily impacts the liver. The failure of the body to eradicate the virus is accountable for roughly 80% of acute HCV infections progressing into chronic infections (Hekmatet al., 2017). Initially, interferons were employed as the initial treatment for HCV, succeeded by direct-acting antivirals (DAAs) and host-targeted antivirals (HTAs).

Considerable work is currently addressed on nanoparticles (NPs) for delivering several treatments, including anti HCV vaccines and diagnosis of therapeutic agents. These drugs are either attached to or enclosed within nanoparticles of different compositions, such as lipid, metallic and polymeric NPs (Hekmatet al., 2017). NPs are designed with specific characteristics to address challenges, such as maintaining the effectiveness of the medication while reducing the dose of administration and minimizing potential side effects. The sustained impact of anti-HCV medications is achieved through Pegylation of nanoparticles, controlling drug diffusion from the NP matrix (Ishihara et al., 2014), and utilizing electrostatic interactions with oppositely charged carriers (Lee et al., 2012).

**Nano-vaccines**

A crucial element of nanotechnology medicine is the advancement of nano immunizations for several infectious illnesses, including HIV and the virus that causes influenza. Nanotechnology has been discovered to have a pivotal impact in the creation of vaccinations, augmenting their efficacy and specificity. Recently, there has been considerable interest in using nano size materials such as virus-like fragments, liposomes, polymeric nanoparticles, and protein nanoparticles as possible carriers for vaccination antigens and adjuvants (Jangraet al., 2021). These materials have several benefits, such as enhanced stability of antigens, precise administration, and continuous discharge, as they are either enclosed within or connected to the exterior of the NP. The obstacles faced in the process of vaccine development may be surmounted by influencing the immune system through the flexible design of nanomedicine (Mamo and Poland, 2012).

**Nano-Vaccine for HIV**

Since the first report of HIV/AIDS, the search for a safe and dependable vaccine has been challenging for almost thirty years. Extensive research has been conducted on lipid-based techniques for administering HIV/AIDS vaccinations. In a prior investigation conducted by Sakaueet al. (2003), it was demonstrated that when the HIV gp160 protein was enclosed in a liposome and delivered nasally to mice, it resulted in the production of significant levels of neutralizing antibodies that specifically targeted gp160. The liposomes were formed by the amalgamation of cholesterol levels, a substance phosphatidylserine, and phosphatidylcholine. In addition, the HIV gp41 protein was administered using liposomes of varying sizes (ranging from 110 to 400 nm), resulting in the mice and rabbits developing strong antibody responses (Letvin, 2006).

These nano-delivery systems need to include a variety of HIV maybe even ones that are meant to boost the production of antibodies and give them access to parts of the HIV forming glycoproteins that aren't activated during acute HIV infection. Though NPs were used as a transport system, the study found that less HIV antigen and flagellin (adjuvant) was needed to get the same cell reaction (proliferation and IFN-γ production) as Freund's adjuvant (Rostami et al., 2017).

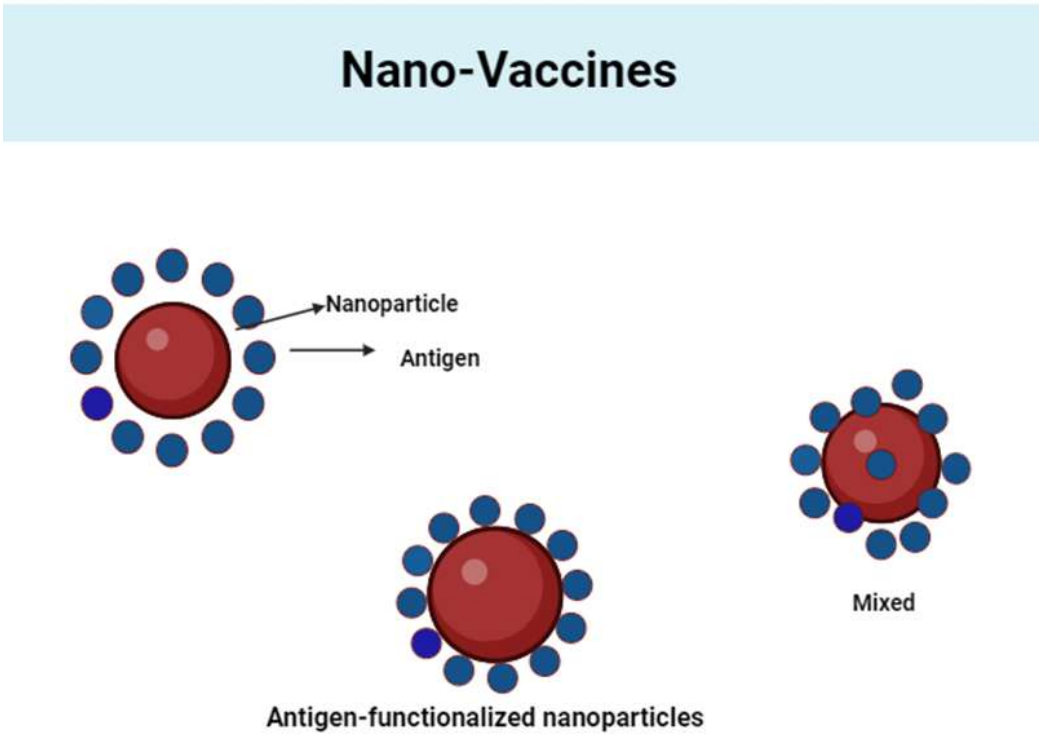


**Fig. 2:** Design of the Nanovaccine

**Nano-Vaccine for HIV**

Complete mucosal respiratory tract influenza protection is excellent. Thus, influenza NP vaccine research is conducted. One of the simplest influenza NP vaccines is a mouse VLP-TIV combo. The VLP/TIV combination induced anti-influenza immunity better than free TIV, especially after intranasal vaccination, based on blood and broncho-alveolar lavage IgG, IgG2a, and IgA titers. Rabbits inoculated with influenza entire virus (WV) encased in chitosan were also able to receive vaccinations via their nasal cavity with NPs (Rioux et al., 2014; Dehghan, 2014). Encapsulating H5 hemagglutinintrimer in polyanhydride NCs created an H5N2 NP vaccination in mice. This method produced high TCD4+ and neutralizing antibody titers. Following an H5N2 nasal challenge, animals were significantly protected (Narasimhan et al., 2014).

**Benefits of Nanomedicine**

Benefits of medicines include: Precise drug delivery to the specific place, to minimize adverse consequences, nano designed devices for molecular targeting, detecting the sickness is pretty straightforward, surgical intervention is unnecessary, the diseases can be readily treated, determine the most effective pharmaceutical medicines for treating the current medical condition or specific infections., conduct diagnostic assessments to identify medical problems and reveal the presence of disease-causing microorganisms, maximize the efficient manufacture of corresponding medications, locate, implant, or affix integrated or target tissue; structures or microorganisms and administer the optimal dosage of a compatible biological agent to the designated target sites.

**Recent Progress in Nano Medicine**

Drug delivery systems have the potential to prevent tissue damage by employing controlled drug secrete methods, lessen drug clearance rates, decreasing distribution volume, and minimizing impact on non-target parts. Nanoparticles can be utilized in therapy to combat antibiotic resistance and take advantage of their antimicrobial properties. Additionally, nanoparticles can be employed to bypass mechanisms of multidrug resistance (MDR).

Some significant applications include the use of iron nanoparticles or gold shells for cancer treatment. Nanotechnology plays a crucial role in discovering new possibilities in drug delivery systems. However, the rapid advancement in this field may lead to challenges related to toxicity, and the effectiveness of drugs may decrease when their concentration falls below the desired levels (Ratner and Ratner, 2002).

**Issue with Nano-medicines**

The current challenges in nanomedicine revolve around understanding the toxicity and environmental impact of materials at the nanoscale, which refers to structures that are billionths of a meter in size (Freitas, 2005). Despite the numerous benefits, the harmful effects of nanomaterial toxicity remain a significant concern for human health and the environment (Gardner and Dhai, 2014). Risks include leakage, spillage, circulation, and agglomeration of nanoparticles. When nanoparticles enter the body through the skin, ingestion, inhalation, or other routes, they can cause damage to vital organs over time (Ali, 2012). The toxicity of nanoparticles also leads to increased reactivity of chemicals and the production of reactive oxygen species.

**Conclusion**

Nano medicine, a novel field of nanotechnology, has had a significant impact on human lives. It encompasses a wide range of medical applications, including drug delivery systems, cancer therapies, and tissue culturing. The potential for nano medicine to enhance health outcomes is vast. However, to fully capitalize on its benefits for both individuals and populations, it is crucial to involve public health expertise. This involvement will aid in identifying areas with the greatest need for technological advancements, optimizing funding allocation, and shaping policies to safeguard human well-being and environmental integrity, thereby promoting better health maintenance.

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Chapter 50

# Nano Particles in the Battle against Disease

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**ABSTRACT**

Nanoparticles have revolutionized the field of medicine with their diverse applications in disease treatment and diagnosis. This study explains different kinds of nanoparticles and how they can be used to fight against diseases. These include organic nanoparticles, polymer nanoparticles, liposomes, micelles, dendrimers, and metal-based nanoparticles like iron, gold, and silver. Organic nanoparticles and polymer nanoparticles are valuable for drug delivery due to their ability to encapsulate and release therapeutic agents in a controlled manner. Liposomes and micelles enhance drug solubility and stability, improving the efficacy of treatments. Due to their highly branched structures, dendrimers provide therapeutic applications with multi-functionality and precise targeting. Silver, gold, and iron nanoparticles are among the special qualities of metal nanoparticles that include targeted therapy, improved imaging, and antibacterial activity. Collectively, these advanced nanoparticles provide new and promising ways to treat a wide range of diseases more effectively and personalized, expanding the possibilities of modern medicine.

**KEYWORDS**
Nanoparticles, Drug delivery, Therapeutics, Metal Nanoparticles, Disease treatment

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## INTRODUCTION

Tiny bits of stuff ranging from one to a hundred nanometers are called ultrafine or nanoparticles. This term can also include really small fibers and tubes in just two directions, or bigger particles up to 500 nm (Suhag et al., (2023). At the smallest scale, metal particles smaller than one nanometer are usually called atom clusters. This study highlights the importance of understanding nanotechnology because it has the potential to be super useful in many areas. Nanotechnology has already made big improvements in things like transportation, security, farming, healthcare, electronics, and more. It's basically making a positive impact on various aspects of technology and the economy. Nanotechnology is a mix of different fields like chemistry, engineering, materials science, and biotechnology. It uses these to make cool materials with special features, all based on their super tiny size. This book explores how nanotechnology has been used a lot recently, and its influence on big tech advances makes it a really groundbreaking science. Nanotechnology is like a problem-solving superhero because it's expected to help fix many issues we have. It does a bunch of things, like making tiny materials and trying to control stuff right at the tiniest level. It even messes around with the physics of things we already use and comes up with new ways to build stuff using molecular self-assembly. Tiny particles called NMs are like important players in today's medicine. They help deliver medicine and genes to tumors and are good for imaging too. Sometimes, they can even do studies and treatments that we couldn't do otherwise. But, they bring challenges for society and the environment, especially in terms of being harmful. So, we need to carefully check how we use them in medicine. Understanding how these tiny particles affect diseases can also help us come up with better ways to diagnose, treat, and prevent illnesses (Malik et al., 2023).

This review highlights how tiny particles (NPs) have been super helpful in today's medicine and delivering medications. It also tries to guess how nanotechnology (NT) will change things. In a newer field called pharmaceutical nanotechnology, there are cool tools and opportunities that might really help with diagnosing and treating illnesses. Recently, medicines based on tiny particles have shown a lot of potential for delivering drugs where needed and for bioactive and diagnostic purposes. Nanotechnology is pretty clever because it gives us special materials for building body tissues. In the field of nano-engineered drugs, we use smart tools for predicting, treating, and delivering medications. There are already some products and ways of delivering drugs based on nanotechnology. In pharmaceutical

nanotechnology, we use tiny technology to make products better by changing them in various ways as shown in Figure 1. Medicines with these tiny changes have special features like staying in the body longer, going to the right place, and working better (Joseph et al., 2023).

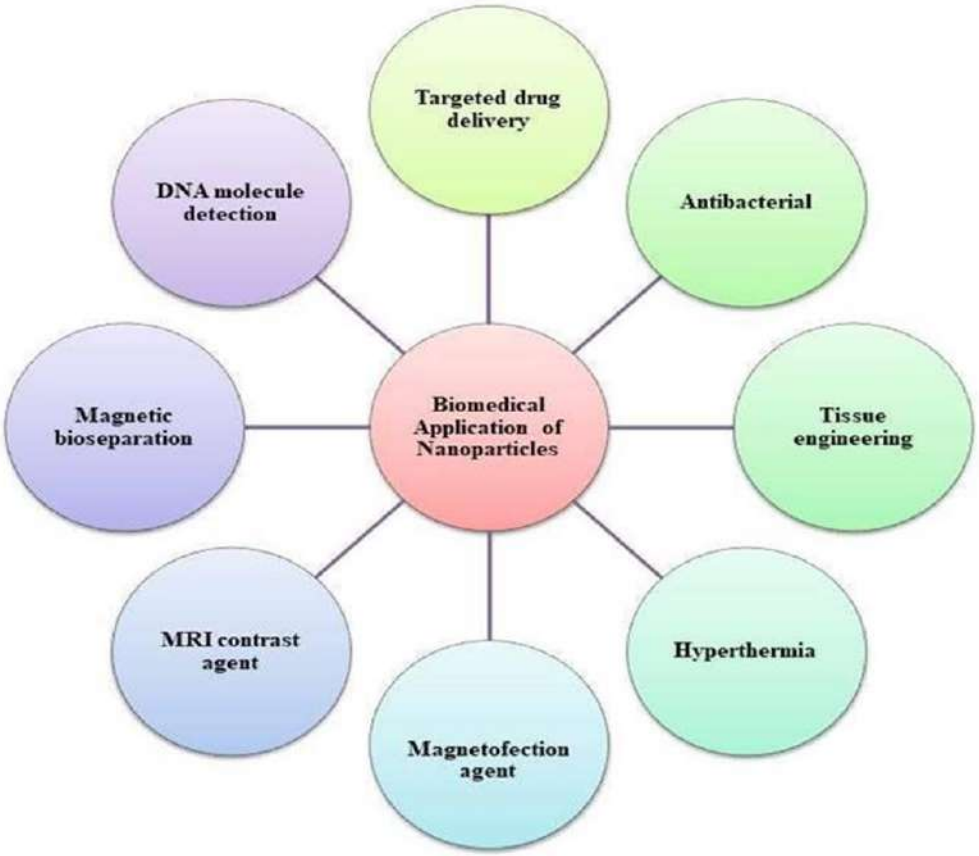


**Fig. 1:** (Flowchart of uses of Nanoparticles)

Tiny medicines, also called nano-pharmaceuticals, have changed the way we give and get drugs in medicine. These include things like tiny particles made of different materials. They have the power to prevent illnesses and help us understand diseases better. But, there are some new health risk details that make it tricky to use them in medicines. Scientists are working hard to find solutions for problems like safety issues, ethical concerns, and other challenges in using these tiny medicines.

Hasan, (2015) explained that Scientists today still don't have enough info and advice on using tiny materials and technologies safely. So, when it comes to using nanotechnology in medicine, we're still in the early stages. This chapter gathers what we currently know about tiny particles and the risks they might pose to health, along with the types of tiny medicines that are most commonly used. Nanotechnology involves making and using materials that are super tiny, up to 100 nm in size. It looks into how things behave at really tiny levels, which can help improve tools and processes in biotechnology and medicine, making them cheaper, easier to carry, safe, and simple to use. Tiny particles, called nanoparticles, are used for many things like labeling in labs, treating certain cancers, making optical devices, and even in everyday stuff like clothes and makeup. They're pretty interesting because they resist damage, conduct heat well, and fight against germs. We can make these tiny particles either through chemicals or by using living things. Different types of metallic nanoparticles, like gold and silver, have big uses in industries. This overview aims to talk about these tiny particles, focusing on the types and how they're made.

**Organic Nano Particles**

Tiny organic particles, like liposomes, polymeric nanoparticles, micelles, and dendrimers, are quite fascinating for many medical uses, such as treating cancer and transporting drugs. Scientists have put in a lot of effort to create these particles with special shapes and features. This review will quickly cover the things used to make these tiny particles and the forces involved. We'll talk about different ways of making them, like self-assembly and microemulsion. After discussing some techniques to change their surfaces, we'll share a vision for better ways to make these tiny particles in the future. We believe people studying medicines, materials, biology, and chemistry will find these topics interesting (Begines et al., 2020).

**Polymeric Nanoparticles**

New advancements in medicine are revolutionizing theranostics (therapy and diagnosis) through the use of newly developed radiolabeled nanosystems. These nanosystems include polymeric nanoparticles, carbon nanotubes, silica

nanoparticles, dendrimers, liposomal carriers, magnetic iron oxide nanoparticles, and inorganic metal-based nanoformulations. Among these, polymeric nanoparticles are gaining attention in the biomedical field due to their favorable characteristics, such as biodegradability, low toxicity, efficient absorption, low surface to mass ratio, and ability to transport other molecules.Polymeric nanoparticles can be attached to radioactive substances, enabling them to carry significant doses of radionuclides for diagnostic, therapeutic, and testing purposes. There are two methods for labeling these nanoparticles: direct labeling and indirect labeling. The choice between these methods depends on the desired qualities of the nanoparticles. One advantage of radionuclide treatment is its ability to spare healthy surrounding tissues while delivering a concentrated dose to the targeted area.In simpler terms, radioactive polymeric nanoparticles show promise in treating and diagnosing various medical conditions, including infectious diseases like tuberculosis, cardiovascular ailments such as cardiac ischemia, and different types of cancer cells or tumors (Wu et al., 2020).

Polymers, which are made up of repeating units called monomers, play a crucial role in drug administration. Polymer nanoparticles come in various forms like nanoshells, polyplexes, nanospheres, and polymersomes, offering a wide range of chemical (biocompatibility, hydrophobicity) and physical (modulus, responsiveness, tunability) characteristics. These polymers can be customized for specific applications by choosing the right monomer, production method, and synthesis approach.In the field of nanomedicine, polymers are versatile and have been employed in vaccine carriers, cancer treatments, pulmonary medication, cardiovascular delivery, antibiotics, and immunological engineering. The flexibility of polymers allows them to be tailored for different medical purposes (Jarai et al., 2020).

**Liposomes**

A big challenge in treating neurodegenerative diseases is getting medications into the brain because of the blood-brain barrier (BBB). We are particularly concerned with advances in drug delivery systems using liposomes to enhance drugs' crossing of the BBB to treat brain illnesses. We focused on Parkinson's and Alzheimer's diseases as examples of frequent chronic neurodegenerative affections (Seo et al., 2021).

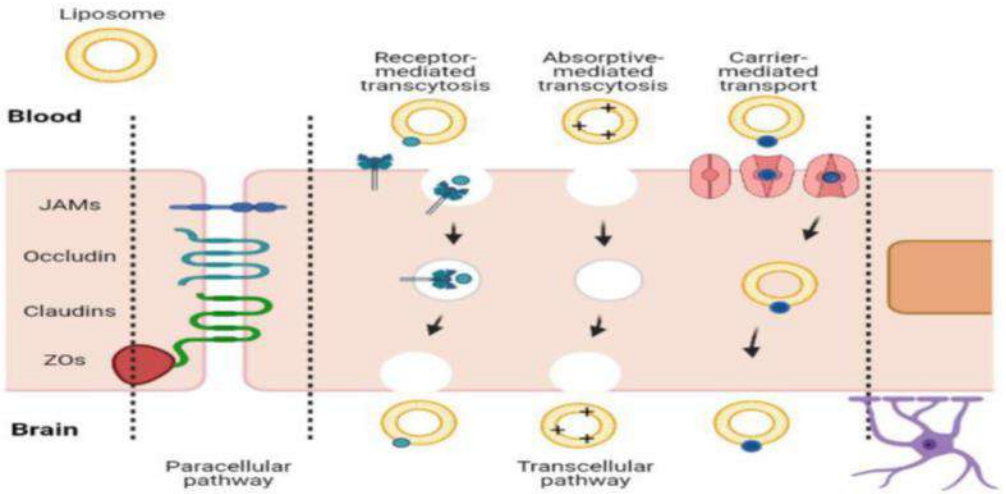


**Fig. 2:** Drug transport mechanism of liposomes in BBB (Seo, and Park, 2021).

For the improvement of these drugs for these diseases various liposomes with modified surfaces have been prepared including BBB targeting agents (Figure 2). These altered liposomes can cross the blood-brain barrier by a process known as transcytosis. Melting development in liposome engineering offer solutions that enhance the way drug is administered making treatments better. Having the knowledge of the alteration of conditions in the barriers at the body's physiology level is important when need be to advance the penetration of liposome.

**Micelles**

Amphiphilic copolymers are discussed as a new class of block copolymers which have received much attention from the scientific community over the past few years as suitable materials for forming polymeric micelles with selective abilities of delivering medicines, proteins, peptides, and genes to the brain. It is for these copolymers that can be described by the following peculiarities: their very small size, the charge switching feature, the ability to all stimulus-triggered cargo release, the fact that they have a flexible structure and are capable of self-assemblying. These characteristics help in the avoidance of the problems inherent in the traditional drug formulations for the management of neurological disorders, such as instability, poor penetration into the brain, rapid dissolution and clearance from the brain. The copolymers have better penetration and retention and are easily tunable and, therefore, suitable for diagnosing various brain diseases. This article discusses other modification of micelles including the inclusion of stimuli-sensitive regions on the copolymers; use of smart linkers between cargo molecules that respond to stimuli; and attaching intercalating agents and imaging moieties for the brain targeting and imaging. Details of how polymeric micelles are vital in delivering neurotherapeutics to the brain is also included in the paper. Some of the patents related to polymeric micelles for brain delivery are also described here for the sake of completeness (Kaur et al., 2022).

**Dendrimers**

Dendrimers as a group of macromolecules that possess certain aspects of long polymers, and also single molecules. They are versatile in many environmental, medical and diagnostic uses because of their ability to incorporate more components into their form and because of the three dimensional shape. Thus the dendrimers have been studied in several subjects including biochemistry, biotechnology and chemistry with respect to their structure and composition. They have shown potential to be used as antiviral, antifungal as well as antibacterial, besides being used as catalysts. For example, peptide dendrimers have been studied for the delivery of genes, antigens, synthetic vaccines and MRI contrast agents. This review mainly focuses on the variety of dendrimers, methods of their preparation as well as brief information on their limitations and potential use in the therapy of infectious diseases (Filipczaket al., 2021).

**Inorganic Nanoparticles**

1. Silver nanoparticles
2. Gold nanoparticles
3. Copper nanoparticles
4. ZnO nanoparticles
5. Iron oxide nanoparticles.

**Silver Nanoparticles**

It is fast developed field with multiple applications and special features mentioned by Almatroudi (2020). Nanomedicine can be described as the clinical application of nanotechnology in the combating as well as management of diseases. Silver nanoparticles in this discipline is one of the most important. The nanoparticles being particles with size between 1 and 100 nm.. They can create different shape when working at the nanoscale level and possess variety features. The best known biomedical applications of Ag NPs include the direct bacterial and cancer cell killing, as well as enhanced wound healing. Also, they are cost-effective to create, and this is a conclusion that can easily be arrived at due to the following reasons. In this work, an attempt has been made to explore the different methods pertaining to physical, chemical and biological synthesis of silver nanoparticles. also is interested in the numerous medical applications of silver nanoparticles considering bone regeneration, tumor therapy, anti-infection, dental implant improvement, and wound recovery. The formation of silver nanoparticles, their mode of action and the techniques for studying the shape and structure of the nanoparticles are other topics addressed by the research in an effort to provide a clearer perspective of the part played by the nanoparticles in illness control and medicinal treatments.

Silver nanoparticles or in short AgNPs, are used in broad range of medical specialties and has been in the focus of attention as antibacterial materials. In developed studies it has demonstrated that the oral microbiota is not simply but rather intricate and the viable strains, which were unknown so far, have been identified. AMP is a small peptide that has been examined because of its invasive capacity of bacteria. In this work, a conjugate of the potent antibacterial peptide indolicidin with AgNPs was synthesised. The size and the optical properties of the AgNPs were analyzed with the help of optical spectroscopy and microscope. Gram positive and Gram negative bacteria were chosen for the experiment to check antibacterial properties of the coated nanoparticles done with oral samples. The results made clear that MIC of the coated nanoparticles was rather low and ranged between 5 to 12 only. Thus, 5µg/mL was effective in preventing the growth of bacteria, used for the experiment. Some examples are said to have suffered this due to the hallmarks of the indolicidin-coated metal surface. In addition, the elongated coated nanoparticles were proved less toxic than the peptide or the naked AgNPs when used individually. The biofilm reducing capabilities are AgNPs coated with AMPs are quite high, especially in oral infections, and require further research (Hamad et al., 2020).

**Gold Nanoparticles**

Thus, vaccinations that strengthen our immune system are perhaps the most effective protection against diseases (Dykman, 2020). As such, it is critically important to choose the right carrier to ensure a strong immune response in the process of manufacturing of antibodies or vaccinations. Determining an appropriate nanoscale particle transporter 'Gold nanoparticles' holds a lot of potential in this field. These are the immunogenic substances referred to as antigens and as Capable of encapsulating or adsorbing these, the microscopic particles are termed as such. In the process of vaccination, we can enhance the immunological effectiveness, using gold nanoparticles as carriers. Depending on which cells they stimulate and how they control the launch of antigens, they can act as adjuvants and enhance the potency of vaccinations (Figure 3). To raise the probabilities of using gold nanoparticles as a base for creating new vaccines against bacterial, viral, and parasitic diseases, one should study the human person's immunity to them as adjuvants and carriers.

Gold is shiny, that is why many people think of it as a royalty metal (Ko et al. , 2022). Some of them are its molecular recognition capacity, ease to process into shapes and forms and biocompatibility.

**ZnO Nanoparticles**

They are colourless powders, and are non-hazardous as they do not dissolve in water or alcohol (Deka et al., 2022). It possesses some attributes such as semiconducting and piezoelectric, which makes them to have many uses. Zinc oxide is enzymatically depositional, almost non-toxic and can be easily grafted with various materials on its surface. Zinc is one of

the important trace minerals that are distributed in muscles, brain, skin, bones, and many other tissues. Nano-ZnO is a very small sized particle and gets easily penetrated and assimilated into the body and is used in food products. It is crucial to know the characteristics of nanoparticles particularly their shape and size and functionality for biological application. Using the biological and non-conventional synthesis of ZnO-NPs in this review, the ensuing applications of the ZnO-NPs include; anticancer, antidiabetic, antimicrobial, anti-inflammatory and antioxidant properties. ZnO-NPs also can be used as drug delivery systems, imaging and biosensors.

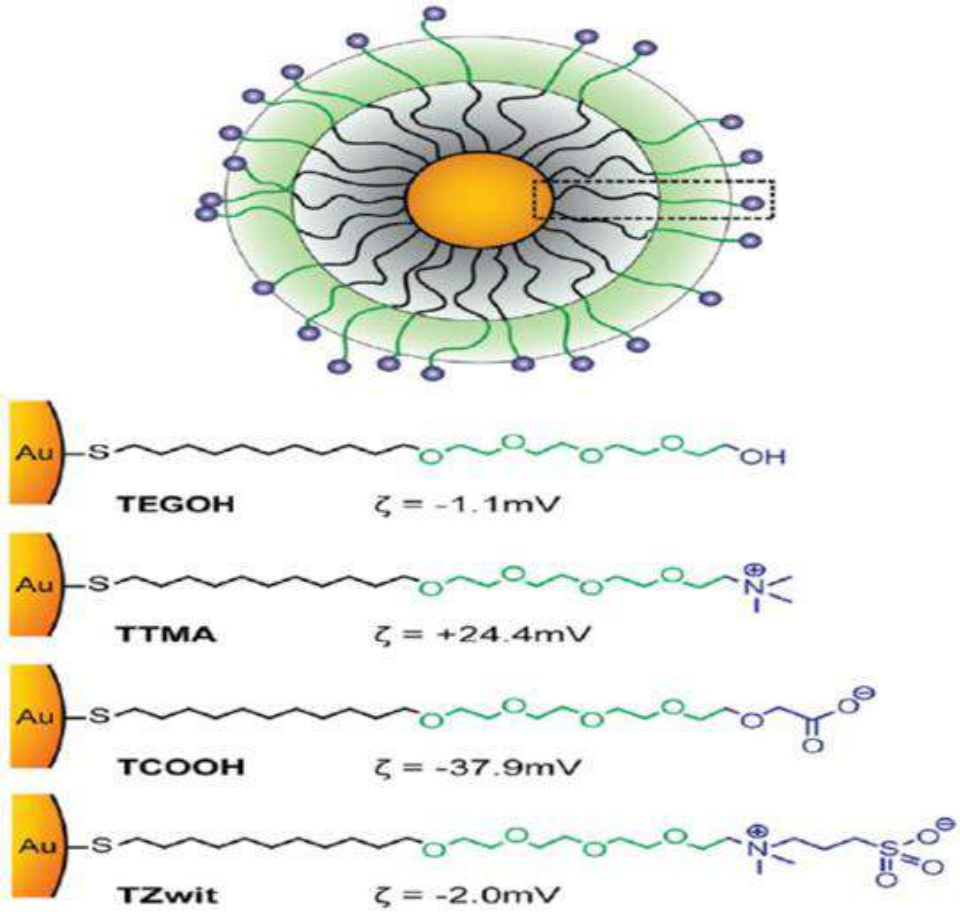


**Fig. 3:** (Structure of Gold Nanoparticle)

This study, thus, Cancer is a life-threatening illness precipitated by the development of new abnormal cells (Anjum et al., 2021). The approaches such as medications and chemotherapy are however restricted by low efficacy, availability of the limited stock and toxic impacts. The branch explores the medical applications of this coming field in nanotechnology, which is still in a state of development. Inorganic particles of very small dimensions, typically in the nanoscale, are prospective in cancer diagnosis and therapy due to the features of ZnO NPs. ZnO NPs cause specific cytotoxicity of cancer cells by producing ROS and causing cellular dysfunctions and final cell death. They also assist in introducing plant medical compounds as well as chemotherapy medicines to the tumor cells. This term paper focuses on the utilization of small zinc oxide particles on recognition of cancer cells and bioimaging. It focuses on the role of ZnO NPs as drug delivery systems and examines these issues as such: surface alteration, drug incorporation, and controlled release. It also summaries the anticancer property of ZnO NPs on various types of cancers and their perspective mechanisms. Also, it overviews the existing drawback and the probable future uses of ZnO NPs in cancer therapy.

**Copper Nanoparticles**

These are some of peculiarities of copper nanoparticles (CuNPs) which are perspective in the field of medicine (Ghasemi et al., 2023). Here, we described our study of the impact of copper nitrate on the oxidative stress and cell death in SW480 human colorectal cancer cell line. To determine the effect of CuNPs on cell viability we performed MTT assay after 24 hours. We quantified Reactive Oxygen Species (ROS) to determine oxidative stress or lack of it and we also determined antioxidant enzyme activity. To detect the cell death, the percentage of Hoechst33258 staining after treatment was assigned and the Bax, Bcl-2 and p53 proteins profiles were detected using qRT. The effect of CuNPs on SW480 viability was assessed by MTT assay and it was observed that CuNPs inhibited cell growth. There was a marked elevation of ROS production in all doses; 31, 68 and 100 µg/mL. CuNPs up regulated Bax and p53 as well as down regulated Bcl-2. Last but not the least the observations made using Hoechst staining that highlighted down the cell death. Taken together, the CuNPs were effective in evaluating the apoptotic effect and so CuNPs may be useful in anticancer effects.

**Iron Oxide Nanoparticles**

Iron oxide particles are the subject of this review which is subcategorized as magnetic nanoparticles (MNPs). MNPs show great promise in various medical applications like using electromagnetic heat, enhancing MRI data, aiding in tissue engineering, and improving drug delivery to challenging microenvironments. Their integration into medical treatments marks a significant growth in using advanced biotechnologies in healthcare. Superparamagnetic nanoparticles (SPNs) can be utilized by doctors to provide localized heat that eliminates bacterial biofilms, and they can also physically disrupt bacterial cell walls, making them more susceptible to antibiotics. Iron oxide nanoparticles (IONPs) enhance the delivery of bactericidal substances to microenvironments, showing potential in treating diseases that require therapeutic intervention, including those crossing the blood-brain barrier. This review thoroughly explores the use of magnetic iron oxide nanoparticles in treating bacterial diseases, focusing on their antimicrobial activity against bacteria (Lamichhane et al., 2021).

**Nanobased Platforms for Cardiovascular Diseases**

Cardiovascular diseases (CVDs) are a significant threat to public health, and traditional drug treatments face challenges like inefficiency and drug resistance. To address these issues, advanced methods for early detection and treatment are crucial. Nanotechnology and nanomedicine offer promising solutions by creating personalized diagnostic and therapeutic agents for CVDs. Nanoparticles act as tiny carriers delivering drugs effectively to damaged areas, addressing problems like bioavailability and solubility. They enable precise medication and gene delivery by binding to specific target molecules on their surfaces. In cardiology, nanoplatforms are popular due to their small size, facilitating easy penetration into heart and artery tissues, higher sensitivity and specificity, and passive or active targeting of cardiac tissues. However, concerns about nanoparticles' immunogenicity and cytotoxicity must be carefully considered (Shariati et al., 2023).

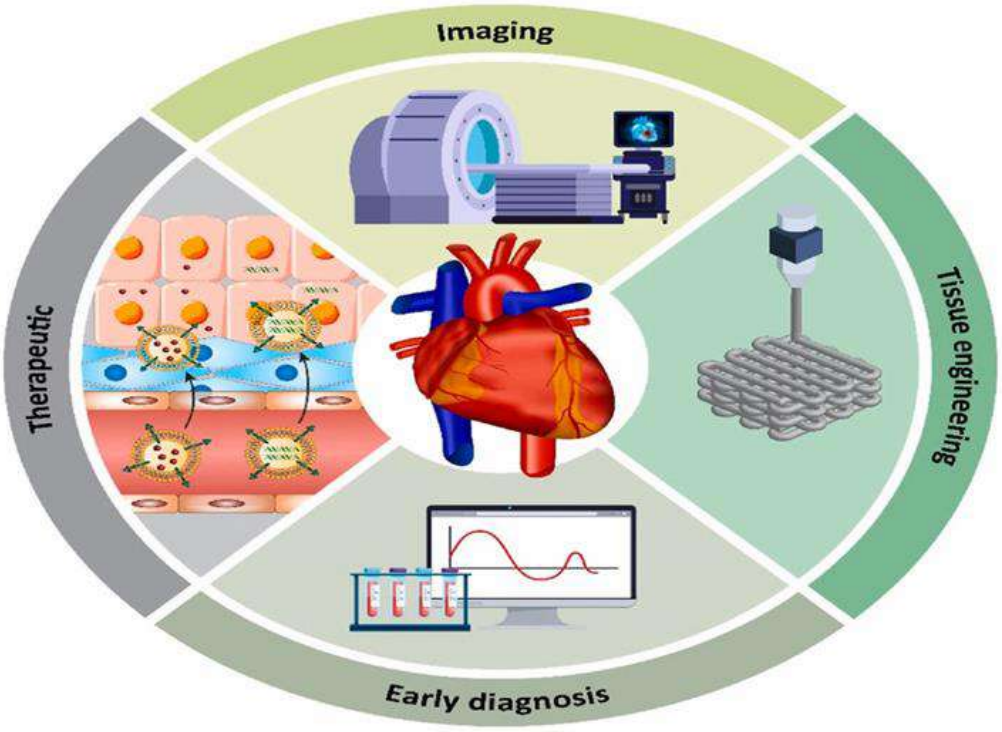


**Fig. 4:** Nanobased Platforms for Cardiovascular Diseases (Shariati et al., 2023)

Nanoparticles also show potential for imaging and diagnosing CVDs, providing simple diagnostic processes and real-time tracking during treatment. Nanotechnology has transformed cardiovascular imaging with multimodal, multifunctional vehicles outperforming traditional techniques. This overview discusses nanomaterial delivery methods, targeting strategies, and current developments in treating, diagnosing, and tissue engineering for CVDs (Figure 4). It explores future applications of nanomaterials in CVDs, aiming to improve cardiovascular care in clinical settings. Improving nanocarriers and delivery techniques could enhance therapy efficiency, reduce adverse effects, and improve patient outcomes (Zhong et al., 2022)

**Antimicrobial Abilities of Metal Oxide Nanoparticles**

The number of infectious diseases worldwide has increased significantly, with bacterial infections being a major cause of potentially fatal illnesses. Although progress has been made in treating bacterial diseases, the risk of death and illness remains high because bacteria are becoming resistant to commonly used antibiotics. In this era of increasing antibiotic resistance, it is crucial to find new ways to detect and develop advanced antibacterial agents to combat harmful bacteria.

Researchers are now exploring the potential of metal oxide-based nanoparticles, such as MgO, Ag2O, CuO, CaO, TiO2, and ZnO, for their antibacterial properties. These nanoparticles have tiny sizes that enable them to effectively interact with the active sites of microbes and disease-causing biomolecules due to their high surface-to-volume ratio. This has sparked interest in using metal oxide nanoparticles as next-generation antibacterial agents. Ongoing research on their antimicrobial properties aims to discover alternative solutions against antibiotic-resistant bacteria, making it a continuously growing and intriguing area of study (Panda et al., 2021).

**Advantages of Nano Biotechnology**

Nanomaterials offer a big boost to biomedical research by easily and strongly connecting with biological samples. This compatibility makes nanomaterials a valuable tool for improving various aspects of biomedical studies (Figure 5).

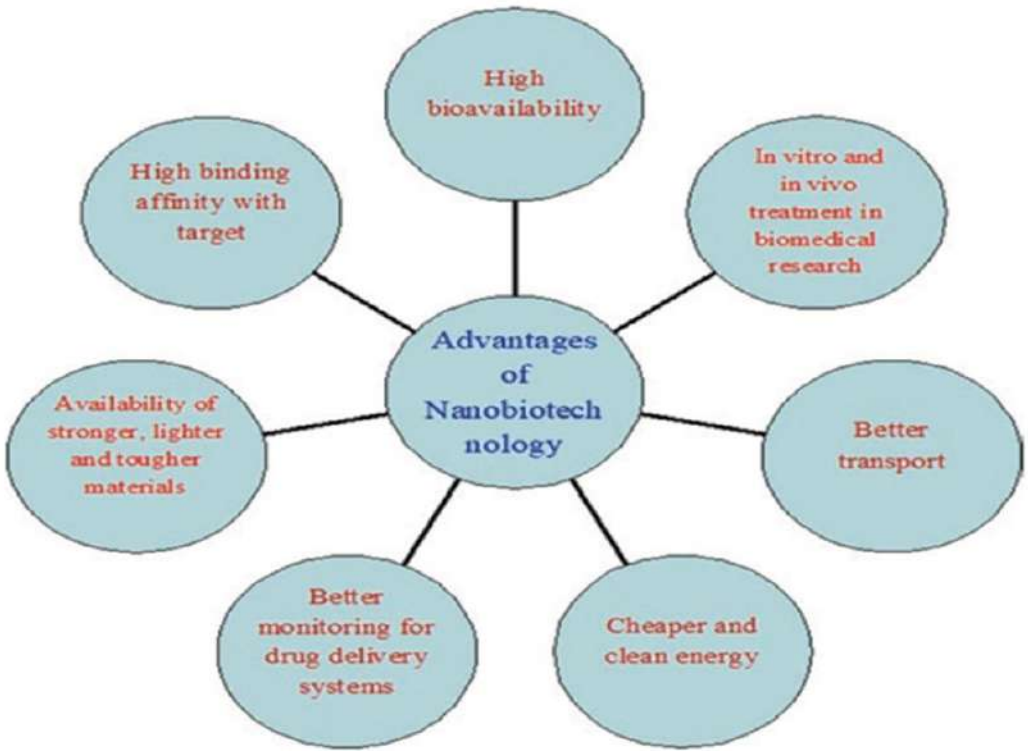


**Fig. 5:** Factors involved in the advancement nanobiotechnology in various research areas (Panda et al., 2021).

**Potential Drug Delivery Pathways for Treatment of Alzheimer's Disease**

Alzheimer's disease (AD), a common neurodegenerative condition affecting mostly those over 65, lacks effective medications despite substantial global spending on its treatment. The Blood-Brain Barrier (BBB) poses a challenge in treating brain diseases like AD as it restricts the entry of most drugs into the brain. After reviewing various publications from PubMed, a biomedical and life sciences journal archive, three potential drug delivery methods have been explored for their effectiveness in getting drug molecules into the brain: inorganic nanoparticles, multifunctional liposomes, and transdermal delivery systems. This research discusses a specific experiment supporting these delivery methods and provides a brief overview of each. It also examines the advantages and disadvantages of each delivery approach (Wang, 2022).

**Nanotechnology against the Novel Coronavirus**

COVID-19, a newly-emerging infectious disease, has significantly impacted society, leading to increased mortality and illness. Currently, there is no authorized vaccine or effective treatment for this pandemic. To tackle the virus, it's crucial to explore innovative methods, especially those involving nanotechnology. This review aims to present various ways to create COVID-19-resistant medicines and diagnostics using nanotechnology. Some promising strategies involve using polymeric materials, inorganic self-assembling materials, and peptide-based nanoparticles to combat and detect COVID-19. The review summarizes the exciting developments in using nanomaterials for preventing, diagnosing, and treating COVID-19 (Rashidzadeh et al., 2021).

**Conclusion**

In conclusion, a wide range of nanoparticle such as organic, polymer, liposome, micelle, dendrimer, and metal-based varieties like iron, gold, and silver, have innovative potential for the treatment of various diseases. Their particular characteristics boost medication methods of administration, increase the effectiveness of treatments, and allow for more precisely targeted therapies. Drug stability is increased by liposomes and micelles, while controlled distribution is facilitated by organic and polymer nanoparticles. Dendrimers offer versatility and targeted delivery, while metal

nanoparticles bring antimicrobial properties and advances in imaging. Collectively, these innovations indicate an enormous development in medicine, opening up new avenues for modern healthcare and offering more effective, individualised treatments for a range of illnesses.

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Chapter 51

# Innovative Nanomaterials: Applications and Challenges in Molecular Diagnostics

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**ABSTRACT**

The field of molecular diagnostics has been revolutionized by nanotechnology as it has provided an opportunity to get precise detection and monitoring of diseases at the molecular level. This book chapter aims to highlight the applications of innovative nanomaterials, while also addressing the limitations and challenges encountered in their implications. Fundamental principles of nanomaterials along with their unique properties are discussed. Various nanomaterials such as quantum dots, gold nanoparticles, and carbon nanotubes are explored with an elucidation of their specific role and functions in diagnostic assays. They have a vast range of applications in nucleic acid detection, protein biomarker analysis, and imaging techniques. Nanomaterials enhance sensitivity, specificity, and multiplexing capabilities. Thus, they have revolutionized disease detection and monitoring. Alongside the advancements in molecular diagnostics due to nanotechnology, issues such as biocompatibility, toxicity, scalability, and reproducibility are thoroughly examined, providing an insight into the current hurdles hindering widespread adoption. Thus, a comprehensive overview of innovative nanomaterials will be provided driving advancements in molecular diagnostics. It will provide a better understanding to researchers and practitioners about the challenges and inherent potential of using nanotechnology for molecular diagnosis.

**KEYWORDS**
Molecular diagnostics, Nanotechnology, Nanoparticles, Diagnostic assays, Biomarkers

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## INTRODUCTION

Molecular diagnostics is a part of a clinical laboratory that includes tests and methods involved in the identification of disease. Advancements in molecular diagnostics have enabled basic research and better results in diagnostics. Still, the use of molecular diagnostics can be improved beyond current nucleic acid testing. Overall, molecular diagnostics has a role in public health, medicine, the pharmaceutical industry, forensics, drug discovery, and biological warfare (Nishonov and Nuriddinova, 2022).

Due to nanotechnology, the limits of molecular diagnostics have been extended to the nanoscale. This increase in precision has improved the flexibility and sensitivity of tests. Each type of nanomaterial has its functions in diagnostics. Magnetic nanomaterials are bound to suitable antibodies and then can be used in labeling specific molecules, microorganisms, or structures. Gold nanomaterials are used for the detection of genetic sequences in a sample by tagging them with short sequences of DNA. Similarly, quantum dots are involved in multicolor optical coding for biological assays. Developments in nanoengineering have increased the use of nanostructures in biosensors. Different types of nanostructures including 0D, 1D, and 2D are being used to enhance sensitivity, selectivity, time, and limit of detection in biosensors (Welch et al., 2021).

### Fundamentals of Nanomaterials in Molecular Diagnostics

Nanomaterials are a group of small-scale substances having structural components less than 1 µm in at least one dimension. They can be categorized into three types based on their source which include: natural, incidental, or engineered. They can be single, fused, aggregated, or agglomerated having tubular, spherical, or irregular shapes.

Nanomaterials can have a natural origin, but engineered nanomaterials are of more interest as they are used in many processes and commercial goods. They are present in cosmetics, sunscreens, electronics, stain-resistant clothing, and medicines etc. (Mohd-Setapar et al., 2022).

**Types of Nanomaterials**

Nanomaterials can be divided into various types. Based on their applications in molecular diagnostics, they have the following types.

**Quantum Dots**

These are semiconductor crystals and were first discovered by a Russian physicist. Quantum dots are composed of groups II-VI or III-V elements of the periodic table. They have physical dimensions smaller than the Bohr radius of exciton (Kargozar et al., 2020). They have unique characteristics of sensitivity and selectivity when used in nano-sized particles. They also possess unique optical properties which enhance their widespread use in biomedical applications. For example, an exhibition of strong and long-lived photoluminescence emission makes it suitable as a luminescent dye having biomedical applications such as biomarker quantification, molecular imaging, and biomolecule targeting (Soldado et al., 2022).

**Metallic Nanoparticles**

Metallic nanoparticle is a new terminology that originated in recent years. Noble metals such as gold, silver, and platinum have beneficial effects on health and are being utilized for the synthesis of nanomaterials thus designated as "metallic nanoparticles". Due to their useful properties, they are used for catalysis, composite-like polymer preparations, disease diagnosis as well as treatment, and labeling of optoelectronic recorded media, etc. (Jamkhande et al., 2019). They can be fabricated, modified in their shape and size, and can be linked to various types of chemical functional groups. Such abilities allow them to bind and attach to ligands which include antibodies, drugs, polymers, and peptides (Mughal, 2022).

**Dendrimers**

A dendrimer is a nanotechnology-based three-dimensional nano-engineered polymeric structure that provides uniform size for surface functionalization thus developing an attractive tool for biomedical applications (Choudhury et al., 2021). A combined advantage of the particle-defined structure and the functionalized surface is provided by optical dendrimers. They improve bloodstream visualization and are used in MRIs. They resemble biomolecules and can be used as antiviral, antibacterial, and anticancer agents.

**Carbon Nanotubes**

They have useful electronic, intrinsic mechanical, and physio-chemical properties. Carbon nanotubes can diagnose and treat neurological pathologies which include Parkinson's and Alzheimer's disease (Waris et al., 2022).

**Polymeric Nanoparticles**

Polymer materials are stable and are safe to use in humans. They improve diagnosis when incorporated with different contrast agents. Polymeric nanoparticles possess the ability to encapsulate different contrast agents within a single matrix which enables multimodal imaging (Srikar et al., 2014). They have a vast range of applications, such as for ocular drug delivery, oncologic treatment, cancer diagnosis and imaging, etc. (Begines et al., 2020).

**Liposomes**

They consist of lipid bilayers and are spherical vesicles. They are highly compatible due to very low toxicity and least side effects in the drug delivery system. They have useful properties of biodegradability, and biocompatibility too. They can be specifically targeted to desired tissues when combined with specific targeting ligands and probes. They can be modified with PEG (polyethylene glycol) chains to prolong blood circulation thus they can enable passive targeting. Active targeting of anticancer drugs to tumor sites can also be done by grafting targeting ligands on liposomes (Allahou et al., 2021).

**Applications of Nanomaterials in Disease Detection**

Nanomaterials possess unique properties such as high surface-to-volume ratio and customizable size and shape, which makes them ideal for biosensing techniques. Their enhanced adsorption and reactivity compared to bulk materials enable surface modification with biological species via bonding, enhancing biosensing characteristics. This customization significantly improves sensitivity, and selectivity, and enables rapid responses to sample analytes, with detection limits reduced by several orders of magnitude (Srivastava et al., 2021).

**Fluorescent Nanomaterials for Imaging**

There has been a growing interest in the utilization of self-assembled fluorescent nanomaterials derived from small-molecule organic dyes for imaging and sensing applications. The increasing appeal of self-assembled fluorescent nanomaterials from organic dyes lies in their ability to blend the spectral tunability and biocompatibility of small organic fluorophores with the brightness and stability of inorganic materials, yielding versatile nanostructures, including core-shell architectures with hyperbranched polymers (Svechkarev and Mohs, 2019).

**Magnetic nanomaterials for separation and detection**

Magnetic nanomaterials' large surface area and strong magnetic response enable easy isolation with external magnets. Their functionalization offers rapid enantioselective separation, surpassing traditional methods (Deng et al., 2020). Magnetic

nanomaterials simplify sample preparation in solid-phase extraction, enhancing selectivity and sensitivity in new analytical methods (Rios and Zougagh, 2016). Surface-modified with antibodies, oligonucleotides, or aptamers, magnetic nanomaterials selectively bind target viruses or biomarkers in biological samples, aiding isolation and detection processes (Rezvani Jalal et al., 2021).

**Quantum Dots and their Applications**

Semiconductor nanocrystals, with nanometer-scale diameters, display quantum size effects, leading to tunable and efficient photoluminescence. Core-shell structures are common, enabling various device applications, including commercially available quantum dots-based displays in everyday life (Cotta, 2020). Quantum dots find diverse applications in biotechnology and biomedicine, including cell sensing, bioimaging, gene therapy, and drug delivery, addressing challenges like off-target effects in cancer treatment (Armăşelu and Jhalora, 2023).

**Successful Nanomaterials-based Diagnostic Techniques**

Nanomaterials have changed demonstrative procedures through convincing contextual analyses in different ways**:**

**Nanoparticle-based Polymerase Chain Reaction (PCR) Assay**

Gold nanoparticles (AuNPs) are key in virus detection, notably enhancing PCR. Recent studies highlight their antiviral activities and applications in diverse analysis techniques and potential therapies, alongside toxicity assessments for respiratory viruses. Magnetic nanoparticles are also being used in PCR assays (Rasmi et al., 2023).

**Nanosensors for Rapid Detection of Biomarkers**

Biosensors with micro- and nanostructured elements are used for frequent and rapid detection of various sepsis biomarkers (Alba-Patiño et al., 2022). Nanosensors are used to detect cardiovascular disease (Tang et al., 2022). Methyl probe, an ultra-sensitive nano-sensor synthesized via ultrashort pulsed ionization, enables direct detection of methylated DNA in plasma for metastasis detection. Integrated with biomarkers and machine learning, it offers scalable clinical adaptability (Ganesh et al., 2024).

**Nanomaterial-enhanced Imaging in Molecular Diagnosis**

Metal-enhanced fluorescence (MEF) boosts fluorescence signal by positioning metal close to a fluorophore, popular for sensitive molecule detection in biosensors. Metal size and structure impact optical properties, enhancing photostability and light characteristics (Goodrum and Li, 2024). These nanoparticles can accommodate imaging agents, enabling real-time treatment monitoring by physicians, and enhancing cancer care effectiveness and personalization through therapy adjustments (Zhang et al., 2018). Biomedical scientists appreciate nanomaterials' extensive surface area, aiding the delivery of therapeutic agents for genetic diseases. Genomics explores genome aspects, while nanotechnology supports gene-editing and stem cell research. Nanomaterials also enhance cell tracking in therapies (Paramasivam et al., 2024).

**Applications of Nanomaterials in Cancer Detection and Treatment**

Nanotechnology has a major role in cancer management. Gene therapy and drug administration are some of its prominent applications. Nanotechnology has a considerable role in diagnostics, monitoring of biomarkers and tracing, histopathological imaging, and medicines. Gold nanoparticles and quantum dots are widely used at the molecular level to diagnose cancer. Various molecular diagnostic techniques are based on nanoparticles such as biomarker discovery which can properly and rapidly diagnose tumours.

Nanotechnology plays a crucial role in solving complications regarding the diagnosis of diseases. Nanoscale drug delivery specifies the target and thus reduces the complications by increasing the sensitivity. Nanomaterials have active as well as passive targeting mechanisms due to which they are used in the treatment and diagnosis of cancer (Kher and Kumar, 2022).

**Nanoparticles in Early Cancer Detection**

Nanoparticles are better than currently used conventional techniques which include ultrasonography, X-ray, MRI (magnetic resonance imaging), PET (positron emission tomography), and CT (computed tomography) scan. Certain morphological changes occur in cells or tissues which help detect or confirm cancer. Conventional techniques involve these morphological changes for results but they are detectable only after certain visible changes in tissues or cells have already occurred. It is the time when cancer has started its proliferation and causes metastasis. Conventional techniques are also unable to differentiate benign and malignant tumors. Histopathology and cytology cannot be utilized as independent tests for cancer detection. Nanotechnology provides rapid and more accurate early diagnosis along with an ongoing assessment of cancer patients' care (Kher and Kumar, 2022).

**Theranostic Applications of Nanomaterials in Cancer Treatment**

Targeted drug delivery systems based on nanomaterials have advanced recently, offering both passive and active targeting options. While passive targeting is accomplished by increased permeability and retention effects, active targeting

is accomplished through the use of antibodies or small molecule-conjugated nanoparticles. Active targeting exhibits significant promise and serves as a viable substitute for passive targeting. Its enhanced efficiency and retention have been shown to improve tumor localization in active targeting. Drugs based on nanomaterials have superior loading capacities, longer half-lives, lower cytotoxicity, better specificity, and better bioavailability than conventional chemical therapies (Zhu et al., 2022).

Nanomaterials have the potential to significantly increase the effectiveness of cancer immunotherapy. Cancer vaccines are an important component of cancer immunotherapy. Remarkably, a recent study described the synthesis of a D-enantiomeric supermolecule nanoparticle and the demonstration of its p53-dependent antiproliferative action, which in turn improved antitumor immunity. Nanomaterials can transport tumor antigens, which will be beneficial for immunotherapy, and because of their unique properties, they can also influence the immune response. Notably, the PC7A nanoparticles triggered the pathway that stimulates interferon genes, which strengthens the anti-tumor immune response (Zhu et al., 2022).

**Infectious Disease Diagnostics with Nanomaterials**

**Nanomaterials in the Rapid Detection of Pathogens**

Contamination of food by various pathogens is a major problem throughout the world. Therefore, quick detection of these pathogens is very important so that infectious diseases can be controlled. Various techniques have been employed in the past to control pathogens, but these techniques require huge apparatus and are very costly. Recently nanomaterials have grabbed attention because of their small size and large surface area Widely used nanomaterials for the detection of food-borne pathogens are peptide nanotubes, magnetic nanoparticles, gold nanoparticles, and quantum dots. Magnetic nanoparticles are used for efficient detection of *Salmonella typhimurium* because the pre-enrichment and isolation of bacteria is not needed using these nanomaterials. The photothermal effects of these nanomaterials have improved the sensitivity of detection also. Similarly, gold nanoparticles can be used for the on-site detection of *Staphylococcus aureus*. It is so rapid that it can be done within 15 minutes (Shen et al., 2021).

**Antimicrobial Nanomaterials for Infectious Disease Management**

Metals such as gold and silver were used in the past because of their antimicrobial properties. These metals can prevent the proliferation of gram-positive bacteria, gram-negative bacteria, and viruses on the surfaces. However, the limitation was that these metals could be toxic, and their bioactivity also decreased over time. With the advancement of technology, nanoparticles have been discovered. These nanoparticles are coated on the surface of biomaterials to prevent adhesion of microbes and biofilm formation (Yılmaz et al., 2023). For example, carbon nanodots, because of the properties of photoactivation and surface charge, can inhibit the growth of *Escherichia coli* O157:H7 and *S. typhimurium*. Therefore, carbon nanodots are used in food packaging materials and wound dressings (Huang et al., 2023).

**Neurodegenerative Disease Diagnostics with Nanomaterials**

Neurodegenerative disorders involve the progressive decline of normal brain functions. Diagnosis of diseases like Alzheimer's disease and Parkinsons' disease is difficult at an early stage. The new hope for the early diagnosis of neurodegenerative diseases is nanotechnology because it is very sensitive to the detection of biomarkers for neurodegenerative disorders (Hanif et al., 2021). Among nanomaterials, two-dimensional nanomaterials have gained interest because of their ultrathin structure and their ability to cross the blood-brain barrier. Two-dimensional nanomaterials include graphene, transition metal dichalcogenides, transition metal carbide and nitride, and transition metal oxide. Among them, graphene nanomaterials have distinct physical and chemical properties. Graphene not only prevents the accumulation of neural proteins but also helps in their removal. Thus, it helps to prevent Alzheimer's and Parkinson's disease (Tiwari and Tiwari, 2023).

**Challenges and Considerations for the use of Nanomaterials in Molecular Diagnosis**

**Biocompatibility and Safety Concerns**

Nanoparticles in *in vivo* applications raise concerns about potential toxic effects, but this is less significant in *in vitro* diagnostics. The environmental impact of nanoparticle release during manufacturing and naturally occurring nanoparticles in the atmosphere are under investigation. Despite advancements, there are still unanswered questions about nanoparticles' fate in living organisms due to the diverse materials and sizes. Nanoparticles smaller than 20 nm have the potential to penetrate cells, and approval for *in vivo* nanomaterials for human diagnostics may be hindered without clear safety demonstrations (Aziz et al., 2021). Nanoparticles play a crucial role in biomedical applications, such as drug delivery, gene delivery, and biosensors, but their blood compatibility is essential for safe use. In vitro studies show moderate biocompatibility, but no aggregation of blood cells with nanoparticle interaction has been observed. Nanoparticles are unstable and undergo agglomeration and dissolution, impacting biological effects. Researchers modify nanoparticles to reduce toxicity and improve biocompatibility, but concerns remain about the potential loss of original features. Continuous efforts are needed to advance the understanding of nanoparticle toxicity and biocompatibility for safe applications (Joglekar and Gajaralwar, 2021).

Even though nanoparticles are the new hope in the field of medicine, surgery, diagnostics, neurodiagnostic, and food, their drawbacks should not be undermined. Nanoparticles can affect different parts of the body by accumulating in them.

The use of nanoparticles offers a promising diagnosis of Parkinson's disease, but it also acts as a contributing factor in the induction of this disease by damaging dopaminergic neurons in the substantia nigra. Thus, before incorporating the use of nanoparticles in different fields, their harmful effects should be extensively studied (Mohammadipour et al., 2020).

Nanotechnology in medical applications has raised concerns about nanoparticle-mediated toxicity and adverse reactions, particularly in Alzheimer's disease treatment. The size, shape, and surface characteristics of nanoparticles influence pharmacokinetics and biodistribution. The benefit-to-risk ratio depends on nanoparticle dose and dosing frequency. Nanoparticles may hinder P-glycoprotein efflux pumps, induce cell death, and modulate gene expression. Hypersensitivity reactions and complement system activation may also cause adverse reactions. Understanding nanoparticle properties and interactions with biological systems is crucial for developing effective and safe treatments, especially in Alzheimer's disease (Halder et al., 2022). The toxicity of gold nanomaterials is a topic of interest due to their potential biomedical applications. However, safety and toxicology studies are crucial before clinical translation. The uptake of gold nanoparticles is influenced by factors such as size, shape, surface functionality, charge, concentration, and cell type. Surface chemistry, particularly charge, plays a crucial role in nanoparticle-cell interactions. Purity assessment is also essential for effective biomedical applications (Balog et al., 2024). Nanotechnology's production of small particles raises toxicological risks due to increased reactivity. Regulations are inadequate, but each system requires thorough investigation for potential health effects. Public awareness and ethical discussions highlight the need for risk assessments and systematic evaluations (Wang et al., 2022).

**Standardization and Regulatory Hurdles**

The Food and Drug Administration (FDA) acknowledges nanotechnology's potential and encourages responsible development. It regulates nanotechnology products under existing statutory authorities, tailoring approaches based on legal frameworks. The FDA addresses scientific gaps and collaborates with other agencies to ensure transparent regulatory pathways. The draft guidance is issued for industry applications, and early consultations are encouraged for safety, effectiveness, and regulatory status questions (Dias et al., 2021). Nanomedicines, a key focus in pharmaceutical research, require rapid advancements to address medical needs. Understanding and characterizing these complex nanomedicines is crucial to prevent adverse effects. Collaboration between scientists, regulators, industry, and patient representatives is essential for successful technology development (Halwani, 2022).

**Integration with Existing Diagnostic Platforms**

Nanotechnology's application in personalized medicine offers unique opportunities for disease treatment. Nanomaterials, with their small sizes, design flexibility, and modifiable surfaces, can be engineered to interact with specific biological components. Understanding nanomaterial interactions is crucial for designing diagnostic imaging and drug delivery (Đorđević et al., 2022).

**Ethical consideration in the use of Nanomaterials for Diagnostics**

The challenge of balancing scientific advancements with human rights and dignity is posed by the rapid evolution of nanotechnology. To address ethical issues, diversifying tests, defining properties, monitoring risk assessments, and conducting continuous studies on nanoparticle interactions with human organisms are crucial (Srivastava and Manjhi, 2023). The ethical discussion surrounding nanotechnologies is hindered by public hype, unclear definitions, and the early stages of research. The term "nano" is broad and vague, making it difficult to pinpoint ethical issues. To address these challenges, scholars recommend critically examining nanotechnology's social context, traditions, stakeholders, and research activities, challenging the current monolithic visions (Wahab et al., 2023).

**Future Perspectives and Emerging Trends for Nanomaterials**

Future perspectives and emerging trends in innovative nanomaterials for molecular diagnostics hold immense promise for revolutionizing healthcare. Nanotechnology offers unparalleled opportunities to enhance sensitivity, specificity, and multiplexing capabilities in diagnostic assays.

Nanomaterial-based biosensors offer the promise of rapid and accurate detection of biomolecules at low concentrations, enabling early diagnosis of diseases such as cancer, infectious diseases, and genetic disorders (Iftikhar et al., 2023). Advancements in nanotechnology will facilitate the development of point-of-care devices that are portable, cost-effective, and user-friendly, enabling decentralized testing in resource-limited settings Here are some anticipated trends and perspectives:

**Enhanced Sensitivity**

Nanomaterials such as quantum dots, gold nanoparticles, and carbon nanotubes possess distinctive optical, electrical, and magnetic properties. Integrating them into diagnostic platforms can significantly enhance sensitivity, enabling the detection of ultra-low concentrations of biomarkers, viruses, or genetic material (Heydari-Bafrooei and Ensafi, 2023).

**Multiplexed Assays**

The simultaneous detection of multiple analytes within a single sample is critical for comprehensive diagnostics. Nanomaterials facilitate the development of multiplexed assays, where different nanoparticles are functionalized to specifically bind to different targets (Jarockyte et al., 2020).

**Point-of-care Testing**

Miniaturized devices integrated with nanosensors can provide real-time results, facilitating early disease detection and personalized treatment strategies. These advancements in point-of-care testing reduce the reliance on centralized laboratory facilities, making diagnostic services more accessible and timely, particularly in remote or resource-limited settings (Biswas et al., 2022).

**Liquid Biopsy**

Liquid biopsy utilizing nanoparticles represents a cutting-edge approach to cancer diagnostics. These tiny particles, engineered for precise targeting, enable non-invasive detection of biomarkers from bodily fluids. Their high specificity and sensitivity offer invaluable insights into disease progression and treatment response, heralding a new era of personalized medicine and improved patient outcomes (Vázquez-Iglesias et al., 2024).

**Emerging Trends in the uses of Nanomaterials in Molecular Diagnostics**

**Functionalized Nanoparticles**

Functionalized nanoparticles are engineered particles with tailored surface properties, enabling precise interactions with biological or chemical entities. Through covalent or non-covalent attachment of functional groups, these nanoparticles exhibit enhanced stability, selectivity, and reactivity. Their diverse applications span drug delivery, imaging, sensing, catalysis, and environmental remediation (Khalili et al., 2022).

**Nanopore Sequencing**

Nanopore-based sequencing technologies offer a promising approach for rapid and cost-effective DNA sequencing. This method involves passing DNA molecules through a nanopore, where changes in electrical conductivity can be monitored, enabling real-time sequencing of nucleic acids. Nanopore sequencing holds great promise for clinical diagnostics, facilitating personalized medicine and the swift identification of genetic mutations (Chen et al., 2023).

**Plasmonic Nanomaterials**

Plasmonic nanoparticles, such as gold and silver nanoparticles, exhibit localized surface plasmon resonance (LSPR), which can be exploited for label-free detection of biomolecules. LSPR-based biosensors offer high sensitivity and allow for real-time monitoring of molecular interactions, making them invaluable tools for molecular diagnostics (Csáki et al., 2018)

**Nanotechnology-enhanced Imaging**

Nanotechnology-enhanced imaging leverages innovative nanomaterials to revolutionize medical diagnostics and research. These cutting-edge materials, manipulated at the nanoscale, enhance imaging resolution, sensitivity, and specificity, enabling unprecedented insights into biological structures and functions (Sikkander et al., 2024)

**Nanoparticle Engineering**

Nanoparticle engineering pioneers the creation of innovative nanomaterials, leveraging meticulous manipulation at the molecular scale. Through precision crafting, it tailors materials with unparalleled properties, revolutionizing industries from medicine to electronics. This interdisciplinary field melds cutting-edge science with engineering prowess, unlocking boundless potential in realms previously unimaginable (Alshangiti et al., 2023).

**Bioconjugation Strategies**

Innovative bioconjugation techniques are emerging to enable stable coupling of biomolecules with nanomaterials. Methods such as click chemistry, DNA origami, and peptide-mediated conjugation offer efficient and site-specific functionalization, minimizing nonspecific binding and enhancing assay robustness (Dubey and Tripathi, 2021).

**Integration of Artificial Intelligence**

Utilizing AI algorithms and machine learning techniques can enhance the interpretation of complex molecular data generated by nanomaterial-based diagnostic assays, enabling accurate disease diagnosis and personalized treatment strategies (Kasture and Shende, 2023).

**Conclusion**

The integration of nanomaterials in molecular diagnostics presents a promising frontier in the field of healthcare and research due to the unique properties of nanomaterials which include a high surface-to-volume ratio. Trends in functionalized nanoparticles, nanopore sequencing, plasmonic nanomaterials, nanotechnology-enhanced imaging, and nanoparticle engineering are emerging nowadays. Overall use of nanomaterials can enhance the sensitivity as well as specificity of diagnostic assays. Still, challenges like standardization, scalability, and safety concerns regarding the use of nanomaterials need to be addressed. Some ethical concerns need to be addressed before using nanomaterials on a commercial scale. With advancements in research, the synergy between nanotechnology and molecular diagnosis is expected to be improved in the future.

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