Nanotechnology: A Broad Spectrum Weapon against Antimicrobial Resistance

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Abstract

Antimicrobial resistance (AMR) is a major public health concern and it exhibits potential threats to environment and health. Due to the AMR, the use of conventional ways of treatment and many therapeutic drugs are of no use which led to the exploration of nanomaterials as an alternative. Nanoparticles are substances with size ranges between 1 nm to 100 nm and are classified into different types based on their size, properties, and shapes. There are different ways of the production of NPs such as top-down approach and Bottom-up approach which are further divided into different processes for the development of NPs. Although the mechanisms of action of nanoparticles are not yet confirmed or poorly understood, the studies show different ways through which these nanoparticles work against pathogens. Different mechanisms of action of NPs include oxidative stress and production of ROS, release of metal ions, disruption of cell membrane of bacteria, and eradication of biofilm etc. This chapter explores different ways of development of NPs, various types of NPs and the mode of action used by NPs to combat AMR as it can be used and explored in future for their amazing characteristics.

Keywords: AMR, Nanotechnology, Nanoparticles, Top-down and bottom-up approach, Membrane disruption, Oxidative stress, Metal ions

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Introduction

The modes of action of antibiotics are either inhibiting bacterial growth or directly killing bacteria (Fischbach & Walsh, 2009). These drugs work by targeting essential components of microbial metabolism, disrupting the formation of functional biological molecules (Linares et al., 2006). In the present situation, the efficacy of antibiotics has reduced significantly due to the developing resistance of microbes (Peschel & Sahl, 2006). Nowadays, the major threat to human health is the continuous rise of drug-resistant microorganisms. According to reports, antibiotic-resistant infections affect over 2 million people annually in the United States, resulting in more than 23,000 deaths (Munir et al., 2020). The motto 'No action today, no cure tomorrow' was used by WHO on World Health Day 2011(Willyard, 2017). It also mentions that the mortality rate from antibiotic resistance is higher in developing nations compared to developed ones (Prestinaci et al., 2015). Long-term drug therapy is crucial for infections caused by multidrug-resistant bacteria, costing approximately 55 billion dollars annually in the USA, according to reports, making it very costly (Laxminarayan et al., 2013). Moreover, the inappropriate use of antibiotics and prolonged treatment for multidrug-resistant bacteria are major causes of resistance. *Enterobacteriaceae, Pseudomonas aeruginosa,* and *Acinetobacter* are some of the major multidrug-resistant bacteria (Chambers, 2001; Perez et al., 2007; Lister et al., 2009). The increasing issue of bacterial multidrug resistance has encouraged research for novel materials that are more effective compared to conventional antibiotics and antimicrobial medications. Researchers increasingly look to nanotechnology as a viable alternative, primarily because of the exceptional physicochemical properties shown by nanoparticles (NPs) (Valodkar et al., 2011; Loza et al., 2020). Nanoparticle (NP) treatments, nanostructured coatings for indwelling and other medical devices, and nano drug delivery systems are some of the possible means to control antibiotic resistance. Nanomaterials, with at least one dimension in the range between 1 and 100 nm, are referred to as nanoparticles (Khan et al., 2019). Because of their extremely high surface area-to-volume ratio and small size, nanoscale materials have attracted a lot of attention. This ratio is improved when materials are shrunk to the nanoscale, giving the resultant nanoparticles (NPs) remarkable chemical activity, solubility, flexibility, and a variety of morphologies with different modes of action (Padmavathy & Vijayaraghavan, 2008). Drug delivery routes, direct antibacterial compounds, and nanostructured layers for medical devices are some of the approaches by which nanoparticles could be used to combat antibiotic resistance. Many types of NPs, such as organic polymers, inorganic metals, and nanostructured materials, are being made for a variety of biomedical applications. They have been established to

exhibit antibacterial activities in laboratory and animal studies, and they could act as a replacement or addition to conventional antibiotics (Tiwari et al., 2012). Nanoparticle efficiency is determined by size, form, and physicochemical character. Scientists discovered that shrinking substances to the nanoscale totally changes their character, such as optical behavior and reactivity. Due to their strong bactericidal action, nanoparticles have emerged in the biomedical field as emerging next-generation antimicrobial drugs. By combining nanotechnology with standard treatments, new means of attacking antibiotic resistance might be found while enhancing patient success (Simon-Deckers et al., 2009; Ssekatawa et al., 2020).

Preparation Methods of NPs

There are different methods used for the production of NPs, but mainly these methods fall under two classes for instance; (1) Top-down approach (2) Bottom-up approach (Wang & Xia, 2004). Based on different properties such as operation, protocols used to synthesize them, and conditions of reaction, these two main classes are further divided into different subclasses (Figure 1) (Tiwari et al., 2012).

Top-down Approach

This method is based on a destructive approach in which larger molecules decompose to make smaller units of these molecules. These smaller units are then employed to make NPs of suitable sizes. Different decomposition techniques are adopted in this method of NPs synthesis which mainly include grinding or milling and physical vapor deposition (Iravani, 2011). Using the top-down approach coconut shell (CS) NPs are produced. In the production method of CN, the milling method is used to finely grind the raw CN powder for a specific interval of time. In this method, ceramic balls and planetary mills are used for milling purposes (Bello et al., 2015). Another example of NPs production from top-down method is the synthesis of spherical magnetite NPs in the presence of organic oleic acid from natural iron oxide ore. The size of these NPs ranges from 20 to 50 nm (Priyadarshana et al., 2015). Another example of top-down approach is the development of colloidal carbon spherical particles in which sizes of the particles are controlled. In this technique, continuous chemical adsorption of polyoxometalates (POM) is done on the interfacial surface of carbon. The continuous adsorption process on the carbon surface results in the aggregation of carbon into smaller spherical particles. These smaller particles have high diapersion capacity and narrow size distribution (Garrigue et al., 2004). Recently another top-down approach called laser fragmentation is employed to make highly photoactive Co_3O_4 Nps. In this method, powerful laser irradiations produced well-uniform NPs with excellent oxygen vacancies (Zhou et al., 2016). The size of these NPs ranges from 5.8 nm ± 1.1 nm.

Bottom-up Approach

In the bottom-up approach, relatively simpler substances are employed for the synthesis of NPs that's why this technique is also known as the building up approach. Sedimentation and reduction techniques are examples of this approach. Different protocols such as sol gel, spinning, biochemical synthesis, and green synthesis are employed in the bottom-up approach (Iravani, 2011). TiO_2 anatase NPs with graphene domains is an example of bottom-up approach (Mogilevsky et al., 2014). Solvent-exchange method is also used recently for the production of limit sized low density lipoprotein (LDL) NPs. These LDL are employed for medical cancer drug delivery applications as they have high hydrophobic capacity (important for drug delivery purposes) (Needham et al., 2016). Another example of bottom-up approach is the synthesis of monodispersed spherical bismuth (Bi) NPs with good colloidal capabilities. The size of these NPs produced from bottom-up approach ranges between 100 nm to 500 nm (Wang & Xia, 2004). Green and biogenic synthesis fall under the category of bottom-up approach which has drawn the attention of researchers as these methods are cost effective, environment friendly, and less toxic. Different biological systems are employed in these techniques for instance plant extracts, Aloe vera, bacteria, yeast, and human cells (Parveen et al., 2016).

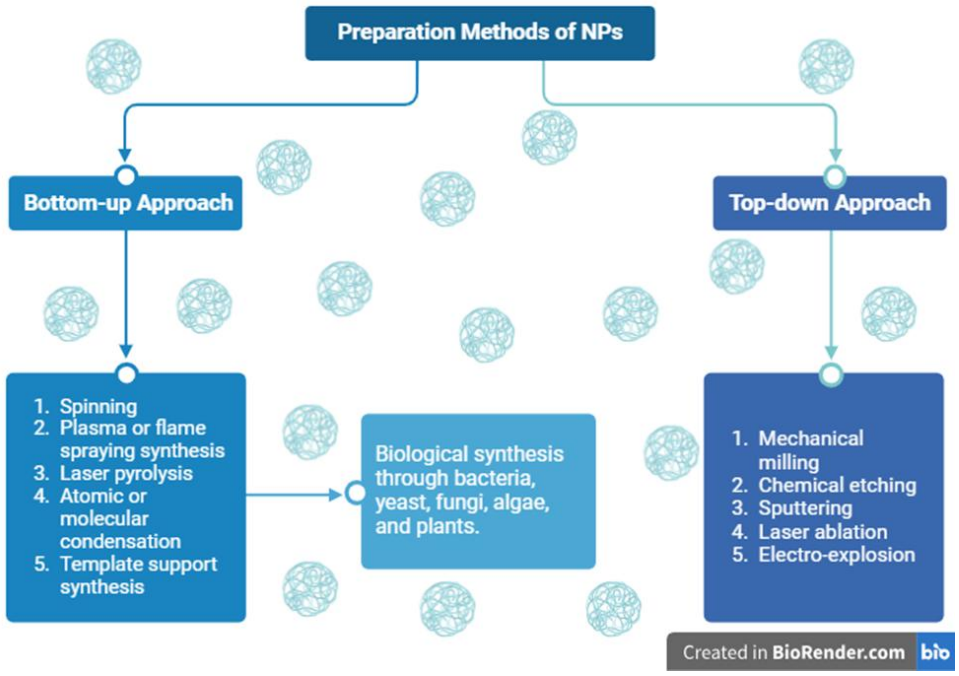

Fig. 1: Methods of preparation of NPs.

Antimicrobial Mode of Action of Nanoparticles

Nanotechnology has become one of the best methods to combat AMR and challenges associated with it. There are a number of ways through which the Nanoparticles work to increase the potency of drugs and decrease the chances of resistance (Figure 2) (Ozdal & Gurkok, 2022).

Disruption of Bacterial Membrane Integrity

The primary physical barrier for any antimicrobial agent is the bacterial membrane so first to penetrate the cell wall of a bacterial cell it is necessary to develop a NP with such effective features. One of the best NPs, AuNP is investigated and considered the best to work as an antimicrobial agent (Bilal et al., 2017; Tao, 2018). The AuNPs attach to the bacterial membrane via electrostatic interactions and finally cause the disruption of the integrity of the bacterial cell membrane (Kundu, 2017). Other than that, Nps changes the membrane potential and hinder the activity of ATP synthase which ultimately result in the reduction of major metabolic activities. AuNPs also cause the disruption of bacterial structure as the cell loses its integrity. Other than AuNPs, GBNs (graphene-based NPs) are also known for their mechanisms of bacterial cell membrane disruption. These GBNs also called as "nano-knives" and MoS2 and MnO2 having sharp edges are also famous for their work as they cause physical harm to lipid bilayer of bacterial cell membrane (Lu, 2017). After cell membrane, biofilm is another way through which bacteria become resistant to various drugs. Different drug delivery systems have been studied which can be used for the destruction of biofilm. In a study, it has been seen that use of silver NPs, capped with rifampicin, reduces the formation of biofilm in different bacteria such as *Klebsiella pneumoniae* and MRSA. It also increases the antimicrobial activity which helps to eradicate the already present biofilm in these strains of bacteria (Farooq et al., 2019).

Formation of Reactive Oxygen Species (ROS)

Oxidative stress due to the production of reactive oxygen species (ROS) is an important mechanism of NPs against bacteria. In normal circumstances, the balance is maintained between the production and clearance of ROS in bacterial cells. While during the excessive production of ROS, the bacterial cell promotes the process of oxidation. The oxidation reaction causes the disturbance of balance and causes oxidative stress which results in the damage of bacterial cell components (Li et al., 2012; Peng et al., 2013). Different types of NPs cause the production of different ROS such as calcium oxide and magnesium oxide NPs produced O-2 and zinc oxide NPs generate H2O2 and OH. Whereas copper oxide NPs produced O-2, OH, H2O2 and O2 (Malka et al., 2013).

Release of Metal Ions

The release of metal ions is another antibacterial mechanism of NPs. In this mechanism, the mental ions are released from the inorganic NPs which are then absorbed in the bacterial cell membrane and cause its disruption. Other than membrane disruption, these metal ions also cause damage to biomolecules of bacteria such as amino, thiol, and carboxylic functional groups. For instance, the antimicrobial activity of AgNPs is attributed to the Ag+ release (Hsiao et al., 2015). These silver ions perform their function by blocking the catalytic activity of enzymes, replication of DNA, and disrupting the synthesis of cell walls of Gram-positive bacteria through their interaction with sulfhydryl groups (Baptista et al., 2018). Zinc NPs work by producing zinc ions which destroy the membrane integrity by making a bond with the thiol group of cell membrane (Stanić & Tanasković, 2020).

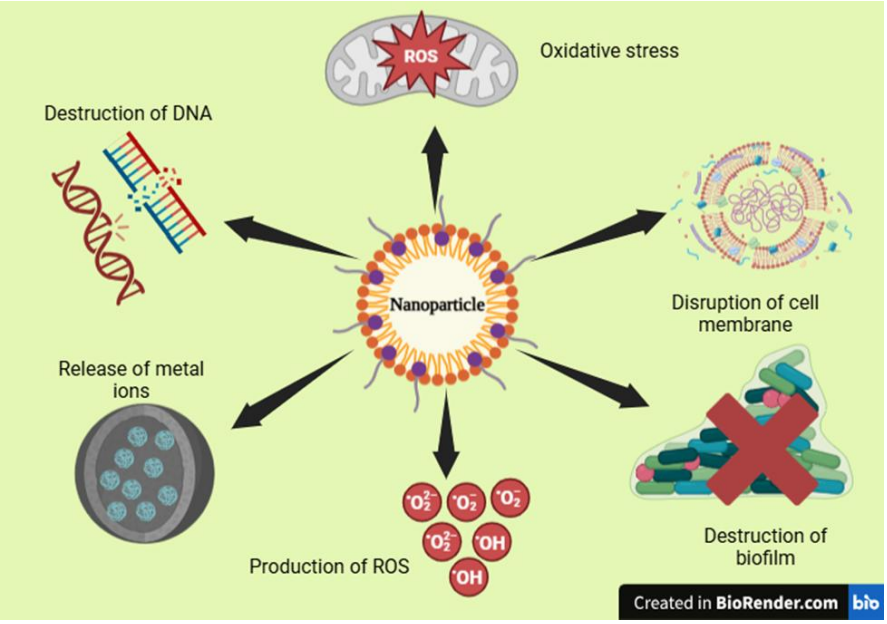

Fig. 2: Mechanism of action of NPs.

Types of Nanoparticles used against AMR

As a potential weapon against AMR, Nanotechnology presents a novel way to fight against AMR. This is done through a number of ways including development of innovative nanoparticles which are then used for drug delivery purposes, antimicrobial agents, and different tools used for diagnostic purposes. Some of the important classes of nanoparticles are mentioned subsequently (Figure 3) (Bharti & Kumar, 2025).

Metallic Nanoparticles

The nanoparticles made of metals are called metallic nanoparticles. Among these metallic nano-sized particles, silver-based nanoparticles (AgNPs) are very famous for killing bacteria. These silver-based nanoparticles (AgNPs) release their charged silver ions that act on bacterial membranes, cut their connection to the outside cell environment, which is essential for life, and become the cause of death of bacteria. Similarly, these silver ions change the structures of the bacteria's protein; due to this, bacteria cannot perfect their functions for the continuity of life. Also, these silver nanoparticles (AgNPs) release reactive oxygen molecules, and bacteria cannot detoxify them, and this ultimately causes the death of bacteria due to oxidative stress (Mukherji et al., 2019). Due to these properties, these silver nanoparticles (AgNPs) are used in medical products like bandages or wound dressings to prevent and control infections. The gold-based nanoparticles (AuNPs) are effective in killing bacteria when used with other antibiotics or antimicrobial peptides. Zinc oxide (ZnO) nanoparticles kill bacteria by releasing harmful reactive oxygen species (ROS). Zinc oxide (ZnO) nanoparticles are used for skin applications to prevent and control infections. Copper-based nanoparticles (CuNPs) also kill bacteria by releasing harmful reactive oxygen molecules. Bacteria form biofilm biotic and abiotic surfaces for their survival. Copper oxide nanoparticles (CuNPs) are specially used in medical instruments to prevent bacterial growth. Both copper and zinc oxide release reactive oxygen molecules, which prevent the formation of bacterial protective coats against microbial agents called biofilm (Applerot et al., 2010; Mousa, 2013).

Lipid based Nanoparticles

The nanoparticles made up of lipids are known as lipid nanoparticles. These lipid-based nanoparticles provide a very efficient drug delivery system into the body. These lipid nanoparticles are highly versatile because all water and fat-soluble drugs can be used with these particles (Yousefi et al., 2019). Famous lipid nanoparticles are liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) (Khalifa et al., 2022). These nanoparticles are biocompatible with the tissues of the body and have an efficient drug release controlled system (Devrim & Bozkır, 2017). Liposomes have two layers, one for water soluble and other for fat soluble drugs. Similarly, solid lipid nanoparticles and nanostructured lipid carriers provide protective structures for drugs that are released by a controlled mechanism. These nanoparticles also increase solubility and bioavailability means they make drugs available and absorbed by the body to its maximum concentration to a specific site to prevent and control infection. Moreover, another benefit of these nanoparticles is that their structures can also be changed according to the infection for reaching that specific site of infection. Lipid nanoparticles have a versatile nature. These can be used as intravenous treatments, as topical ointments, and in specific areas for effective treatment to prevent and control infections (Md et al., 2017).

Polymeric Nanoparticles

The nanoparticles that are made up of polymers are known as polymeric nanoparticles. The polymer-based nanoparticles are compatible with the tissues of the body and are biodegradable, meaning they easily break down in the body and are removed by the body. So, these types of nanoparticles are very helpful for the transportation of antimicrobials into the body (Agnihotri et al., 2012). These polymer-based nanoparticles also offer a targeted approach and controlled drug release mechanism for antimicrobials to combat specific infection. Polymeric micelles are a type of polymer based nanoparticles which are small structures through which water insoluble drugs can be delivered into the body easily. Dendrimers are another type, which are loaded with many branches that help them incorporate many drugs at a time. Therefore, at a time, these nanoparticles are effective at multiple infection sites. And its unique, highly branched structure makes them able to penetrate and degrade biofilm, which is a protective barrier of bacteria against many antimicrobials (Vassallo et al., 2020). Chitosan nanoparticles are also polymer-based. Chitosan is a polysaccharide made up of chitin from the exoskeleton of crustaceans. These nanoparticles are positively charged, which attach to the negatively charged membrane of bacteria and cause damage by disturbing its arrangement of molecules, leading to the death of the bacteria cell. This property of Chitosan is from inheritance. Due to their unique structure and properties, these nanoparticles are most commonly used for healing purposes and controlled and targeted drug delivery into the body (Ansari-Asl et al., 2022).

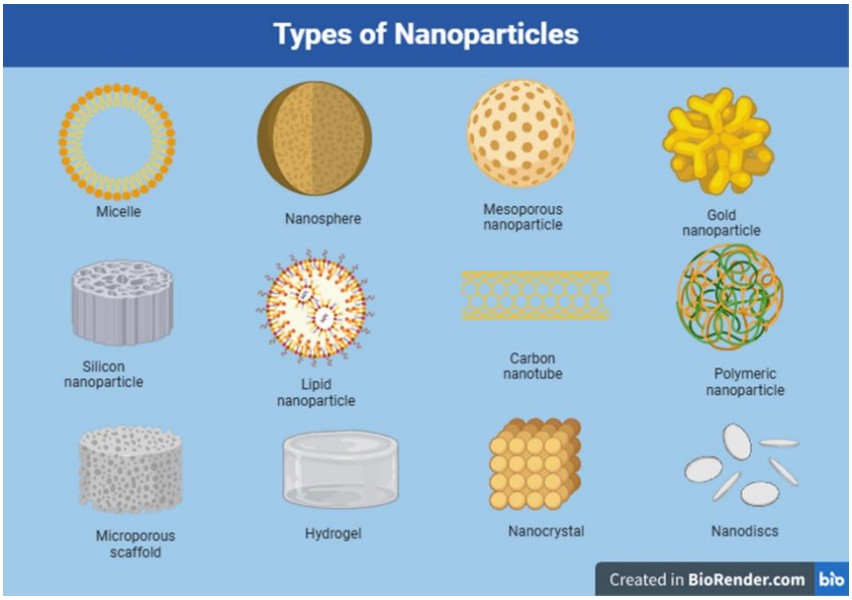

Fig. 3: Different types of Nanoparticles.

Carbon based Nanoparticles

Carbon also used for the manufacturing of NPs such as graphene nanotubes and carbon quantum dots are made up of carbon. These NPs have shown significant antibacterial activity. The carbon NPs cause minimum chemical and physical harm which is not avoidable as they perform their bactericidal activity. The exact mechanism of action of carbon NPs is not confirmed yet but some studies show that carbon nanotubes (multi-walled) helps to destruct the formation of biofilms by different bacteria such as *Klebsiella oxytoca, Pseudomonas aeruginosa and Staphylococcus epidermidis* (Prestinaci et al., 2015; Jit et al., 2024).

Conclusion

Infections caused by bacteria, virus, and fungi are common and increase at a high speed which also causes the issue of AMR, a major challenge for public health. AMR is the result of misuse of antimicrobial drugs, overuse of antibiotics and this situation demands novel strategies to handle these crises. Nanotechnology is the answer to all these crises as the success rate of treatment with nanotechnology is high as compared to conventional treatment. Decreasing the use of antibiotics, enhancement of drug release, change the target of antibiotics, and modification of enzymes in pathogens, are the various methods to stop the development of AMR. All the functions are performed by nanoparticles as the nanotechnology rises up as a beacon of hope and it provides a large number of solutions against the complexities of AMR. Nanotechnology as a weapon against AMR serves as a leverage for the researchers to explore nanoparticles as an innovative approach against AMR. There is a need of comprehensive understanding of potential of these nanoparticles such as metals, lipids, and polymers to address the challenge of AMR. This will empower the development of understanding of nanotechnology having great advantages to be used as a significant treatment against bacterial infections and AMR.

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