Chapter 01

The role of Nanoparticles in Vaccine Development

Javeria Arshad[1*], Savaira Tariq[2], Khadija Babar[2], Maha Khalid[3], Syed Sharf ud Din[3], Munazza Habib[3], Gulab Ali[2], Mohsan raza[4], Wajeeha Sultan[5] and Aimen Khalid[6]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Government College University, Faisalabad-38000, Punjab, Pakistan
[2]Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Government College University, Faisalabad-38000, Punjab, Pakistan
[3]Faculty of Pharmaceutical Sciences, University of Central Punjab, Lahore-54000, Punjab, Pakistan
[4]Department of Pharmacy, University of Agriculture Faisalabad-38000, Punjab, Pakistan
[5]Department of Pharmacy, Faculty of Pharmaceutical sciences, Government College University, Faisalabad-38000, Punjab, Pakistan
[6]Department of Microbiology, Faculty of Biological Sciences, Government College University, Faisalabad-38000, Punjab, Pakistan
*Corresponding author: pharmacistj.arshad@yahoo.com

ABSTRACT

Conventional vaccines come with low immunogenicity, an increased potential for toxicity, and a need for multiple administration. To overcome these problems associated with conventional vaccines, researchers have sought to incorporate nanotechnology in the science of vaccine development. Nanoparticles have addressed major issues concerned with the traditional and subunit vaccines. The advancements have increased the vaccine efficacy and effectiveness. Types of nanoparticles include Solid-Lipid Nanoparticles, Polymeric Nanoparticles, Liposomes, Virus-Like Particles. Nanoparticles have emerged as a promising platform for enhancing the immune response, either through innate immune potentiation or through improved delivery of antigens or other immune stimulants. This can be accomplished through the use of virus-mimetic nanostructures, which are designed to mimic viral assembly and elicit a strong immune response in the body. Nanoparticles can encapsulate antigenic material, protecting it from degradation and improving its stability during storage and transportation. This is particularly important for vaccines that require refrigeration, as nanoparticles can extend their shelf life and reduce the need for cold chain storage. Furthermore, obstacles are being removed by advances in intracellular delivery, which makes it easier for immune cells to absorb antigens and mount a strong defence. Stability is still a crucial component, and research is being done to create vaccines that can be stored at ambient temperature without the need for the laborious cold chain. Personalized vaccines, a novel frontier, leverage nanoparticle technology to tailor immunization to individual immune profiles. This not only maximizes efficacy but also minimizes adverse reactions, offering a paradigm shift in vaccination strategies. Thus through innovation, collaboration, and ethical practice, they hold the potential to revolutionize immunization worldwide.

KEYWORDS
Conventional Vaccines, Nanotechnology, Nanoparticles, Immunogenicity, Personalized Vaccines.

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INTRODUCTION

Conventional vaccines proved to be a great success in the field of disease prevention and alleviating human health. But these conventional vaccines are short of many good things and raise concerns. Conventional vaccines come with low immunogenicity, an increased potential for toxicity, and a need for multiple administration. To overcome these problems associated with conventional vaccines, researchers have sought to incorporate nanotechnology in the science of vaccine development (Kheirollahpour et al., 2020). Previously, under nanotechnology, nanoparticles have already been used to deliver drugs such as cytotoxic drugs or immune-suppressive drugs (in case of transplantation). Nanoparticles can ensure a target-specific and controlled delivery of medications. Nanoparticles not only provide a site-specific delivery of medications but also protect the drug from degradation (Diaz-Arévalo and Zeng, 2020). Nanoparticles come with some diverse compositions, these versatile compositions help researchers to develop novel and innovative strategies and platforms for vaccine development. Nanoparticles ensure high effectiveness of vaccines (Lung et al., 2020a). Owning to the unique structure of nanoparticles, they can be used as adjuvants in vaccines. Their structure help to accommodate various

loading antigens. It also prevents degradation and prolongs in-vivo antigen exposure. Size of nanoparticles help to induce specific immune response and action at a particular target site. Furthermore, the response or impact of nanoparticles to the immune system can be modulated, if their physical and chemical properties are properly controlled and modified as per the desired action (Mao et al., 2021). As mentioned earlier, nanoparticles are capable of inducing site specific responses and vaccines require a site specific response to overcome concerns related to their effectiveness. Therefore, nanoparticles are what we need to improve the safety and effectiveness of vaccines. Nanoparticles provide benefit in two ways; they act as delivery systems and also as adjuvants. Their use as adjuvants or carriers enhance stability of antigens, decrease the rate of degradation, improve immunogenicity and therapeutic effectiveness, and enhance membrane permeability (Bezbaruah et al., 2022a). As discussed, nanoparticles have potential to be used as immune-stimulating adjuvants in vaccines, therefore they are referred to as nano-adjuvants. They work by encapsulating or absorbing an antigen or DNA in the formulation, and thus increasing stability, immunogenicity, and cellular uptake (Garg and Dewangan, 2020).

In this advancing world, new therapeutics are rapidly introduced by the researchers. Messenger RNA (mRNA) is also a novel therapeutic marking its importance in disease treatment and prevention. mRNA and DNA vaccines also present their therapeutic importance in the vaccination system taking it to new heights. They are cheap, quickly adaptable to changing pathogenic strains, and are cheap to manufacture. These nucleic acid therapeutics require stable and effective in-vivo delivery systems that protect against in-vivo degradation and enable cellular uptake and release. Therefore, instead of using viral vectors for nucleic acid delivery, non-viral vectors especially nanoparticles are rapidly used in the vaccine development. Nanoparticles provide effective targeted therapies with needed pharmacokinetics, bioavailability, efficacy, and bio-distribution. One such nanoparticle (Lipid nanoparticle-loaded mRNA vaccine) has been in clinics against COVID-19 and validates the importance of the use of nanoparticles in the vaccines (Ho et al., 2021; Hou et al., 2021).

Types of Nanoparticles used in Vaccines

In immunization, nanoparticles have made their mark as shown in Fig. 1. Nanoparticles have addressed major issues concerned with the traditional and subunit vaccines as in Table 1. The advancements have increased the vaccine efficacy and effectiveness (Vasudevan et al., 2024).

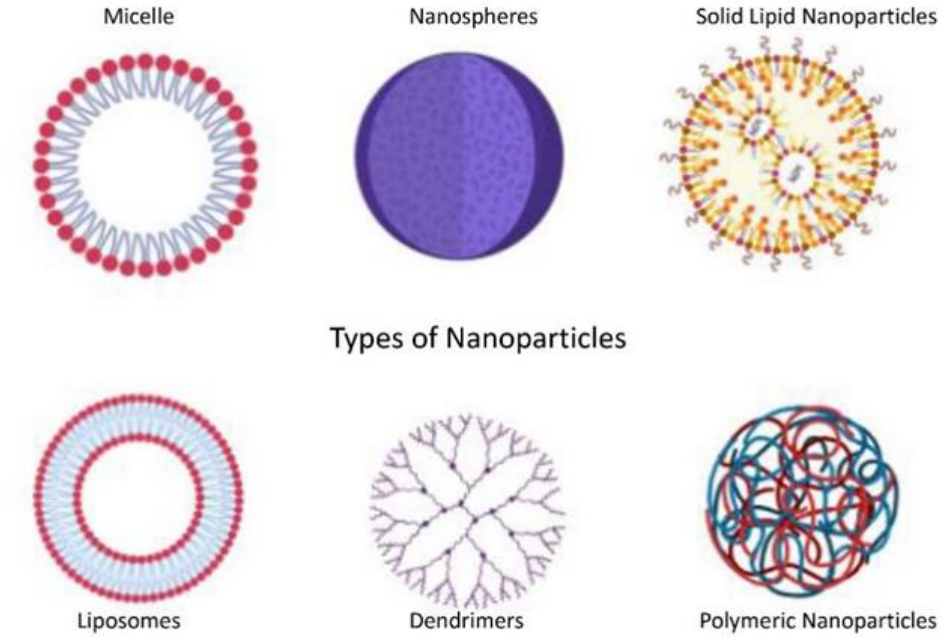

Fig. 1: Types of Nanoparticles.

Enhanced Immune Response with Nanoparticles

Nanoparticles have emerged as a promising platform for enhancing the immune response, either through innate immune potentiation or through improved delivery of antigens or other immune stimulants. This can be accomplished through the use of virus-mimetic nanostructures, which are designed to mimic viral assembly and elicit a strong immune response in the body (Bros et al., 2018). To increase immunogenicity, these nanostructures can be decorated with proteins or recombinant antigens. Nanoparticles' immunogenicity can also be affected by their size and surface charge; smaller particles and those with a positive surface charge are generally more immunogenic. Nanoparticles can be engineered to boost immune responses in the treatment of inflammatory and autoimmune disease (Gause et al., 2017). Nanoparticles have the potential to change the treatment paradigm for these diseases by interacting with various cellular and molecular

components of the immune system. Several strategies have been used to optimize vaccine immunogenicity and generate a strong B-cell response. Nanoparticles have been shown to improve the antigenicity of conjugated antigens, and this effect is dependent on particle size and surface charge. Particle size has been identified as a significant factor in determining whether antigens loaded into nanoparticles elicit type I or type II immune responses, thereby influencing the type of immune response (Glass et al., 2016). A leading hypothesis for why nanotechnology-driven compositions are effective in vaccine development is that non-soluble antigens release slowly, forming a depot at the injection site and providing protection in the destabilizing in vivo environment. The antigenicity of the nanoparticles themselves is less well understood. Two studies found that when C60 fullerene derivatives conjugated to a BSA were used for immunization, particle-specific antibodies were produced, implying that some water-soluble nanoparticles might act as haptens, i.e., they are not antigenic unless they bind to a protein carrier (Moni et al., 2023).

Table 1: Types of Nano-Particles with Theirs Specifications

Serial Number	Type of Nanoparticle	Explanation	Fabrication Technique	Characterization	References
1	Solid-Lipid Nanoparticles.	Lipid nanocarriers having a solid core. They are capable of holding both hydrophilic and hydrophobic drugs	1. Ultrasonication 2. Solvent emulsification evaporation 3. Solvent emulsification diffusion 4. Supercritical fluid extraction 5. High pressure homogenization 6. Hot homogenization 7. Cold homogenization	1. Particle size, charge analysis, polydispersity index (Photon Correlation Spectroscopy (PCS). Dynamic Light Scattering (DLS)) 2. Crystallinity (Differential Scanning Calorimetry) 3. Size, surface topography, stability (Scanning Electron Microscopy (SEM)) 4. Zeta potential (Phase Analysis Light Scattering (PALS))	(Duan et al., 2020; Paliwal et al., 2020; Fan et al., 2021; Khairnar et al., 2022)
2.	Polymeric Nanoparticles	They have substances entrapped or adsorbed into the polymeric core.	1. Solvent Evaporation 2. Emulsification/Diffusion 3. Emulsification/Reverse salting-out 4. Nanoprecipitation 5. Supercritical fluid technology 6. Membrane reactor method 7. Sono-precipitation	1. Particle size (laser Scattering (LS), Field Flow Fractionation (FFF), Electron Microscopy (EM)) 2. Size, polydispersity, zeta potential (Dynamic Light Scattering (DLS))	(Zielińska et al., 2020; Gagliardi et al., 2021; Castro et al., 2022; Sakhi et al., 2022)
3.	Liposomes	Spherical-lipid vesicles composed of one or more lipid bilayer structures. They are the most widely considered nanocarriers for the targeted drug delivery.	1. Thin film hydration method/ Bangham's method 2. Reverse-phase evaporation method 3. Solvent injection method 4. Detergent removal method 5. Heating method 6. Supercritical fluidic method 7. Packed-bed assisted hydration method 8. Curvature tuning method	1. Size (Dynamic Light Scattering (DLS)) 2. Shape (Optical Microscopy (OM), Electron Microscopy (EM), Scanning Electron Microscopy (SEM)) 3. Polydispersity Index and zeta potential (Dynamic Light Scattering (DLS))	(Has and Sunthar, 2020; Miere et al., 2020; Walunj et al., 2020; Nsairat et al., 2022)
4.	Virus-Like Particles	Multimeric structures having one or more non-genetic material viral structural proteins. They are formed by the self-assembly of viral structural proteins either in vivo or in vitro.	1. Use of yeasts to express virus-like particles 2. Cell-free protein synthesis	1. Electron Microscopy (EM) 2. Transmission Electron Microscopy (TEM)-negative staining 3. Super-resolution Florescence Microscopy (SRFM) 4. Nanoparticle Tracking Analysis (NTA) and Flow virometry	(Dondapati et al., 2020; González-Domínguez et al., 2020; Qian et al., 2020; Srivastava et al., 2023)

Immunological Mechanism of Nanoparticle Vaccines

Nanoparticle vaccines are a diverse category of vaccines used to prevent or treat a variety of diseases. Vaccines are

administered intramuscularly, and nanoparticles are coated with interstitial fluid proteins (Tursi et al., 2023). The composition and mass of this corona are influenced by the nanoparticle's physiochemical properties, such as size and surface chemistry, which may result in enhanced or diminished cellular interactions and immune system recognition (Wilson et al., 2017). Proteolytic analyses of coronas formed on nanoparticles after administration by injection or serum incubation consistently identify opsonins such as fibrinogen, complement, and immunoglobulin proteins. Nanoparticles quickly become coated in soluble factors such as complement and immunoglobulins (Zhang et al., 2021). This marks nanoparticles for comprehension and uptake by local innate immune cells like neutrophils, monocytes, macrophages, and dendritic cells, which are a subset of antigen presenting cells (Kapczynski et al.). Pathogen recognition receptors (PRRs), such as Toll-like receptors (TLRs), found on the cell surface, inside of the endosome, or in the APC's cytoplasm, can enhance this uptake. These PRRs recognize molecular patterns linked to pathogens (PAMPs) on nanoparticles (Zhao et al., 2023). The motions of particle trafficking to the lymph node (LN) are largely determined by nanoparticle size. Smaller particles can freely drain into the LN, whereas larger particles require cellular transport from APCs. In addition to improved LN drainage, nanoparticles are retained in the LN for longer than readily soluble antigens, providing more opportunities for interactions with immune cells (Zhao et al., 2018). A recurrent display of the most effectively spaced antigen on the nanoparticle surface promotes B cell receptor (BCR) crosslinking, which leads to B cell activation. T cell activation is also increased after nanoparticle vaccine administration. A portion of activated CD4+ T cells differentiate into T follicular helper (TFH) cells and migrate to the B cell follicle. In this case, TFH cells drive sustained germinal center reactions, resulting in the filtering of B cell clones with high antigen affinity into persistent memory B cells and/or plasma cells that produce antibodies (Diaz-Arévalo and Zeng, 2020).

Nanoparticles for Targeted Vaccines Delivery

Using nanotechnology to transform vaccine development, the field of nanovaccinology has grown quickly in the last few years. Because of their special qualities and abilities, nanoparticles have become important players in boosting immune responses. The capacity of nanoparticle-based vaccines to function as adjuvants that is to both act as antigens and increase the antigenicity of conjugated or adsorbed antigens is a key benefit. This dual function strengthens both the innate and adaptive immune responses, resulting in longer-lasting and more powerful immunity (Vu et al., 2021). When compared to traditional vaccination delivery methods, nanoparticles have a number of advantages. They make it possible for antigens to be delivered locally and specifically; they enhance the presentation and processing of antigens; they sustain and raise antigen concentrations at mucosal surfaces; they improve bioavailability; and they can either stimulate or suppress the immune response (Fan and Moon, 2015). In addition, nanoparticles have the ability to effectively target immune cells, cross physiological barriers, and regulate the kinetics of antigens, which makes them excellent choices for boosting vaccine efficacy. Antigen size plays a crucial role in the uptake by antigen-presenting cells (APCsLarger antigens have a greater ability to interact with APCs because of their varied surface characteristics, including charge, hydrophobicity, and receptor interaction potential. Examples of these include nano- or microparticles and whole-pathogen vaccines. Small protein antigens, on the other hand, are less effectively absorbed and presented by APCs, underscoring the significance of antigen size in the stimulation of the immune response (Trabbic et al., 2021). The antigens are exposed to the stomach's acidic environment and are broken down by enzymes in the gastrointestinal tract when immunized orally. The mucosa of the upper airways, saliva, and nasal secretions all experience an increase in antibody responses as a result of nasal immunization.

Nanoparticles Encapsulated Vaccine Antigen

Nanoparticles can encapsulate antigenic material, protecting it from degradation and improving its stability during storage and transportation. This is particularly important for vaccines that require refrigeration, as nanoparticles can extend their shelf life and reduce the need for cold chain storage (Fan and Moon, 2015).

Nanoparticles can also improve antigen presentation to the immune system, leading to enhanced immune responses. This is achieved by protecting antigens from degradation, enabling prolonged release, and modulating immune responses by entering antigen-presenting cells through various pathways (Fan and Moon, 2015). Nanoparticles can also be designed to target specific cells or tissues, such as mucosal surfaces or lymph nodes, further enhancing their ability to stimulate an immune response.

Different types of nanoparticles have been explored as effective delivery systems for vaccine antigens, including virus-like particles, liposomes, ISCOMs, and polymeric nanoparticles. Virus-like particles, for example, are non-infectious particles that mimic the structure of viruses but lack the viral genome (Fan and Moon, 2015). They can be used to deliver antigens from various pathogens, such as H1N1 influenza virus, hepatitis B surface antigen, and HPV. Liposomes, on the other hand, are spherical vesicles made of lipid bilayers that can encapsulate hydrophilic antigens. They have been used to deliver antigens from various pathogens, such as Leishmania infantum, Streptococcus equi, and M. tuberculosis.

ISCOMs are cage-like structures made of saponins, cholesterol, and phospholipids that can encapsulate antigens and adjuvants. They have been used to deliver antigens from various pathogens, such as influenza, HIV, and HCV (Zhao et al., 2014). Polymeric nanoparticles, such as PLGA-encapsulated SIV vaccine, have also been explored for vaccine delivery. They can protect antigens from degradation, enable prolonged release, and shape the immune response.

Recent advancements in chemical and biological engineering have allowed precise regulation of nanoparticle characteristics, leading to enhanced antigen presentation and robust immune responses in nanovaccines (Zhang et al., 2023). Self-healing encapsulation technology, for example, has been used to improve the stability and release kinetics of nanoparticle-based vaccines. Polymeric nanoparticles have been used to deliver DNA vaccines, and nanoparticles have been used to co-deliver multiple antigens or adjuvanting molecules.

Regulatory Considerations for Nanoparticle Vaccines

Nanoparticle vaccination regulations require careful assessment at different phases of research and implementation. Comprehensive preclinical studies are used to examine safety, and they look into the possible toxic effects, immunity, and distribution of particle materials (Banoun, 2023). Before moving on to clinical trials, these investigations give regulatory body's vital information about the risk profile of vaccines made with nanoparticles. Nanoparticle vaccines undergo extensive efficacy assessments in clinical trials (Bezbaruah et al., 2022b). Research designs are carefully thought out in order to assess how well nanoparticle formulations defend against traditional vaccines and how well they elicit immunogenic responses. Trial protocols incorporate markers and surrogate endpoints to expedite vaccine development schedules and enable efficacy assessments (Knezevic et al., 2021; Ali et al., 2023; Lu et al., 2023).

A key factor in guaranteeing the reliability and Caliber of vaccines containing nanoparticles is the application of manufacturing standards. Strict regulations control production sites, tools, and procedures to reduce unpredictability and preserve product integrity (De Jong et al., 2022). To guarantee uniformity and stability from batch to batch, nanoparticle characterization—including dimensions, form, and surface characteristics—is crucial. For continuous evaluation of the overtime safety and efficacy of nanoparticle vaccinations in real-world environments, post-marketing surveillance is essential (Lung et al., 2020b). Utilizing surveillance systems makes it possible to promptly intervene and manage risks by identifying and assessing unfavorable occurrences that occur after vaccination. Regulatory bodies, medical professionals, and vaccine producers working together to guarantee ongoing surveillance and assessment of vaccination safety profiles as shown in Figure 2 (Liu et al., 2020; Lung et al., 2020b; Naik and Peden, 2020; Ali et al., 2023).

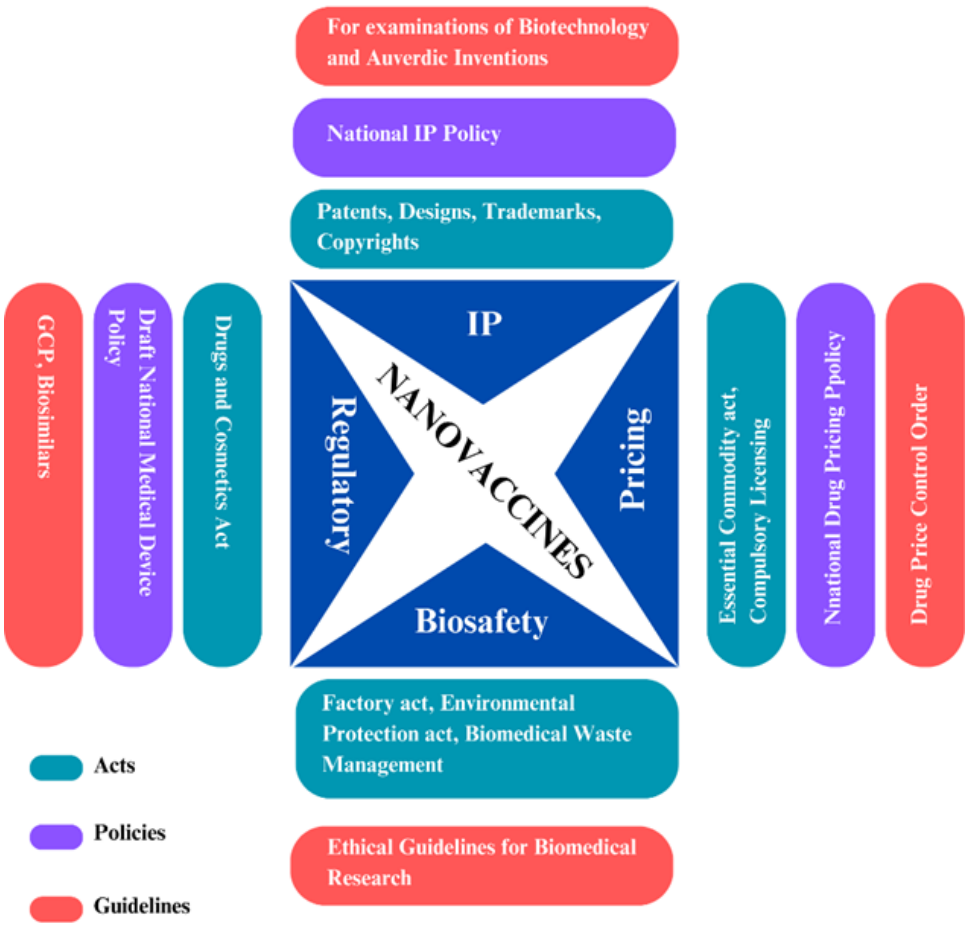

Fig. 2: Regulatory Considerations of Nano Vaccines.

Future Perspectives and Emerging Trends in Nanoparticle Vaccines

Nanoparticle vaccines have great potential in the field of vaccine development. Precision medicine techniques, which

allow vaccinations to be customized to a person's immunological profile and genetic composition, are being made possible by developments in vaccine design (Parupudi et al., 2022). Multivalent nanoparticle vaccines represent a significant advancement in the fight against complicated illnesses since they may target numerous pathogens or strains at once. With nanoparticles designed to target certain organs or tissues, tailored delivery systems are transforming the way vaccines are administered, increasing effectiveness while reducing adverse effects (Prasanna et al., 2021). Furthermore, obstacles are being removed by advances in intracellular delivery, which makes it easier for immune cells to absorb antigens and mount a strong defence. Stability is still a crucial component, and research is being done to create vaccines that can be stored at ambient temperature without the need for the laborious cold chain (D'Amico et al., 2021; Pippa et al., 2021).

Ethical and Societal Implications of Nanoparticle Vaccine Technology

A key component of moral behaviour in the field of nanoparticle vaccination research is informed permission. Because these technologies are complex, it is important to be extra careful to make sure clinical trial participants understand the advantages and disadvantages of new nanoparticle formulations (Chauhan et al., 2021). The need for fair access is even more urgent because vaccine nationalism is a major threat in today's world. Fairness and justice are major concerns that call for coordinated efforts to make sure that the benefits of scientific advancement are distributed fairly throughout communities and nations. With nanoparticle vaccinations, safety and risk evaluation present significant difficulties because of the possibility of unanticipated outcomes. The need to protect public health necessitates a close examination of the long-term impacts that nanomaterials have on people and ecosystems (Nath et al., 2021). Furthermore, the possibility of augmentation looms large, provoking moral discussions about the appropriate limits of scientific interference with human biology (Babatunde et al., 2019; Nath et al., 2021; Van de Voorde and Vlerick, 2021).

The adoption trajectory of nanoparticle vaccines is significantly influenced by public perception. It is critical to address issues with safety, effectiveness, and confidence in scientific institutions in order to promote broader use of these revolutionary technologies. Moreover, the incorporation of vaccines containing nanoparticles into healthcare systems requires a reevaluation of infrastructure and resource allocation in order to account for their distinct features (Van de Voorde and Vlerick, 2021). As policymakers grapple with issues of accessibility and affordability in the midst of rising healthcare expenditures, economic factors play a significant role. As vaccination technology utilizing nanoparticles advances, environmental sustainability becomes an increasingly important problem (Nath et al., 2021). The creation and elimination of nanomaterials give rise to complex inquiries about their environmental impact and enduring effects on the environment.

Future Directions in Nanoparticles Vaccine Research

Nanoparticle vaccines offer a game-changing approach to immunization, boasting enhanced efficacy and versatility. With their tiny structures, spanning lipid-based to viral-like particles, they hold immense promise in tackling a broad spectrum of pathogens (Tay et al., 2021).Efforts in nanoparticle vaccine research are particularly crucial in the face of emerging infectious diseases. By targeting specific pathogens, these vaccines can swiftly adapt to evolving threats, bolstering our defenses (Hu et al., 2023).Critical to their success is overcoming production and distribution challenges. Innovations in formulation and manufacturing are underway, aiming to ensure stability, scalability, and cost-effectiveness, essential for global deployment (Liu et al., 2019; Schuemann et al., 2020).

Personalized vaccines, a novel frontier, leverage nanoparticle technology to tailor immunization to individual immune profiles. This not only maximizes efficacy but also minimizes adverse reactions, offering a paradigm shift in vaccination strategies (Jain et al., 2021).Next-generation delivery systems, such as microneedle patches and inhalable nanoparticles, promise improved vaccine uptake and effectiveness, enhancing patient compliance. Nanoparticle-based adjuvants play a pivotal role in boosting vaccine efficacy by fine-tuning immune responses (Schuemann et al., 2020). Their targeted action ensures optimal vaccine outcomes. The fusion of nanotechnology and artificial intelligence accelerates vaccine development, streamlining processes from antigen prediction to immune response modelling (Liu et al., 2019).Yet, regulatory and ethical considerations must be carefully navigated. Establishing robust pathways and addressing ethical implications are vital for ensuring safety, efficacy, and public trust in nanoparticle vaccines. In conclusion, nanoparticle vaccines represent a transformative approach to global health challenges. Through innovation, collaboration, and ethical practice, they hold the potential to revolutionize immunization worldwide (Jain et al., 2021; Tay et al., 2021; Hu et al., 2023).

Conclusion

For a number of reasons, nanotechnology presents a huge possibility to enhance various diseases prevention and treatment. Firstly, complex antiviral and antibacterial designs can be realized by flexibly functionalizing nanomaterials with numerous compounds. Second, due to their diverse antiviral and antimicrobial properties, nanomaterials prevent infection in a number of ways. Third, because they share similar underlying processes, nanoparticles exhibit broad-spectrum effects. One effective measure to guard against microbial infection is vaccination. Compared to conventional vaccines, nano-based vaccines offer numerous clinical benefits, such as the capacity to deliver high antigen concentrations to B cells and elicit robust immune responses. The application of nanotechnology to vaccinations against bacteria and viruses, including

coronaviruses, HIV, and FMDV, is progressing, indicating that this method is promise for vaccine development, even though many nano-based vaccines are still in the pre-clinical phases. It is also necessary to conduct more research on the distribution, buildup, and removal of nanomaterials from the human body.

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