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Review

Nanomaterials: The New Antimicrobial Magic Bullet

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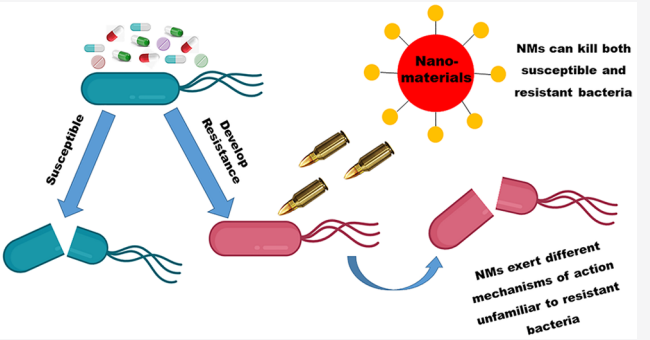
ABSTRACT: Bacterial infections are a significant cause of mortality and morbidity worldwide, despite decades of use of numerous existing antibiotics and constant efforts by researchers to discover new antibiotics. The emergence of infections associated with antibiotic-resistant bacterial strains, has amplified the pressure to develop additional bactericidal therapies or new unorthodox approaches that can deal with antimicrobial resistance. Nanomaterial-based strategies, particularly those that do not rely on conventional small-molecule antibiotics, offer promise in part due to their ability to dodge existing mechanisms used by drug-resistant bacteria. Therefore, the use of nanomaterial-based formulations has attracted attention in the field of antibiotic therapy. In this Review, we highlight novel and emerging nanomaterial-based formulations along with details about the mechanisms by which nanoparticles can target bacterial infections and antimicrobial resistance. A detailed discussion about types and the activities of nanoparticles is presented, along with how they can be used as either delivery systems or as inherent antimicrobials, or a combination of both. Lastly, we highlight some toxicological concerns for the use of nanoparticles in antibiotic therapies.



KEYWORDS: *nanomaterials, antibiotic alternatives, antimicrobial resistance, MRSA*

1. INTRODUCTION

Bacterial infections are an increasing menace to human well-being worldwide, especially in the developing world.[1,2] The emergence of bacterial resistance to conventional antibiotics has made the matter even worse, becoming a growing crisis in public health. Resistant infections lead to an increase in mortality and morbidity, with adverse impact on the clinical outcome of many types of patients, especially those undergoing surgery, transplantation, cancer treatment or those in intensive care units.[3] A report by the WHO suggests that global mortality due to antibiotic-resistant bacteria is already around 700 000 people annually,[4] and it is predicted that this number will climb to 10 million by 2050, with an estimated cumulative loss of 100 trillion USD to the global economy,[5] if efforts are not made to curtail resistance or develop new antibiotics.

Most antimicrobials have been developed from metabolic biochemical products that have been used by microbes to fight each other for millions of years.[6] The evolution of these offensive molecules throughout this continuous warfare has in turn created defense responses;[7,8] through intrinsic resistance such as reduced outer membrane permeability and/or increased efflux pumps of antimicrobial agents,[9] or through evolutionary resistance, where the resistance is acquired through biochemical pathways, which include destruction of antimicrobial agents or modification of their targets (Figure 1).[7,8] For example, genes encoding for resistance to the clinical antibiotics tetracycline, vancomycin, and penicillin have been detected in bacteria isolated from 30 000 year old permafrost.[6] Therefore, to eradicate these microorganisms, doctors often use multiple and high doses of antibiotics and/or may need to rely on so-called "last-resort" antibiotics.[10] In addition, other bacterial survival mechanisms, such as biofilms, provide barriers to effective therapies. Biofilms may require physical treatment via debridement (removal of necrotic tissues) as well as high doses of antibiotics.[11,12] These treatments can be expensive and extended, with uncertain outcomes and possible long-term adverse effects.

Antibiotic-free strategies or combination therapies where antibiotics are used in conjunction with other strategies are emerging approaches that are being investigated in order to counter this antimicrobial resistance challenge.[13] Some "non-antibiotic" methods such as phage-based therapies and

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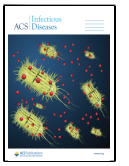

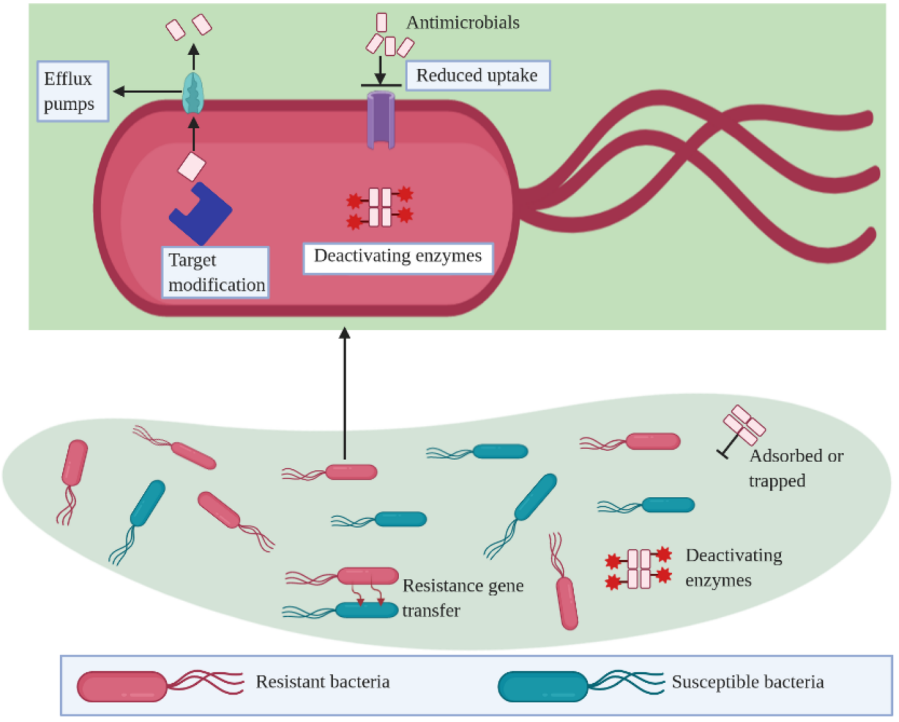

Figure 1. Antimicrobial resistance mechanisms toward conventional antibiotics. The resistance can happen in different ways: (1) bacteria mutate, communicate, and share resistance genes with one another, which results in spread of resistance genes across bacteria population; (2) resistant bacteria can harbor intracellular/extracellular enzymes that limit the binding of, degrade, or deactivate the antibiotics; (3) entry of antimicrobials into bacterial cells is limited; (4) efflux pumps, which in most cases are upregulated in resistant bacteria, actively transport antimicrobials outside the bacteria; and (5) the complex nature of the bacteria extracellular polymeric substances restricts the penetration of antimicrobials into bacterial cells.

vaccines have already been utilized against bacterial infections. However, these therapies only work selectively on specific bacterial strains, so they do not cover the spectrum of potential pathogens.[14,15] Furthermore, while vaccines have proven to be very effective as preventatives, they cannot be used to treat an acute infection. Other strategies, such as targeting virulence factors or harnessing the immune system response, have had limited success to date. Therefore, new and emerging approaches for antimicrobial chemotherapy are needed to accomplish better outcomes.

The use of nanomaterials in antimicrobial therapy, as antimicrobial alternatives or as a complement to existing antibiotics, is one research area that shows potential and may provide a window of opportunity to intervene where conventional antibiotic therapies fail.[16] Nanoparticles (NPs) might be used alone or as delivery systems (Figure 2 and Table 1), and the bacteria may not be able to quickly develop resistance to NPs since the resistance mechanisms needed are not already in the bacteria's natural offensive/defensive arsenal. However, the rapid evolution of new resistance mechanisms is still possible due to selection for beneficial mutations during the rapid exponential growth of bacterial populations. In recent years, the development of new antimicrobial NPs-based systems, where the NPs can act as nanocarriers for the efficient delivery of antimicrobials, possess inherent antimicrobial activity on their own, or work using a combination of both properties, gives new opportunities to confront bacterial infections and antimicrobial resistance.[17] The physicochemical properties of NPs, such as shape, size, and surface chemistry and porosity are very unique, and these parameters impact their therapeutic activity.[18] NPs generally vary in size from 10 to 1000 nm (vs 1 μm for bacteria), and the size can be engineered depending on the targeted bacteria and the mechanism of action.[19−21] Also, NP−bacteria cell interactions can be further modulated or regulated by functionalizing the NPs surface.[22−24] Therefore, nanotechnology can provide a novel toolkit to combat not only bacterial infections but also antimicrobial resistance.

In this Review, the types of NPs that have potential as antibiotic therapy are described, along with their mechanism of action. Moreover, we discuss the application of NPs as antibiotic alternatives, providing insight into how NPs have been applied in different ways for antibiotic therapy. Lastly, we highlight the potential toxicity issues associated with NPs, both as carrier systems and as inherent antimicrobials.

2. TYPES OF NANOPARTICLES

The continuing interest in developing NPs for therapeutic and diagnostic applications has largely focused on the field of cancer therapies. However, the challenges associated with antimicrobial resistance and the recognition that we need some alternatives to conventional antibiotics have led to increased studies on the application of NPs as substitutes for, or complements to, conventional antibiotics. There are a range of NP-based systems and constructs that have been studied for antimicrobial therapies.[28,29] Figure 3 shows five examples of NPs prototypes that have been investigated for antimicrobial applications. Silica-based NPs are made of silica polymers formed from repeating silicate tetrahedron units. There are various subclasses of silica NPs, such as nonporous silica NPs, mesoporous silica NPs, hollow mesoporous silica NPs, and

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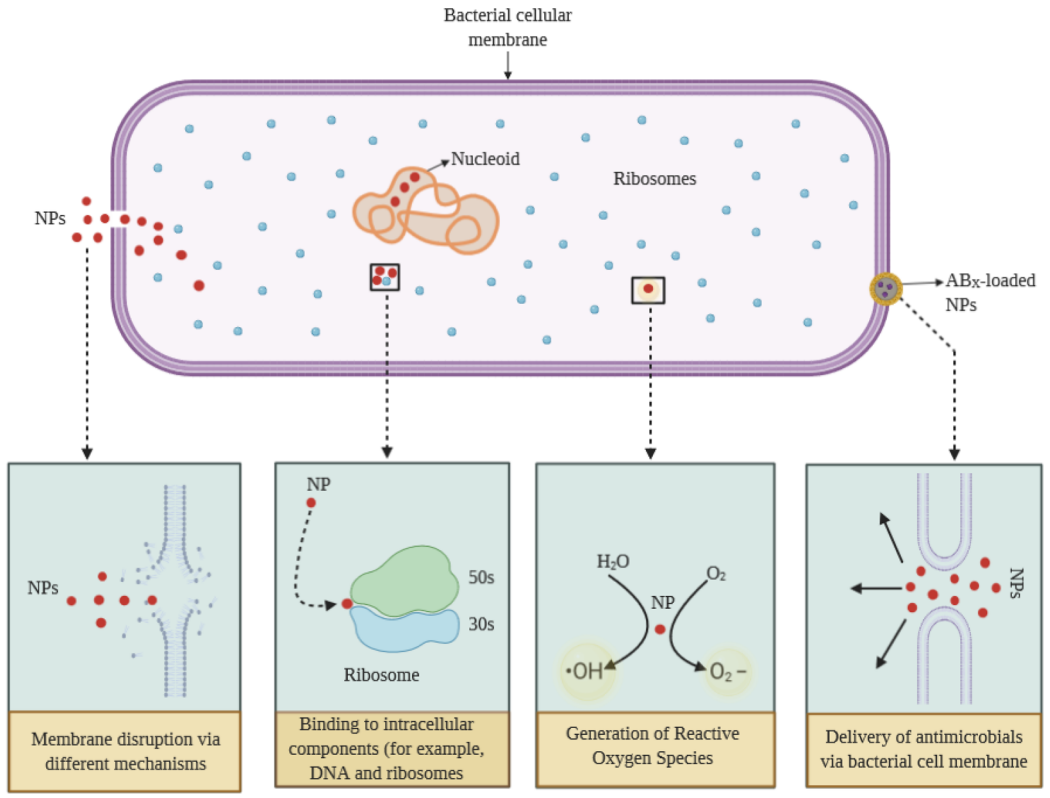

Figure 2. Simplified diagram showing the role of NPs when used in the treatment of infections. Electrostatic interactions between anionic groups on the bacteria outer surfaces and engineered NPs can cause bacterial cell membrane damage and cytoplasmic leakage. Bacteria metabolism can be disrupted or compromised with NPs binding to different intracellular components (like DNA, ribosomes, and proteins). NPs with catalytic activities can induce and/or increase reactive oxygen species (ROS) production, causing oxidative stress in the bacteria. Moreover, NPs (for example, polymeric/liposomes based) can be used as delivery vehicles of antimicrobial agents by readily entering bacteria cell membranes. [Note that in this figure and in all figures in this Review the sizes of NPs, molecules, and bacteria are not to scale. The detailed mechanisms of action of NPs against bacteria are discussed in section 3.]

Table 1. Antimicrobial Resistance Mechanisms and How Nanoparticles Can Circumvent Those Mechanisms[a]

antibiotic resistance	What can NPs offer?	refs
1. Transfer of resistance gene and modification of target	Since NPs can have numerous bactericidal mechanisms due to their access to various targets, the emergence of bacterial resistance is less likely in comparison to conventional antibiotics.	25
2. Deactivating enzymes	The various and complex mechanisms of action of NPs and their non-biological nature give them the ability to evade deactivation by bacterial intracellular and/or extracellular enzymes.	25
3. Reducing antibiotic uptake	Some Gram-negative bacteria have evolved to limit the entry of antimicrobial agents through porin gene mutations. Some NPs, on the other hand, penetrate the bacteria cells via non-porin pathways, such as membrane fusion or endocytosis.	25
4. Efflux pumps	NPs can prevent efflux pump mechanisms, which increases penetration and accumulation of antimicrobials inside bacteria cells.	26, 27
5. Extracellular polymeric substances limit antibiotic penetration	The unique particle size, morphology, and surface chemistry of NPs permit them to interact with and penetrate the bacteria cells. The ability to engineer the polarity of NPs can help them exert different interactions with extracellular polymeric substances, including electrostatic and hydrophobic interactions, which maximizes adsorption on bacteria outer surfaces and diffusion across bacterial cells.	18, 25

[a]Bacteria have developed different survival mechanisms to avoid killing by antibiotics. NPs are new to bacteria and can overcome and circumvent antimicrobial resistance mechanisms that affect conventional antibiotics.

core−shell silica. Further diversity is possible, as their surfaces can be either modified or unmodified.

2.1. Mesoporous Silica Nanoparticles. Among the nanomaterials for delivery systems and other pharmaceutical applications, mesoporous silica NPs have received considerable attention for their potential to deliver antimicrobial agents, as they possess desirable properties such as high drug loading capacity, ease-of-functionalization, and biocompatibility. The porous NPs enable the adsorption and entrapment of antimicrobial agents within the pores, and there is considerable capacity for optimization through tunable pore size and volume as well as variation in particle size and surface functionalization.[30−33] Mesoporous silica NPs can be synthesized and utilized as inherent antimicrobial NPs and/or as delivery vehicles for antimicrobial agents. For direct activity, mesoporous silica NPs-based antimicrobial nanosystems are

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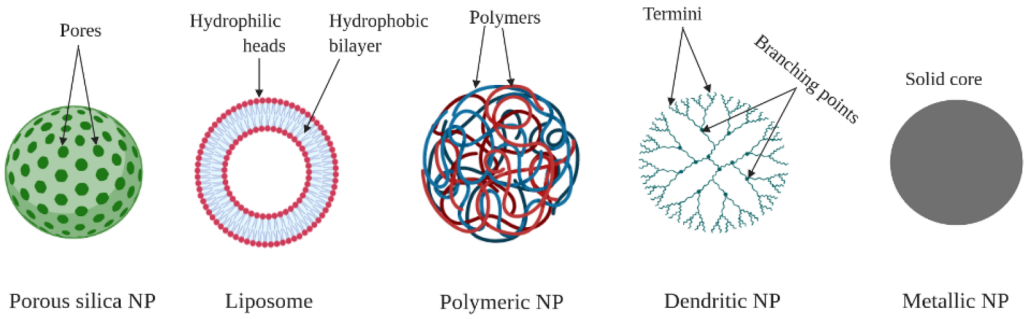

Figure 3. General types of NPs used for antimicrobial applications.

designed and tuned to exert their antimicrobial activities by, for instance, adsorbing metal ions or ultrasmall NPs onto the silica NPs matrix or by encapsulating metal/metal oxide NPs into the pores of silica NPs. In this way, the antimicrobial activity of metal/metal oxide NPs, like metal ion release, oxidative stress, and other non-oxidative mechanisms, can be imparted on the mesoporous silica NPs-based composites. Various design approaches, such as a porous silica shell coating on metal or metal oxide NPs, can be employed to overcome problems related to the aggregation of metal or metal oxide-based NPs that sometimes hamper their performance. For delivery of antimicrobials, the high surface area and porosity of silica NPs are essential, and together with the encapsulated antimicrobial agent, they can provide synergetic effects for the treatment for infections.[30−34] The antimicrobial agents, such as vancomycin, have been delivered by loading the antimicrobial agents into the mesopores of silica NPs,[35,36] by incorporating the antimicrobial agent of interest into the mesoporous silica NPs matrices, and/or by adsorbing/conjugating them onto the surfaces of mesoporous silica NPs surfaces.[37] When using the mesoporous silica NPs as the carrier systems for antimicrobial agents, their surface functionalization as well as their shape and size are important, and those parameters need to be optimized in order to have the desired release profiles of the drug.[38] Therefore, the mesoporous silica NPs characteristics including high surface area, tunable pore size, and large pore volume, their easy surface functionalization, as well as the permeation capacity of mesoporous silica NPs through physio-biological barriers make them good candidates for the design of effective carrier systems for antimicrobials.[30−33,36]

2.2. Liposomes. A very different type of NPs are liposomes, spherical-shaped vesicles that are comprised of an aqueous solution core bounded with phospholipid bilayers. These bilayers separate the continuous aqueous phase on the outside from the aqueous phase media on the inside. It is possible to load the liposomes with either hydrophilic and/or hydrophobic drugs with hydrophobic drugs becoming encapsulated in the hydrophobic lipid bilayer, while hydrophilic drugs become sequestered inside the aqueous core.[39] Liposomes are among the most used nanosystems in drug delivery.[40,41] The mechanism of liposomal drug delivery is based on the liposomes interacting with the cell membrane via a passive or a mediated pathway that allows release of the loaded drug (antibiotic in this case) into the cellular membrane of the targeted cell—usually mammalian cells for most liposomal delivery research (e.g., lipid nanoparticles have played a key role in the successful delivery of mRNA vaccines),[42] but obviously selectivity for bacterial cells is required for antibiotic delivery. This fusion is driven by the similarity of the liposome composition with the biological constituents of the cell membranes.[43] Moreover, it is possible to functionalize the liposomal exterior surface with different functional groups that add additional properties, such as a "stealth" ability in the human body where they are modified to avoid rapid clearance by the reticulo-endothelial systems and increase their circulation time in the blood.[44] The surface modification and/or coating can convey an additional advantage of creating a well-ordered hydrated layer around the particle, inhibiting the formation of undesired plasma protein coronas. However, the instability and osmotic fragility of liposomal formulations have always been one of the hurdles to overcome in their practical use, since the encapsulated drugs can then escape through the bilayers.[45]

2.3. Polymeric Nanoparticles. Polymeric NPs are another type of NP-system that can be used to deliver antimicrobial agents, which are either adsorbed onto the surface or encapsulated within the polymeric core. They are hyper-branched and perforated NPs based on polymeric skeletons that allow drugs to be entrapped within the crevices of the polymeric matrices, via either covalent or non-covalent interaction. Polymeric NPs have been used in the clinic for drug delivery due to their biodegradability and biocompatibility.[46−49] They have many unique characteristics, such as narrow size distribution and structural stability. Properties such as surface charge, release behaviors of loaded drug, and size can be tuned and optimized by changing the length and size (between 10 and 1000 nm) of the polymer, the solvent used in synthesis, and the surfactant, among many other parameters. Polymer-based NPs are normally stable in blood, and elimination can be delayed by modifying their surfaces with different functional groups.[16,28]

Polymeric NPs can be categorized based on the routes of their synthesis, as self-assembled polymer NPs, star polymer NPs, inorganic polymer hybrid NPs, and other core−shell polymer NPs.[50] Self-assembled polymer NPs are of the most used nanostructured materials in drug delivery applications.[51] Thermodynamically stable molecular and supramolecular complexes are formed by non-covalent and weak interactions between the individual chemical building blocks.[51] Self-assembly has been utilized for the synthesis of nanosystems for different applications, where polymer micelles and polymer vesicles have been explored for use as novel antimicrobial agents. Polymer micelles are formed through the self-assembly of amphiphilic block copolymers,[52] whereas polymer vesicles are spherical polymeric capsules with an inner hollow compartment confined by a bilayered membrane that is composed of amphiphilic block copolymers.[53] In comparison with polymer micelles that have an internal lipophilic core and

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a lipophobic outer layer, polymer vesicles have a hydrophilic cavity inside the polymer shell, with the outside layer in contact with the medium. This structure enhances the compartmentalization and increases versatility in functionalization for polymer vesicles[54] and has structural similarity to biological cells. Polymer vesicles proven to be useful in the delivery of antimicrobial agents and, more generally, in drug delivery.[55] Despite their versatility and potential for antimicrobial applications, the dynamic and reversible behavior of self-assembled NPs might render these nanosystems susceptible to disassembly.[51] In contrast, star polymer NPs are unimolecular nanostructures formed by covalent bonds with a branched architecture that links a number of linear chains to a central inner core, leading to the formation of 3-D globular structures.[56] Star-shaped polymer NPs can be synthesized with high molecular weight while still maintaining their viscosity and solubility.[57] Other characteristics of star polymers NPs, such as their loading/encapsulation abilities, and their internal, peripheral, and compartmentalized functionalities,[56] have made them attractive for pharmaceutical applications. A final type of polymer NPs expands the application of inorganic NPs such as metal/metal oxide-based NPs, by coating them with a polymer to reduce potential particle aggregation, improve their oxidative stability, and improve the biocompatibility toward mammalian cells and tissues—enhancing or reducing protein binding as desired.[58]

2.4. Dendritic Nanoparticles. A fourth class of NPs used for drug delivery are dendrimers.[59,60] They are highly branched and normally symmetrical around the coreand have spherical 3-D morphologies that create interior crevices, with several arms coming from a central core. These NPs have demonstrated unique structural characteristics including their globular architectures, multivalency, and high degree of branching, and this makes them promising systems for delivery of active molecules of interest.[61,62] The hydrophobicity of dendrimers can be tailored by functionalization of their outer shells with groups of different polarities and/or with groups of different charges.[61] Antimicrobial agents can successfully be loaded in dendrimers either by covalent chemical bonding or through physical interactions in order to ameliorate their aqueous solubility and bioavailability.[63] Dendrimers have also been used as effective antimicrobial agents on their own.[64] For example, dendrimers with cationic surfaces can interact with anionic bacterial cell membranes, whereas those possessing metal cores can release metal ions and/or produce ROS, resulting in the bacterial cell death.[65]

Dendritic NPs are usually synthesized by either a convergent or a divergent approach.[66] In the divergent strategy, the growth of dendrimers begins from a multifunctional core. Then via a series of reaction and purification stages, the dendrimers grow radially outward.[67] Dendrimers are identified by generations, and the size of the dendrimer, molecular weight, and the number of functional groups increase as the generation increases.[67] At a certain stage of the synthesis, steric hindrance prevents the continuation of the process, and the highest generation is obtained at that stage.[67] In the convergent strategy, dendrons (as parts of dendrimers) are formed in similar fashion to the divergent method but these dendrons are then coupled to a multifunctional core. The benefit of the convergent strategy is that the chemical properties of each dendron can be different and unique, and distinct functional groups with distinct properties can be inserted into the dendrimers at specific sites. Due to their characteristics, dendrimers have drawn attention as possible antimicrobial agents.[66] Both specific and non-specific interactions can be employed to design and synthesize novel antimicrobials. If one of the functional groups is able to interact with the target, all the other groups are in such close vicinity that one might hope for synergies or cooperative interactions. If specific interactions are used, the dendritic structure might offer another advantage due to its inherent polyvalent nature. The existence of internal cavities in the dendrimers can be exploited to design controlled delivery systems, where drug concentrations in the body can be modulated as desired.[68] Dendrimers also offer unique opportunities in these applications since the structure of the dendrimer can be specifically tuned to the requirements of the delivery system. For example, some drugs are very hydrophobic and not very soluble in water. Here the dendritic NPs interior can be tuned to be hydrophobic so that a lipophilic drug can be loaded, while still retaining their hydrophilic exterior for optimum circulation time, dissolution, and solubility.[67,69]

2.5. Metal-Based Nanoparticles. Finally, there are solid core NPs, where metals and metal oxides are used. These NPs are the most common NPs that have direct antimicrobial activity. It is generally not possible to load antimicrobial agents into these metallic-based NPs although their surfaces can be adorned with antimicrobial agents. Different types of metal-based NPs, including silver, copper, titanium, zinc, nickel, and their oxides, have shown antimicrobial activity[70] and it has been demonstrated that their activity depends on their composition, intrinsic characteristics, surface functionalization, and the targeted bacteria.[71] The methods of synthesis can be categorized into physical, chemical, and green synthesis.[72] Regarding physical methods, the evaporation/condensation method is the physical method often used to synthesize metal-based NPs, where NPs are directly synthesized from a metal source. By modulating the synthesis conditions, the physical characteristics of the NPs can be controlled through adjustments in the length, diameter, and size of the NPs.[72] For chemical methods, methods like the atomic layer deposition method, where metal oxide and metallic 3-D nanomaterials are synthesized using different techniques and templates;[73,74] the chemical reduction method, where a metal ion precursor is reduced by a reducing agent in the presence of a stabilizer (protecting agent);[75] and the cryo-chemical method, where metallic and volatile components (for example, an organic monomer) are simultaneously evaporated, which is followed by co-condensing the vapors on the cold surfaces of the vacuum reactor,[76] have been used. Although physical and chemical methods have been predominately used for the synthesis of metal-based NPs, both methods have been shown some drawbacks and are not eco-friendly.[77,78] The physical methods are done at high temperatures and pressures and require expensive equipment, making them expensive and non-scalable[79] On the other hand, the chemical methods both utilize and can generate toxic chemicals that can be cytotoxic and carcinogenic.[80,81] This has led to substantial interest in eco-friendly methods for the synthesis of metal-based NPs, also called "NPs green synthesis" methods. Here besides their eco-friendly nature, these green approaches can also show higher performance, reduced energy cost, and improved safety and scalability.[80,82]

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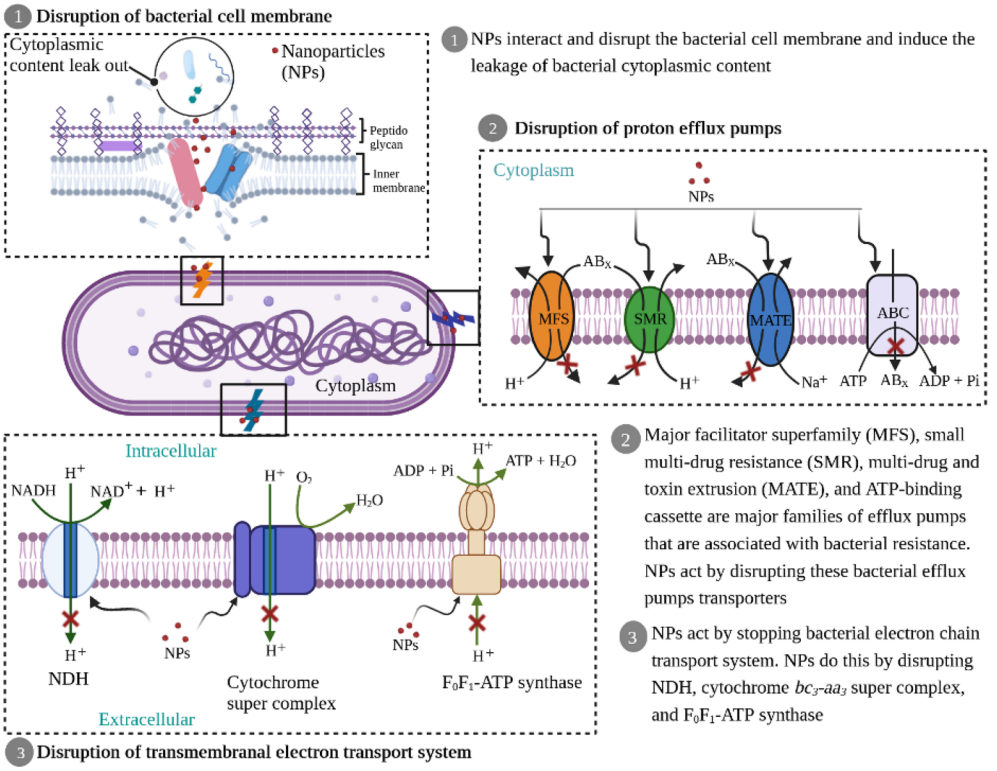

Figure 4. Detailed scheme showing how NPs can exert their antimicrobial activity by disrupting bacterial cell membrane integrity and inhibiting other bacterial mechanisms. NPs can interact and disrupt the bacterial cell membrane, and this can cause the leakage of content of bacteria protoplasm (such as enzymes, nutrients, etc.), which can lead to bacteria death. NPs can also inhibit the proton efflux pumps, one of the mechanisms of bacteria resistance, which can cause the bacteria death. Moreover, they can disrupt the bacteria transmembrane electron transport chain.

3. NANOPARTICLES FOR ANTIMICROBIAL APPLICATIONS AND THEIR MECHANISM OF ACTION

The range of sizes, shapes, and other characteristics of NPs offers a very unique ability to target bacterial infections.[83] Different types of NPs possess varying levels of innate antimicrobial activity, using several antimicrobial mechanisms. This includes (1) immediate damage of the bacteria cell wall and/or cell membrane which leads to the disruption of cell membrane integrity; (2) generation of reactive oxygen species (ROS); and (3) binding and damage to bacterial intracellular components, which leads to inhibition of RNA/DNA synthesis, protein synthesis, and/or other bacterial metabolism. These mechanisms come from the unique physicochemical properties of NPs, particularly their multivalent interactions with bacterial cells. Also, van der Waals forces along with electrostatic, receptor−ligand, and hydrophobic interactions play a big role in the interfacial interactions between NPs and bacteria.[84] Below, we discuss in detail different mechanisms that NPs use in killing bacteria.

3.1. Disruption of Bacterial Cell Membrane and Other Bacterial Processes. The bacteria cell wall and membrane serve as a protection from external invasion and a physical barrier to antimicrobial agents. Lipopolysaccharides and teichoic acids, present in the outer membrane and in the cell wall of Gram-negative and Gram-positive bacteria, respectively, possess phosphate groups that make the outer surfaces of bacteria hydrophobic and negatively charged. This environment restricts the permeation of many antimicrobial agents across bacterial cell membranes, which limits their efficacy.[84] Moreover, bacterial cell wall surfaces have more negative charges than those in mammalian cells, which can preferably favor the electrostatic interactions with NPs that are positively charged.[85] Here NPs can exert their antimicrobial activity (Figure 4) by binding, interacting, and disrupting the bacterial cell membrane.[86−90] Also, polarity and other surface chemistry characteristics of the NPs are crucial factors for the selective binding and disruption of bacterial cell membranes by NPs without affecting other mammalian cells.[91,92] For example, Huo et al.[93] designed and synthesized zwitterionic gold NPs-based antimicrobials. Three sets of zwitterionic gold NPs with different core sizes (2, 4, and 6 nm) were synthesized by reducing chloroauric acid in the presence of zwitterionic ligands. Zwitterionic headgroups (from acylsulfonamide groups) were conjugated to gold NPs. These zwitterionic ligands had different charge orientations; one had positive charge inside the ligand terminus, whereas the other had positive charges in the outermost layer. Improved selectivity and effectiveness was were through modulating the NPs' size and orientation of NPs' surface charges. Increasing the NPs particle size of NPs (from 2 to 6 nm) augmented their antimicrobial effectiveness via bacterial cell membrane disruption. Also, these zwitterionic coated gold NPs exhibited improved antimicrobial activity with low hemolytic activity. NPs with cationic surfaces can bind to the surface of bacterial cells and disrupt the membrane due to their selective targeting of the negatively charged nature of bacterial surfaces. Similarly, NPs with hydrophobic surfaces have shown to be effective at interacting with bacterial cell membranes.[94−97] Here, though, a good polarity/charge balance (i.e., the adjusted balance

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between cationic charge and hydrophobicity) on the surfaces of NPs should be achieved in order for NPs to give effective antimicrobial activity with lower hemolysis of red blood cells and cytotoxicity of mammalian cells.[91] Moreover, the possibility of antimicrobial resistance to these NPs-based systems is minimal as the bacterial membrane composition must be fundamentally altered, making these approaches promising for long-term solutions in the fight against superbugs.[97,98] Apart from interacting and disrupting the bacterial cell membrane, NPs can also exert their antimicrobial activity through inhibiting other mechanisms that bacteria use for resistance and/or for metabolic activities (Figure 4). These involve for instance the inhibition of bacterial proton efflux pumps (which compromises the ability of the bacteria to expel substances),[99−101] and inhibition of bacterial electron transport chains (which results in the bacteria inability to continuously create and maintain an electrochemical gradient over a membrane).[102−104]

3.2. Generation of ROS. ROS are highly reactive chemical byproducts resulting from oxidative metabolic activities in the cell that affect many cell processes such as cell signaling and cell differentiation, subsequently affecting bacterial cell survival.[105] ROS accumulation in bacterial cells results in lethal oxidative stress. ROS can disturb, damage, or destroy the bacterial cell through various mechanisms, specifically via reaction of ROS on cellular proteins, lipids, and nucleic acids.[106,107] A range of antibiotics have been proposed to act via production of ROS,[108,109] though the importance of the role ROS is controversial.[110,111] NPs can generate ROS through different mechanisms (Figure 5). These may include direct ROS generation from the NP surfaces due to interaction with bacterial cell organelles or by oxidation due to interaction with cellular redox-active biomolecules.[112] One example of antimicrobial activity of NPs based on the generation of ROS is the production of copper (Cu^+) ions from copper iodide (CuI) NPs, generating ROS that are able to kill both Gram-positive and Gram-negative bacteria. CuI NPs (in size range of 1−100 nm) can induce bacterial death through ROS production and lipids peroxidation.[19] Pramanik et al. synthesized (by co-precipitation) copper iodide (CuI) NPs with an average particle size of 8 nm and average charge of −21.5 mV. Studies revealed that CuI NPs generated ROS in both Gram-positive and Gram-negative bacteria. which led to ROS-induced DNA damage by suppressing the DNA transcription, as was shown by reporter gene assay. It was hypothesized that ROS might form on the surface of the CuI NPs in the presence of amine functional groups of various biological molecules.[113] Likewise, zinc oxide−silver composite NPs have shown antibacterial activity against resistant *Escherichia coli* and *Staphylococcus aureus*, and this was attributed to the generation of ROS and release of zinc (Zn^{2+}) and silver (Ag^+) ions. The combination of ROS generation and release of those ions generated a flow of bacteriostatic and bactericidal effects.[114] ROS production in bacteria has been shown to be increased by silver and other Fenton-inactive metals since they have the ability to interfere with cell donor ligands coordinating with iron, such as cysteine, and to prompt the release of iron from iron−sulfur clusters; which then augments the formation of ROS.[105] In another approach, mesoporous silica NPs were used to support and improve the catalytic activity and stability of gold NPs.[115] The gold NPs, attached on the functionalized mesoporous silica NPs, exhibited oxidase and peroxidase-like activities, killing both Gram-negative and Gram-positive bacteria, by offering dual enzyme-like activity which enhanced the production of ROS, causing massive oxidative stress to bacteria.[116]

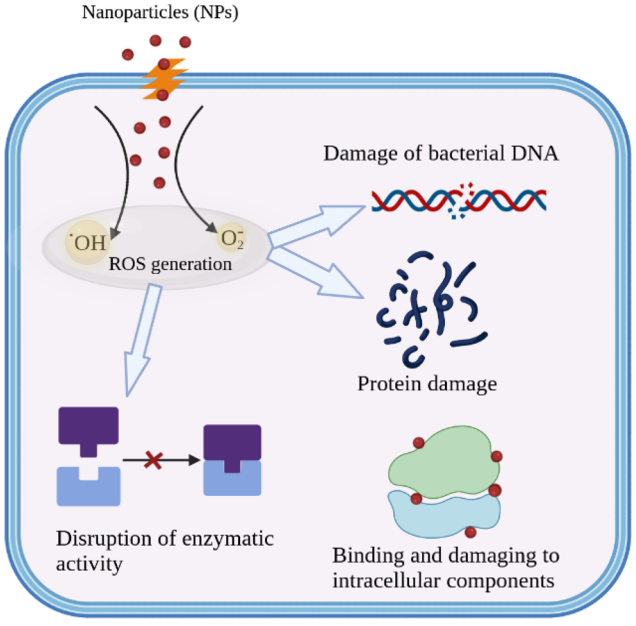

Figure 5. Some NPs (silver NPs, titanium oxide NPs, etc.) exert their antibacterial activity by producing (for example, through Fenton reactions) reactive oxygen species (ROS) inside the bacterial cell, which through different mechanisms cause bacteria death. Meanwhile NPs can directly bind to other bacterial intracellular components (like ribosomes, bacterial chromosomes, and plasmid) and cause the inhibition of bacterial intracellular activity.

3.3. Damage to Intracellular Components. Homeostasis (where the bacterial cell membrane acts as a barrier separating the cellular internal environment from its surroundings) and intracellular signaling pathways are key processes to the survival and function of bacteria. NPs can be tailored to obstruct these processes, eventually causing bacterial cell death. These interferences happen due to inhibition of protein synthesis, changes in gene expression, and DNA damage.[117,118] Gold NPs have shown to act by disrupting the internal metabolic activities of bacteria. The normal size of these gold NPs is 5−400 nm, and they can decrease the attachment of tRNA to ribosome units and cause bacterial death.[19] For instance, 4,6-diamino-2-pyrimidine thiol was used to surface-functionalize gold NPs, producing pyrimidine-capped gold NPs[119] which exhibited activity against resistant strains of *Pseudomonas aeruginosa* and *E. coli*. The mechanisms of action of these NPs were explored with gel electrophoresis that showed the NPs were binding to bacterial DNA, while images from electron microscope showed leakage of nucleic acids. An *E. coli*-free transcription/translation system displayed inhibition of protein synthesis, and colorimetric analyses demonstrated the NPs selectively chelated Mg^{2+}, leading to the destabilization of the bacterial cell membrane. In the same way, silver NPs coated with polyvinylpyrrolidone killed *E. coli* by hindering both amino acid and citric acid cycle metabolic pathways.[120] This was investigated by Ashmore et al., who studied and compared the antimicrobial activity of synthetic polymer-coated silver NPs (Ag 10%) and polyvinylpyrrolidone-coated silver NPs (Ag 99%) in relation to

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Table 2. Nanoparticles and Their Applications against Different Bacteria and Their Characteristics (References are given in brackets)

nanoparticles	mode of application	targeted bacteria	particle size characteristics	role of NPs/antibacterial mechanisms	factors affecting antimicrobial activity/delivery ability
liposomal	delivery vehicle[a] [121−124]	MRSA	250 nm	The lipid bilayer can fuse with other bilayers in the bacterial cell membrane, thus permitting the delivery of antimicrobials into the bacteria. [128, 129]	particle size, stability, and chemistry of liposomes, pH of the medium, and membrane composition [130, 131]
	combination[c] 125−127	MRSA	∼100 nm		
Polymeric NPs					
PLGA	delivery vehicle [132−134]	MRSA	250 nm, 150 nm	by diffusion, solvent-controlled, chemical interaction, and stimulated release [139, 140]	particle size, surface chemistry and charge, stability, solid state nature, and pH of medium [141, 142]
chitosan	antimicrobials[b] [135]	MRSA	100 nm		
	combination136−138	*P. aeruginosa*, MRSA	−		
silica-based NPs	delivery vehicle143−148	*S. aureus*, *E. coli*, *P. aeruginosa*, *M. tuberculosis*	140−190 nm, 300 nm	disruption of cell walls through ROS [152−154]	size, shape, surface charge and chemistry, surface area [152−155]
	combination [149−151]	*E. coli*, *S. aureus*, MRSA	130 nm		
silver-based NPs	antimicrobials [156, 157]	*S. aureus*, *E. coli*, *P. aeruginosa*, *E. faecalis*, *E. faecium*	−	generation of ROS, lipid peroxidation, cytochromes inhibition in the electron transport chain, disintegration of bacterial cellular membrane, bacterial cell wall synthesis inhibition, increase in cell membrane permeability, cell lysis resulting in dissipation of proton gradient, protein and lipid damage due to NPs adhering to bacterial cell surfaces, destabilization and damage of ribosome, and intercalation between DNA bases [152−154]	particle size and shape of particles [152−154]
	combination [138, 158, 159]	MRSA, *P. aeruginosa*	−		
copper-based NPs	antimicrobials [160, 161]	MRSA	−	generation of ROS, DNA damage, protein and lipid oxidation, and dissipation of bacterial cell membrane potential [118, 153, 154]	particle size and concentration [118, 153, 154]
	combination [162, 163]	*E. coli*, *E. faecalis*, *A. baumannii*, *S. aureus*	−		
zinc oxide-based NPs	antimicrobials [164, 165]	MRSA	−	ROS generation, adsorption to cell surface, bacterial cell membrane disruption, and protein and lipid damage [152, 166]	particle size and concentration [152, 166]
	combination [165]	MRSA	−		
titanium oxide-based NPs	antimicrobials167, 168	*P. aeruginosa*, *S. aureus*	−	adsorption to the bacterial cell surface, and production of ROS [152, 153, 169]	crystal structure, shape, and size [152, 153, 169]
	combination94, 165	MRSA	−		
gold-based NPs	antimicrobial [170, 171]	*S. aureus*, MRSA	−	disrupting the respiratory chain, loss of membrane integrity, reduction in ATPase activity, reduced tRNA binding to ribosome subunit, and generation of holes in the cell wall [152, 153]	Roughness and particle size [152, 153]
	combination [127, 172−174]	MRSA	−		

[a]NPs used as delivery systems. [b]NPs used as inherent antimicrobials (on their own), [c]NPs used as a combination of both delivery system and antimicrobials, or which combine two NPs to produce a hybrid NPs.

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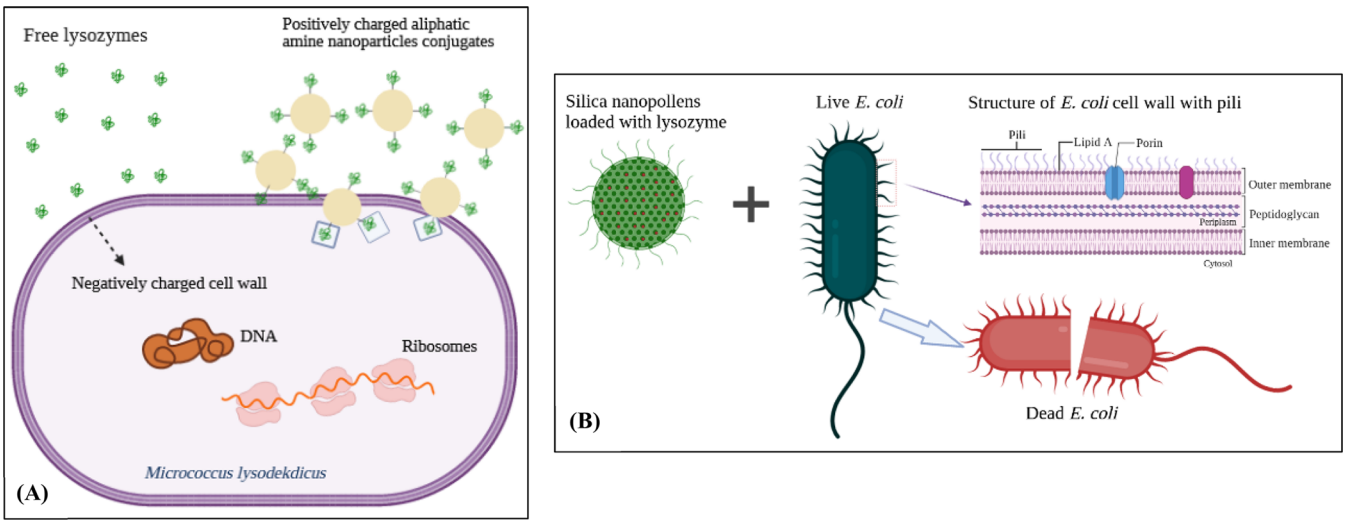

Figure 6. Nanomaterials-based delivery systems. (A) Polystyrene NPs functionalized with cationic amine groups enhanced the NPs interaction with bacterial membranes and helped the delivery of lysozyme into the bacteria, which increased the antibacterial activity of lysozyme. (B) Silica nanopollens with hair-like structures were loaded with lysozyme. Here the purpose was because the Gram-negative bacteria have pili on their surfaces, those hair-like structures on lysozyme-loaded silica nanopollens would adhere with bacteria pili and enhance the delivery of lysozyme into the bacteria, and its antibacterial activity would be improved.

uncoated silver NPs. Uncoated silver NPs inhibited the growth of *E. coli* only at double the concentration required for both coated silver NPs. Likewise, bacterial growth was hampered as early as 8 h with polymer-coated NPs showing lower MIC as compared to uncoated silver NPs. Electron microscope data demonstrated that NPs caused damage bacterial cell, and this resulted in the lysis of bacterial cell, expulsion of bacterial intracellular components, and total disintegration of bacterial cells. The expression of genes related to amino acid metabolism (*argC*, *metL*, and *gadB*) and the tricarboxylic acid cycle (*frdB* and *aceF*) were downregulated in the bacteria, and the diminution in the Ag^+ ions concentration of polymer-coated silver NPs did not impact their antimicrobial activity.

4. NANOPARTICLES AND THEIR ANTIBIOTIC THERAPY APPLICATIONS

Depending on the role played by NPs or the mechanisms of antimicrobial activity, NPs for antimicrobial applications can be divided into different categories. First, the NPs may act as an inert nanodelivery system of an antibiotic agent, where they facilitate the loading, stability, and release of antimicrobial agent. Here the antibacterial activity comes from an adsorbed, complexed, or encapsulated antimicrobial agent that is attached to the NP's surface (physically or chemically) or entrapped inside the NP, with the purpose of using the nanoparticle to enhance delivery of the antibiotic agent. Second, they may have antibiotic activity on their own, where they act as antimicrobial agents themselves. Finally, they may act via a combination of both roles, where the NPs act as a delivery system and add their own antimicrobial activity, in either an additive or a synergistic manner. This section will discuss these three modes of NPs applications in antibiotic therapy, and Table 2 highlights previous studies about NPs applications in antibiotic therapy.

4.1. Nanoparticles as Delivery Systems for Antibiotics. Attempts to improve the effectiveness and potency of different therapeutic agents have been made for many decades using NP delivery systems. Here scientists use NPs morphology, size, surface charge, or protective internal environment as a way to better deliver the therapeutic agent into a cell, tissue, or organ, or through a delayed/sustained release, in such way that the antimicrobial agent is able to more successfully exert its activity.[84] There is a range of possibilities to achieve this. For instance, the antimicrobial agent can be entrapped or encapsulated, adsorbed, or chemically attached to the NP matrices. The intention here is of course to improve the pharmacokinetics and pharmacodynamics of the antimicrobial agent by engineering the size, shape, surface chemistry, and charge of NPs and improving their sensitivity and stability toward biochemical degradation. Compared to other means of drug formulations, NP systems can have many advantages including improved antibiotic solubility, controlled and sustained release of the loaded antibiotic, and prolonged systemic circulation. Also, NPs can be used to improve antibiotic efficacy against intracellular infections since they can enter the host cells via endocytosis or other mechanisms.[175]

Many NP-based delivery systems have been studied for effective delivery of antibiotics, such as polymeric NPs. For instance, rifampicin-loaded PLGA NPs were manufactured by a solvent-based method to treat *Mycobacterium* and MRSA infections. The average particle size of the NPs was 250 nm, but with a low loading capacity. Release studies demonstrated a release profile with an initial burst release (>14% of the loaded rifampicin), probably due to diffusional release of antibiotic near or on the NPs surface. Their antibacterial activity was tested against a clinical strain of MRSA, and they exhibited 4-fold more potent MIC in comparison to free rifampicin (0.002 μg/mL vs 0.008 μg/mL), which suggested that the NPs may have facilitated the delivery of rifampicin into the bacteria.[132] Martins et al.[133] incorporated violacein, a natural bis-indole pigment with antimicrobial activity, into PLGA NPs by nanoprecipitation as a way to possibly improve its hydrophilicity and decrease its cytotoxicity to human cells. The average particle size of the NPs was around 130 nm, homogeneously size distributed, with a high loading capacity and sustained release for over 5 days. In antimicrobial assays against MRSA strains, the MIC values (1.2−2 μg/mL) for violacein-loaded NPs were significantly lower than that of free

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violacein (5.1 μg/mL), and the loaded NPs were more effective at inhibiting the bacterial growth over longer time. Satishkumar and Vertegel[176] investigated how to improve delivery of an antimicrobial enzyme, lysozyme, using polystyrene latex NPs and whether this could increase its antibacterial activity. Lysozyme (hen egg) was attached covalently to polystyrene NPs. Polystyrene NPs modified either with amine groups for positive charge or with sulfate groups for negative charge. When tested on Gram-positive bacteria *Micrococcus lysodeikticus*, the antibacterial activity of lysozyme conjugated to cationic polystyrene NPs was about 2-fold higher in comparison to that of free (unconjugated) lysozyme, whereas lysozyme conjugated anionic polystyrene NPs significantly lowered the activity, and the explanation for this was that due to stronger electrostatic attraction of cationic polystyrene NPs to negative bacteria cell membrane (Figure 6A), the delivery of lysozyme into the bacteria was better compared to free lysozyme. Lysozyme-conjugated anionic polystyrene NPs showed lower antimicrobial activity since there might have been an electrostatic repulsion from the negatively charged bacterial cell membrane, which did not help the delivery of lysozyme into the bacteria.

Silica-based NPs have also been extensively used for delivery of antibiotics. Pedraza et al.,[143] designed a "nano-antibiotic" system made of mesoporous silica NPs with levofloxacin. They functionalized the NP surface with a cationic functional group (*N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane) to induce affinity toward anionic bacterial membrane and biofilms. These NPs loaded with levofloxacin exhibited activity against *S. aureus*, and they showed a high activity against the biofilm after treatment. Braun et al.[144] loaded a cationic antimicrobial peptide onto negatively charged, surface functionalized silica NPs through strong electrostatic interactions. They found that the loading capacity of the cationic LL-37 onto anionic mesoporous silica NPs (with charge of −35 mV) was high compared to cationic amino-functionalized mesoporous silica NPs or non-porous silica NPs. A lower loading capacity onto non-porous silica NPs even if they had higher negative charge (around −55 mV) was ascribed to their lower surface area. Moreover, the authors showed that there was a link between loading capacity and surface charge and the way the LL-37-loaded NPs interacted with the bacterial cell membrane. The studies about the interaction between bacterial cell membrane and LL-37-loaded NPs demonstrated that anionic mesoporous silica NPs (even with high loading capacity) were not able to interact with bacterial cell membrane while non-porous silica NPs did interact with bacterial cell membrane with a slow release of LL-37. Also, cationic aminated mesoporous silica NPs (even unloaded NPs) were able to interact with bacterial cell membrane, and this was attributed to their positive charge. Gounani et al.[151] loaded vancomycin and polymyxin B on mesoporous silica NPs modified with carboxyl groups with the aim of achieving a simultaneous targeted delivery of both antibiotics against Gram-negative and Gram-positive bacteria. Both unmodified and modified silica NPs loaded with antibiotics were potent against the tested bacteria, where carboxyl-modified mesoporous silica NPs showed higher potency in comparison to free (unloaded) antibiotics. Also, the combination of vancomycin and polymyxin B demonstrated the synergistic effect with lower toxicity, and their loading into mesoporous silica NPs enhanced their potency compared to individual antibiotic, which indicates that combining and loading these antibiotics or other conventional antibiotics into NPs systems can enhance their antimicrobial efficiency, and this can be advantageous for infections that require high concentrations of multiple antimicrobials. Song et al. designed novel drug delivery nanocarriers that mimic the bacteria pili in order to deliver lysozyme into the bacteria.[177] They developed silica nanopollens with rough surfaces and hair-like structures, which upon contact with Gram-negative bacteria would enhance both the adhesion toward bacteria surfaces and the delivery of antimicrobial agent into the bacteria (Figure 6B). Lysozyme was loaded into these NPs and showed prolonged release, effective antimicrobial activity, and long-term antibacterial activity up to 3 days toward *E. coli*. The activity of lysozyme-loaded silica nanopollens was further shown in *ex vivo* studies using a small intestine infection model, in which they showed significantly higher activity compared to free lysozyme. The ability of silica NPs to deliver and improve the activity of an FDA approved antibacterial and antifungal triclosan was investigated by Makarovsky et al.[149] Triclosan was covalently attached to the silica NPs surfaces using 3-(triethoxysilyl)propyl isocyanate as a coupling agent. Upon interacting with bacteria cells, some cellular membrane enzymes ensued the slow release of triclosan from silica NPs, and as a consequence the antibacterial activity was increased. In another study exploring the use of silica NPs to deliver and enhance the potency of a photosensitizing molecule (Rose Bengal), this molecule was covalently conjugated to amine-functionalized silica NPs to effectively inhibit the growth of Gram-positive bacteria, including MRSA.[150] A light source with a 525 nm bandpass filter, which has a measured ∼14 mW cm^{-2} output was used for illumination. Rose Bengal-decorated silica NPs effectively inhibited MRSA (up to eight times in effectiveness compared to silica NPs or Rose Bengal alone). In a recent study,[178] the antitubercular agents Pretomanid and MCC7433, both possessing high cell permeability but poor aqueous solubility, were loaded into functionalized and unfunctionalized mesoporous silica NPs in order to improve their oral bioavailability. The encapsulation of those antitubercular agents into silica NPs improved their solubility, and their activity was retained when tested against *Mycobacterium tuberculosis* using these drug-loaded silica NPs. Also in that study, amine-modified silica NPs were shown to enhance the systemic exposure of orally administered MCC7433 in mice (1.3 times higher C_{max}) in comparison to unloaded MCC7433, and this showed the potential of using mesoporous silica NPs as a carrier for oral delivery of those poorly aqueous soluble antibacterial agents against tuberculosis. Similarly, toward improving the oral delivery of vancomycin, Ndayishimiye et al.[36] investigated the ability of silica NPs to enhance the epithelial permeability of vancomycin. Silica NPs were first surface functionalized with different groups in order to change the surface charge and polarity. Vancomycin-loaded silica NPs, especially hydrophilic and with negative charge, significantly enhanced the permeability of vancomycin across an epithelial cell monolayer (Caco-2 cell model) by up to 6-fold compared to unloaded vancomycin, and the permeation enhancing effect of Silica NPs was ascribed to their ability to transiently open the tight junctions of Caco-2 cell monolayers. This capability opens up the potential of a new route to orally administer these formulations to treat systemic infections.

Liposomes are another delivery system that have been used for delivering antimicrobial agents. MRSA can live and persist in alveolar macrophages, and this can affect the clinical success of intravenously delivered vancomycin for treatment of

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pneumonia. In order to overcome this, Pumerantz et al.[179] formulated vancomycin in liposomes in order to increase its antimicrobial activity and overcome resistance. Here the encapsulation of vancomycin in liposomes was done by a hydration−dehydration method, and they were tested for activity against intracellular MRSA in macrophages. When treated with vancomycin-loaded liposomes (vancomycin concentration of 200 and 800 μg/mL), a significant reduction in MRSA was noticed, while free (unencapsulated) vancomycin was not able to kill MRSA inside infected macrophages, probably due to poor uptake of vancomycin into the macrophages. In another study, Sande et al.[180] studied the role of encapsulating vancomycin within liposomes and whether this could enhance its antibacterial activity against MRSA. Vancomycin-loaded liposomes were obtained by a rehydration−dehydration method, and their MICs and MBCs were investigated against strains of MRSA. The efficacy was also examined in a time−kill assay *in vitro* and in a murine infection model. The MIC for MRSA strains was around 2-fold more potent, and vancomycin liposomal formulation enhanced killing of MRSA *in vitro*. In the systemic murine infection model, intraperitoneal injection of vancomycin-loaded liposomal formulation (treatment of a 50 mg/kg) improved kidney clearance of an ST8:USA300 MRSA strain by 1 log in comparison to injection of free (un-encapsulated) vancomycin.

4.2. Nanoparticles as Inherent Antimicrobial Agents. In addition to NPs explicitly designed for the delivery of antimicrobial agents, some NPs have also been explored to be used as antimicrobial agents on their own. Here the material to be used to develop the NPs is critical. Many types of materials have been studied for their antimicrobial applications.[29] Biologically, the vast majority of antimicrobial-active NPs act via different antimicrobial mechanisms compared to conventional antibiotics. Their physicochemical properties can give them a unique propensity to enter the bacterial cell membrane bilayers, enabling them to reach the cytoplasm, while simultaneously disrupting the function and integrity of the membrane.[84,181] For example, dendrimers functionalized with positively charged quaternary ammonium salts have high antibacterial potency due to their ability to disrupt of bacterial cell membranes.[182] The strong electrostatic physisorption of polycationic groups (functionalized on the surface of dendrimers) onto the negatively charged bacterial lipid bilayer increases their cell membrane penetration and leakage of K^+ from inside of the bacteria cell to the outside.[183]

Metal NPs have similarly been studied as antimicrobial agents, with silver NPs the most studied metal NPs.[184] Apart from being used in other NP composites, silver NPs have been investigated for their antimicrobial potency on their own. When tested against MRSA, silver NPs with sizes of 10, 30−40, and 100 nm were effective with an MIC of around 10 μg/mL, and they exhibited similar activity when tested on methicillin-sensitive *S. aureus*, showing how resistance against traditional antibiotics has little effect on silver NPs activity.[185] However, resistance to silver has been described.[186,187] Regarding other metals, NPs of copper and its oxide have been studied *in vitro* and also showed activity against MRSA.[160] However, in comparison to silver NPs, higher concentrations of copper NPs were necessary in order to have a bacteriostatic and bactericidal effect. This was interesting since they are thought to have a similar mechanism of action, in this case, by releasing Cu^{2+} ions that change conductivity and local pH, and consequently the disruption of cell membranes and alteration of some key intracellular components and other metabolic activities. Zinc oxide NPs have also been tested *in vitro* against clinical isolates of methicillin-sensitive *S. aureus* and MRSA but showed less potency compared to silver or copper NPs.[164]

Combining organic and inorganic materials to make hybrid NPs, Huang et al.[136] amalgamated silver NPs and chitosan acetate to produce nano-antimicrobials for burn dressings. The chitosan-silver NPs were tested for their effectiveness in *in vivo* murine burn models, and these NPs were shown to combine synergistically to inhibit the growth of Gram-positive MRSA and Gram-negative bacteria. This synergistic effect was ascribed to the permeation of NPs into the bacteria cells, where chitosan mediated the effective penetration of silver ions into the bacterial cells. Bresee et al.[172] investigated the anti-MRSA activity of ligand-coated gold NPs using thiol exchange reactions to attach organo-thiol ligands of different properties of H-bond donor/acceptor and polarity on the gold NPs surfaces. Here their aim was to adjust the binding affinity of NPs to the targeted bacteria and increase their cellular internalization capacity. The ligand-conjugated gold NPs were examined for their activity against MRSA and methicillin-sensitive *S. aureus*, with some ligand-conjugated gold NPs exhibiting a 99.9% bacterial growth inhibition at 10 μM for both MRSA and methicillin-sensitive *S. aureus*. This activity was attributed to the improved bacterial cell internalization and the ability of the NPs to aggregate inside the cells, or also to the targeted binding to the outer surfaces of the bacteria.

Investigating the antimicrobial effect of organic NPs, Dugal and Mamajiwala[135] prepared and functionalized chitosan NPs by ionic gelation, producing amino-functionalized chitosan NPs with a zeta potential of +88 mV and particle size of approximately 100 nm. *In vitro* essays showed that these chitosan NPs were potent against MRSA biofilms and the functionalized NPs were more active than non-functionalized ones (MIC values: 400 μg/mL vs 3000 μg/mL). The authors concluded that the enhanced activity for functionalized chitosan NPs was due to positive charges on the surface of the functionalized NPs.

4.3. Nanoparticles Used as a Combination of Both Delivery Systems and Antimicrobial Agents. Several studies have employed a strategy that combines both approaches described previously. The encapsulation, adsorption, and/or decoration of antimicrobial NPs with antimicrobial agents or combination of two NPs has shown a synergistic antimicrobial effect.[188−190]

The antimicrobial potency of silver NPs conjugated to antimicrobial peptide antimicrobials has been investigated.[158] This conjugation was done with the purpose of decreasing the toxicity of the free antimicrobial peptide toward other healthy cells and improving their targeted delivery. It was shown that the peptide-conjugated silver NPs exhibited high potency toward MRSA and *P. aeruginosa* (resistant to conventional antimicrobials) without causing a significant membranolytic effect, which is inherent in peptides. Moreover, it was revealed that the characteristics and behavior of the conjugates differed significantly from the properties of the constituent silver NPs and antimicrobial peptides. Moreover, a β-lactam antibiotic ampicillin was chemo-physically adsorbed onto the silver NPs surfaces.[159] These NPs exhibited an increase of 2-fold in *in vitro* anti-MRSA activity in comparison to the silver NPs (alone) or free ampicillin. In another study, the synergistic effect of gold−silver NPs (with size of around 27 nm) in

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combination with doxycycline was tested in burn infections.[173] The antimicrobial activity of doxycycline mixed with gold−silver NPs was investigated against *P. aeruginosa*, *E. coli*, *S. aureus*, and *M. luteus*. The antimicrobial activity of the gold−silver−doxycycline NPs was significantly higher compared to gold−silver NPs or doxycycline alone. The increase of antimicrobial activity was attributed to the synergy between those bimetallic NPs and doxycycline, and the antibacterial mechanism was revealed to be through the formation of reactive oxygen species.

The synergistic effect of titanium oxide NPs in combination with the antibiotic cefotaxime sodium was tested against implant-associated infections.[191] In this study, titanium oxide NPs were coated by dopamine (via a one-step pH-induced polymerization), and cefotaxime sodium was immobilized onto the polydopamine-coated titanium oxide NPs. These cefotaxime sodium-decorated titanium samples were then investigated on their ability to prevent adhesion and proliferation of *E. coli* and *S. mutans*. The results showed that cefotaxime sodium-grafted titanium samples possessed enhanced antibacterial activity, and they maintained the activity for a longer time compared to ungrafted cefotaxime sodium.

4.4. Nanoparticles Used for the Treatment of Biofilms. In addition to the above-mentioned microbial resistance mechanisms, bacteria may also show community-derived resistance by forming biofilms. Microbial biofilms, also metaphorically referred to as "cities for microbes", are community structures where microbial cells stick to each other and are surrounded by a hydrated matrix which is secreted by microbes residing in that community to protect themselves from outside threats or other unfavorable conditions.[192] Stress conditions prompt microbes to transition and attach to a substratum, making them phenotypically distinct from their planktonic counterparts.[192] After substratum attachment, these microbes make extracellular polymeric substances, which are the primary component of microbial biofilms (extracellular polymeric substances comprise nearly 90% of the whole biofilm mass).[193] This complex web of extracellular polymeric substances comprising abundant hydrophilic polysaccharides helps to retain water and moisture in biofilms and increases the chances of cell-to-cell communication, as well as the possibility of horizontal gene transfer, which often lead to community-derived resistance in biofilms.[193,194] The matrix of extracellular polymeric substances surrounding the bacterial cells can act as a "shield" to protect them from the action of antibiotics, which can lead to antimicrobial resistance.[194,195] Bacterial or other microbial cells in biofilms can endure up to 1000 times higher antibiotic concentration than free-living microorganisms.[192] Therefore, microbial biofilms have been considered as one of the major challenges in treating microbial infections. Different new strategies have been reported to inhibit and kill bacteria contained in biofilms. Some of these strategies include bacteriophages, quorum sensing inhibitors, enzymes, antimicrobial photodynamic therapy,[196−198] diguanylate cyclase inhibitors, and ethnopharmacology.[195]

Another antibiofilm strategy that has been used to treat biofilms is the use of NPs.[199] Reports have demonstrated that NPs can be used either to eliminate pre-formed microbial biofilms or to hinder their formation on the surfaces. NPs are designed to interact with and disrupt the biofilm extracellular polymeric substances matrix and then reach and kill the bacterial cells in the biofilm.[200] Based on their material nature, NPs antibiofilm agents might include metal-based NPs, polymeric-mediated antibiofilm agents, non-metallic inorganic NPs, or NPs resulting from a combination of natural compounds with metal NPs.[192,201]

NPs obtained from different classes of metals, including silver NPs, silver-based nanocomposites, copper NPs, iron NPs, magnesium NPs, and zinc NPs, have shown antibiofilm activity.[201,202] Among the investigated metal NPs, silver NPs are often reported to have antibiofilm efficacy.[202,203] For instance, Taglietti et al.[204] studied the antibiofilm activity of silver NPs adsorbed/anchored to an amino-silanized glass surface. Amino-silanized glass was immersed in a silver NPs colloidal suspension, with the antibiofilm activity tested against the biofilm-producing *S. epidermidis* RP62A. Silver NPs glasses had excellent stability in aqueous medium and prolonged release behavior. They also showed high local concentration of silver ions (without detachment of Ag NPs), and they demonstrated high antibiofilm activity against *S. epidermidis* RP62A.

Polymeric NPs are another category of NPs that have been used for biofilms, due to their tunable physicochemical characteristics. Makambeta et al.[205] studied the activity of poly(oxanorborneneimide)-based polymeric NPs against biofilms from multiple bacterial species. The NPs demonstrated good penetration behaviors in dual-species biofilms with high bacterial viability reduction and high reduction in overall bacterial biofilm mass. They also showed minimal toxicity to fibroblast cells and exhibited high antimicrobial efficacy in *in vitro* co-culture model consisting of fibroblast cells and biofilms of *E. coli* and *P. aeruginosa*. In another study, Nguyen et al.[206] developed novel antimicrobials in the form of single-chain polymeric NPs that exhibited high antimicrobial activity against Gram-negative bacteria and were able to kill (within an hour) both free-living bacteria cells and bacterial biofilm. In aqueous media systems, the linear copolymers (with oligoethylene glycol, lipophilic, and amino groups) underwent self-folding, due to intramolecular hydrophobic interactions, to give the single-chain polymeric NPs. The authors demonstrated that, by systematically modifying the hydrophobic property of the polymer, the extent of bacterial cell membrane wall disruption could be tuned, which in turn governed their microbial inhibition capacity. They also showed that the attachment of oligoethylene glycol groups into the polymer design was crucial in avoiding complexation of the NPs with proteins or the formation of protein coronas in biological systems, hence maintaining their antimicrobial activity even in *in vivo* simulating environments. Compared with colistin (a clinical antibiotic used as the last-resort for highly resistant infections), the lead NPs agents had higher therapeutic index (of about 2−3 times) and better biocompatibility.

Non-metallic inorganic NPs have also been explored as antibiofilm agents. They exert their activity by imparting mechanical damage to the biofilm structures, and they can also release compounds that inhibit biofilm formation. Nitric oxide is an antibacterial molecule, and many nitric oxide-releasing NPs have been used for this purpose.[207,208] Slomberg et al.[209] investigated the NO-releasing silica NPs and their antibiofilm activity. Here, the activity of NO-releasing silica NPs (with sizes of 14, 50, and 150 nm) with the same total NO release (~0.3 μmol mg^{-1}) was evaluated against *P. aeruginosa* and *S. aureus* biofilms. To investigate the impact of NPs shape on their antibiofilm activity, the aspect ratio of the NO-releasing silica NPs was varied from 1 to 8 while maintaining particle

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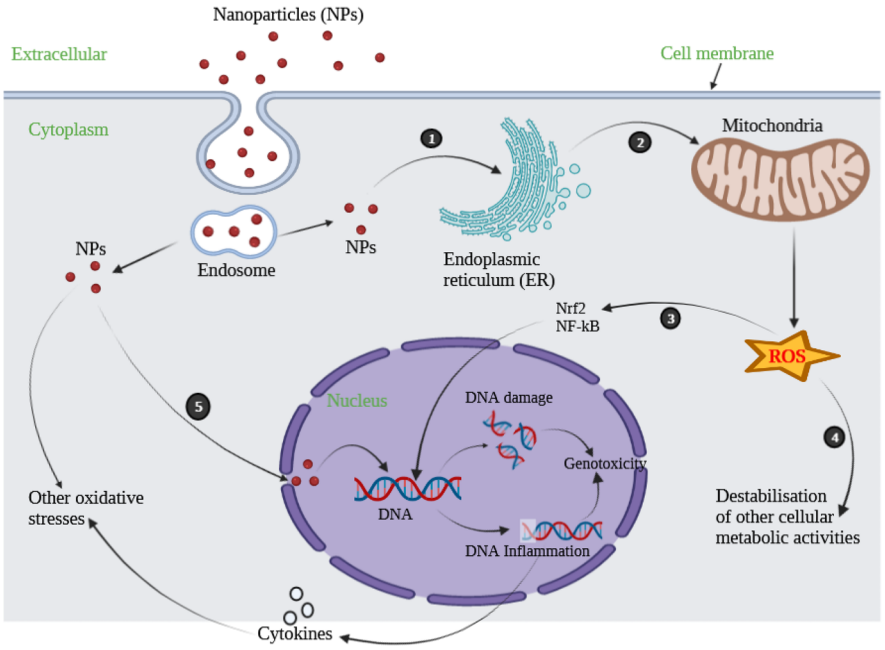

Figure 7. Nanoparticles (silver NPs, titanium oxide NPs) can cause toxicity to mammalian cells through different mechanisms. Different mechanisms and events are involved: (1) After the NPs are taken by the cell by phagocytosis or other mechanisms, this induces the activation of the ER stress pathway. (2) ER stress triggers the activation of mitochondria to produce reactive oxygen species (ROS). (3) The released ROS directly cause DNA damage and/or inflammation, which lead to genotoxicity and/or cell death. (4) They can also disrupt other cytoplasmic metabolic activities, and this can lead to cell death. (5) Meanwhile, NPs can directly enter the nucleus, bind to DNA, and cause some damage. Moreover, the inflamed DNA can produce and release cytokines (for example, IL1, IL6, and TNFα), which can cause other oxidative stresses to other cell organelles.

volume (~0.02 μm (3)) and NO-release totals (~0.7 μmol mg^{-1}). NO-releasing silica NPs with decreased particle size and high aspect ratio were more effective against both *P. aeruginosa* and *S. aureus* biofilms, with Gram-negative species showing higher susceptibility to NO. NO-releasing silica NPs with size of 14 nm showed better NO delivery and increased biofilm killing in comparison to NO-releasing silica NPs with sizes of 50 and 150 nm. Similarly, the rod-like NO-releasing silica NPs were more effective in delivering NO compared with spherical ones, and they induced greater antibiofilm activity.

5. TOXICITY CONSIDERATIONS IN THE APPLICATION OF NANOPARTICLES

One important aspect to consider when applying NPs-based antibiotic therapies relates to the possibility of cytotoxicity and systemic toxicity.[181] Systemic and local toxicity, as well as the possible bactericidal effects on beneficial human microbiota, are of concern and have to be taken into consideration when using NPs.[41,210] Toxicity mechanisms of commonly used NPs for antibiotic application to mammalian cells are summarized in Figure 7, and Table 3 shows some general toxicological effects for various types of NPs.

Investigations of silver NPs, commercially available inorganic-based NPs commonly used as bacteriostatic coatings for controlling infections, have shown that silver NPs have toxic effects in several cell lines. For example, Carlson et al.[211] studied the impact of size-dependent reactive oxygen species production of silver NPs on cells. They examined cellular interactions of silver NPs (size: 15, 30, and 55 nm) and the effect of ROS on cell mitochondria and cell membrane viability. After 24 h of exposure, there was a significant decrease of cell viability with increasing concentration (from 10 to 75 μg/mL) of silver NPs (15 and 30 nm). For example, there was >10-fold increase of ROS levels in cells treated with 50 μg/mL of silver NPs (15 nm), and this suggested that the cytotoxic effect of these silver NPs was likely due to oxidative stress. Moreover, activation of the release of inflammation

Table 3. Some Toxicological Effects of Different Nanoparticles

types of NPs	possible toxicological effects	refs
chitosan	induce cytotoxicity, malformations, and physiological stress in zebra fish embryos	217
	induce toxicity in mice embryos	218
silica NPs	moderate toxicity to kidneys, lungs, liver, and brain depends on the route of administration in mice	219
silver NPs	histopathological lesions and cytotoxicity in mice	220
	inflammatory response and accumulation of the NPs in several tissues in rats	221
gold NPs	inflammatory response in rats and mice	222
copper NPs	toxicity to liver, spleen, and kidney in mice	223
iron oxide NPs	histopathological lesions in spleen and liver in mice	224
titanium oxide NPs	toxic to brain and liver in mice; adverse effects on mouse embryos	225
zinc oxide NPs	inflammation and damage to different tissues dependent on the mode of administration in rats or mice	226, 227

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mediators was investigated by examining the levels of chemokines/cytokines, which include cell necrosis factor (TNF-α), interleukin-6 (IL-6), and macrophage inhibitory protein (MIP-2), released into the culture medium. After 24 h of exposure to silver NPs (15 nm), there was a significant inflammatory response with the release of TNF-α, IL-1β, and MIP-2. Overall, a size-dependent cytotoxic effect of silver NPs was observed, and the main mechanism of toxicity was found to be through oxidative stress. Regarding genotoxicity, NPs can damage DNA by producing ROS or by diminishing ATP production (associated with mitochondria damage), which as a result impairs energy-dependent DNA repair mechanisms and other cellular activities (Figure 7). DNA damage by silver NPs was investigated by Ahamed et al.[212] They examined the impact of polysaccharide (acacia gum)-coated and uncoated silver NPs on DNA damage response in two mammalian cells: mouse embryonic fibroblasts and mouse embryonic stem cells. Both silver NPs up-regulated the cell cycle checkpoint protein p53 and DNA damage repair proteins Rad51 and phosphorylated-H2AX expression. Also NPs induced cell death as examined by MTT assay and the annexin V protein expression. Moreover, coated silver NPs exhibited more severe DNA damage than uncoated silver NPs, and it was suggested that this was probably due to the fact that coated NPs are more individually distributed while the uncoated silver NPs can agglomerate, which might limit their availability and access into the cell.

In vivo toxicity studies have also been conducted. The subchronic inhalation toxicity of silver NPs was studied in Sprague−Dawley rats by Sung et al.[213] Rats were exposed to silver NPs (18−19 nm) in different doses (low dose of 0.6×10^6 particles/cm^3, equivalent to 49 μg/m^3, middle dose of 1.4×10^6 particles/cm^3, equivalent to 133 μg/m^3, and high dose of 3.0×10^6 particles/cm^3, equivalent to 515 μg/m^3) for 13 weeks in a whole-body inhalation chamber. A dose-dependent augmentation in bile duct hyperplasia was observed in rats. Histological and pathological evaluations also demonstrated a dose-dependent increase in lesions associated with exposure to silver NPs, including small granulomatous lesions, chronic alveolar inflammation, and mixed inflammatory cell infiltrate, and generally the targeted organs for silver NPs were lungs and liver.

Titanium oxide NPs are another type of metal NPs that have been investigated for their possible toxic effects. In one study, the toxicity of titanium oxide NPs of different sizes (25, 80, and 155 nm) was investigated on adult mice.[214] A dose of 5 g/kg body weight of titanium oxide NPs suspensions was administrated by an oral gavage. In 2 weeks, the changes in hepatic biochemical and pathological characteristics showed that the liver injury was induced after exposure to titanium oxide NPs. Moreover, nephrotoxicity, like increased blood urea nitrogen level and renal pathology change, was observed. Titanium oxide NPs of size 25 and 80 nm showed significant myocardial damage in comparison to 25 nm NPs, and the biodistribution experiments demonstrated that titanium oxide NPs were mainly retained in the spleen, liver, lungs, and kidneys. Azim et al.[215] also studied the toxicity of titanium oxide NPs in mice. Titanium oxide NPs (size of 21 nm) were administered (150 mg/kg/day) for 2 weeks, and there was a significant increase in hepatic serum function enzyme activities, hepatic coefficient, and level of malondialdehyde in liver tissue. Also, titanium oxide NPs suppressed the glutathione level in the liver and induced an inflammation response through the activation of macrophages and the increase of interleukin-6 level and tumor necrosis factor-α level. Furthermore, following treatment with titanium oxide NPs, there was an up-regulation of mRNA expression of nuclear factor-erythroid-2-related factor 2, nuclear factor kappa B, and Bax, while that of Bcl-2 was down-regulated. Titanium oxide NPs also efficiently activated caspase-3, which is involved in cell apoptosis, and caused liver DNA damage. Studying the possible neurotoxic effect of titanium oxide NPs, Hong et al.[216] examined whether titanium oxide NPs induce neurotoxicity by affecting neurite outgrowth of hippocampal neurons. It was found that titanium oxide NPs, in a concentration-dependent behavior, considerably suppressed dendritic growth of hippocampal neurons and enhanced nitric oxide and nitric oxide synthase while reducing the activities of Ca^{2+}-ATPase and Na^+/K^+-ATPase and elevating the ADP/ATP ratio. The authors concluded that titanium NPs inhibited neurite outgrowth of hippocampal neurons by interfering with glutamate metabolism and impairing *N*-methyl-D-aspartate receptor function.

Overall, although nanomaterials have shown to be promising in killing resistant bacteria, the possible toxicological effects (especially metallic NPs) on the healthy cells and tissues cannot be ignored. Investigations into possible toxicity of NPs at cellular and systemic levels are important for a possible and successful clinical translation. Parameters such as the type of NPs and their characteristics as well as route of administration and the nature of how NPs interact with cells and tissues have to be more extensively investigated.[210] Other phenomena associated with NPs, such as precipitation, aggregation, accumulation, and agglutination phenomena of NPs within blood vessels, fatty tissues, bone tissues, or other organs, can be issues, and it is important to consider that when using NPs.[181] Finally, the accumulation and persistence of NPs in the environment must also be considered.

6. CONCLUSION

Bacteria have developed multiple mechanisms of resistance to antibiotics, becoming a substantial threat to global human health. Alternative therapeutic options with different mechanisms of action are needed to combat these emerging bacterial strains. Nanomaterials offer an alternative to conventional antibiotics due to their potential for innate bactericidal activity, coupled with unique characteristics that offer distinct differences from conventional antibiotics. Nanomaterials can serve as delivery vehicles to improve the effectiveness and lifetime of existing antibiotics or act as antimicrobial agents on their own. Both of these properties can be combined, giving an additional window of opportunity to explore for the development of effective antibiotic therapeutics. While the application of nanomaterials to combat microbial/resistant microbial infections is an exciting concept, this enthusiasm must be tempered by potential issues of toxicity that need to be addressed before any successful translation to the clinic. Therefore, formulation of appropriate guidelines and standardization of assays to study of nanotoxicity are required, as are protocols to study and compare results from *in vitro*, *ex vivo*, and *in vivo* models. Furthermore, most reports on the antimicrobial activity of nanoparticles are limited to *in vitro* assays looking at antibacterial activity, with limited *in vivo* data. In many cases it is not clear how nanoparticles would be delivered clinically—with intravenous delivery likely required.

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REFERENCES

(1) WHO. The top 10 causes of death. Dec 9, 2020; https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death (acessed Dec 18, 2020).

(2) Reardon, S. Antibiotic resistance sweeping developing world. *Nature* **2014**, *509* (7499), 141−2.

(3) Michael, C. A.; Dominey-Howes, D.; Labbate, M. The antimicrobial resistance crisis: causes, consequences, and management. *Front. Public Health* **2014**, *2*, 145.

(4) WHO. Global Antimicrobial Resistance Surveillance System (GLASS), 2017; https://www.who.int/glass/en/ (accessed Dec 18, 2020).

(5) O'Neill, J. Tackling drug-resistant infections globally: final report and recommendations. The Review on Antimicrobial Resistance, May 2016. https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf.

(6) D'Costa, V. M.; et al. Antibiotic resistance is ancient. *Nature* **2011**, *477* (7365), 457−461.

(7) Blair, J. M.; et al. Molecular mechanisms of antibiotic resistance. *Nat. Rev. Microbiol.* **2015**, *13* (1), 42−51.

(8) Munita, J. M.; Arias, C.A. Mechanisms of Antibiotic Resistance. *Microbiol. Spectrum* **2016**, *4* (2), 4.2.15.

(9) Aminov, R. I. A brief history of the antibiotic era: lessons learned and challenges for the future. *Front. Microbiol.* **2010**, *1*, 134.

(10) Ventola, C. L. The antibiotic resistance crisis: part 2: management strategies and new agents. *P&T* **2015**, *40* (5), 344−352.

(11) Arciola, C. R.; Campoccia, D.; Montanaro, L. Implant infections: adhesion, biofilm formation and immune evasion. *Nat. Rev. Microbiol.* **2018**, *16* (7), 397−409.

(12) Wu, Y. K.; Cheng, N. C.; Cheng, C. M. Biofilms in Chronic Wounds: Pathogenesis and Diagnosis. *Trends Biotechnol.* **2019**, *37* (5), 505−517.

(13) Namivandi-Zangeneh, R.; Wong, E. H. H.; Boyer, C. Synthetic Antimicrobial Polymers in Combination Therapy: Tackling Antibiotic Resistance. *ACS Infectious Diseases* **2021**, *7* (2), 215−253.

(14) Cattoir, V.; Felden, B. Future Antibacterial Strategies: From Basic Concepts to Clinical Challenges. *J. Infectious Diseases* **2019**, *220* (3), 350−360.

(15) Matsuzaki, S.; et al. Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. *J. Infection Chemotherapy* **2005**, *11* (5), 211−9.

(16) Huh, A. J.; Kwon, Y. J. Nanoantibiotics": A new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. *J. Controlled Release* **2011**, *156* (2), 128−145.

(17) Wang, L. S.; Gupta, A.; Rotello, V. M. Nanomaterials for the Treatment of Bacterial Biofilms. *ACS Infectious Diseases* **2016**, *2* (1), 3−4.

(18) Gupta, A.; Landis, R.F.; Rotello, V.M. Nanoparticle-Based Antimicrobials: Surface Functionality is Critical. *F1000Research* **2016**, *5*, 364.

(19) Hosseini, S. M.; et al. Nano drug delivery in intracellular bacterial infection treatments. *Biomedicine & Pharmacotherapy* **2022**, *146*, 112609.

(20) Singh, R.; Lillard, J. W. Nanoparticle-based targeted drug delivery. *Experimental Molecular Pathology* **2009**, *86* (3), 215−223.

(21) Gao, W.; et al. Nanoparticle approaches against bacterial infections. *WIREs Nanomedicine Nanobiotechnology* **2014**, *6* (6), 532−547.

(22) Sanità, G.; Carrese, B.; Lamberti, A. Nanoparticle Surface Functionalization: How to Improve Biocompatibility and Cellular Internalization. *Front. Molecular Biosciences* **2020**, *7*, 587012.

(23) Yeh, Y.-C.; Huang, T.-H.; Yang, S.-C.; Chen, C.-C.; Fang, J.-Y. Nano-Based Drug Delivery or Targeting to Eradicate Bacteria for Infection Mitigation: A Review of Recent Advances. *Front. Chemistry* **2020**, *8*, 286.

(24) Daima, H. K.; et al. Fine-Tuning the Antimicrobial Profile of Biocompatible Gold Nanoparticles by Sequential Surface Functionalization Using Polyoxometalates and Lysine. *PLoS One* **2013**, *8* (10), e79676.

(25) Pelgrift, R. Y.; Friedman, A. J. Nanotechnology as a therapeutic tool to combat microbial resistance. *Adv. Drug Delivery Rev.* **2013**, *65* (13−14), 1803−15.

(26) Gupta, A.; et al. Synergistic antimicrobial therapy using nanoparticles and antibiotics for the treatment of multidrug-resistant bacterial infection. *Nano Futures* **2017**, *1* (1), No. 015004.

(27) Gupta, D.; Singh, A.; Khan, A. U. Nanoparticles as Efflux Pump and Biofilm Inhibitor to Rejuvenate Bactericidal Effect of Conventional Antibiotics. *Nanoscale Res. Lett.* **2017**, *12* (1), 454.

(28) Abeylath, S. C.; et al. Nanobiotics to combat bacterial drug resistance. In *Antibiotic resistance: causes and risk factors, mechanisms and alternatives*; Nova Science Publishers: New York, 2009; pp 425−465.

(29) Makabenta, J. M. V.; et al. Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. *Nat. Rev. Microbiol.* **2021**, *19* (1), 23−36.

(30) Blanco, E.; Shen, H.; Ferrari, M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat. Biotechnol.* **2015**, *33* (9), 941.
(31) Son, S. J.; Bai, X.; Lee, S. B. Inorganic hollow nanoparticles and nanotubes in nanomedicine: Part 1. Drug/gene delivery applications. *Drug Discovery Today* **2007**, *12* (15−16), 650−656.
(32) Wang, Y.; et al. Mesoporous silica nanoparticles in drug delivery and biomedical applications. *Nanomedicine: Nanotechnology, Biology Medicine* **2015**, *11* (2), 313−327.
(33) Tang, L.; Cheng, J. Nonporous silica nanoparticles for nanomedicine application. *Nano Today* **2013**, *8* (3), 290−312.
(34) Selvarajan, V.; Obuobi, S.; Ee, P. L. R. Silica Nanoparticles—A Versatile Tool for the Treatment of Bacterial Infections. *Front. Chemistry* **2020**, *8*, 602.
(35) Khan, M. A.; et al. Adsorption and Recovery of Polyphenolic Flavonoids Using TiO2-Functionalized Mesoporous Silica Nanoparticles. *ACS Appl. Mater. Interfaces* **2017**, *9* (37), 32114−32125.
(36) Ndayishimiye, J.; et al. Engineering mesoporous silica nanoparticles towards oral delivery of vancomycin. *J. Mater. Chem. B* **2021**, *9* (35), 7145−7166.
(37) Mosselhy, D. A.; et al. Silica-Gentamicin Nanohybrids: Synthesis and Antimicrobial Action. *Materials* **2016**, *9* (3), 170.
(38) Radin, S.; et al. Silica sol-gel for the controlled release of antibiotics. I. Synthesis, characterization, and in vitro release. *J. Biomed. Mater. Res.* **2001**, *57* (2), 313−20.
(39) Sercombe, L.; Veerati, T.; Moheimani, F.; Wu, S. Y.; Sood, A. K.; Hua, S. Advances and Challenges of Liposome Assisted Drug Delivery. *Front. Pharmacology* **2015**, *6*, 286.
(40) Pinto-Alphandary, H.; Andremont, A.; Couvreur, P. Targeted delivery of antibiotics using liposomes and nanoparticles: research and applications. *Int. J. Antimicrob. Agents* **2000**, *13* (3), 155−168.
(41) Zhang, L.; et al. Development of nanoparticles for antimicrobial drug delivery. *Curr. Med. Chem.* **2010**, *17* (6), 585−594.
(42) Hou, X.; Zaks, T.; Langer, R.; Dong, Y. Lipid nanoparticles for mRNA delivery. *Nat. Rev. Materials* **2021**, *6*, 1078.
(43) Daraee, H.; et al. Application of liposomes in medicine and drug delivery. *Artificial cells, nanomedicine, biotechnol.* **2016**, *44* (1), 381−391.
(44) Mei, L.; et al. Increased tumor targeted delivery using a multistage liposome system functionalized with RGD, TAT and cleavable PEG. *Int. J. Pharm.* **2014**, *468* (1−2), 26−38.
(45) Pattni, B. S.; Chupin, V. V.; Torchilin, V. P. New developments in liposomal drug delivery. *Chem. Rev.* **2015**, *115* (19), 10938−10966.
(46) Soppimath, K. S.; et al. Biodegradable polymeric nanoparticles as drug delivery devices. *J. Controlled Release* **2001**, *70* (1−2), 1−20.
(47) Ulbrich, K.; et al. Targeted drug delivery with polymers and magnetic nanoparticles: covalent and noncovalent approaches, release control, and clinical studies. *Chem. Rev.* **2016**, *116* (9), 5338−5431.
(48) Mora-Huertas, C. E.; Fessi, H.; Elaissari, A. Polymer-based nanocapsules for drug delivery. *Int. J. Pharm.* **2010**, *385* (1−2), 113−142.
(49) Adelnia, H.; Blakey, I.; Little, P. J.; Ta, H. T. Hydrogels Based on Poly(aspartic acid): Synthesis and Applications. *Front. Chemistry* **2019**, *7*, 755.
(50) Lam, S. J.; et al. Antimicrobial polymeric nanoparticles. *Prog. Polym. Sci.* **2018**, *76*, 40−64.
(51) Baek, K.; et al. Self-Assembly of Nanostructured Materials through Irreversible Covalent Bond Formation. *Acc. Chem. Res.* **2015**, *48* (8), 2221−2229.
(52) Croy, S. R.; Kwon, G. S. Polymeric micelles for drug delivery. *Curr. Pharm. Des.* **2006**, *12* (36), 4669−84.
(53) Brinkhuis, R. P.; Rutjes, F. P. J. T.; van Hest, J. C. M. Polymeric vesicles in biomedical applications. *Polym. Chem.* **2011**, *2* (7), 1449−1462.
(54) Bäumler, H.; Georgieva, R. Coupled Enzyme Reactions in Multicompartment Microparticles. *Biomacromolecules* **2010**, *11* (6), 1480−1487.
(55) Janib, S. M.; Moses, A. S.; MacKay, J. A. Imaging and drug delivery using theranostic nanoparticles. *Adv. Drug Delivery Rev.* **2010**, *62* (11), 1052−1063.
(56) Sulistio, A.; et al. Peptide-Based Star Polymers: The Rising Star in Functional Polymers. *Aust. J. Chem.* **2012**, *65* (8), 978−984.
(57) Wiltshire, J. T.; Qiao, G. G. Recent Advances in Star Polymer Design: Degradability and the Potential for Drug Delivery. *Aust. J. Chem.* **2007**, *60* (10), 699−705.
(58) Ghosh Chaudhuri, R.; Paria, S. Core/Shell Nanoparticles: Classes, Properties, Synthesis Mechanisms, Characterization, and Applications. *Chem. Rev.* **2012**, *112* (4), 2373−2433.
(59) Gillies, E. R.; Frechet, J. M. Dendrimers and dendritic polymers in drug delivery. *Drug Discovery Today* **2005**, *10* (1), 35−43.
(60) Kesharwani, P.; et al. Dendrimers in targeting and delivery of drugs. *Nanotechnology-Based Approaches for Targeting and Delivery of Drugs and Genes*; Elsevier, 2017; pp 363−388.
(61) Nanjwade, B. K.; et al. Dendrimers: Emerging polymers for drug-delivery systems. *European J. Pharmaceutical Sciences* **2009**, *38* (3), 185−196.
(62) Cheng, Y.; et al. Design of biocompatible dendrimers for cancer diagnosis and therapy: current status and future perspectives. *Chem. Soc. Rev.* **2011**, *40* (5), 2673−2703.
(63) Svenson, S. Dendrimers as versatile platform in drug delivery applications. *Eur. J. Pharm. Biopharm.* **2009**, *71* (3), 445−62.
(64) Alfei, S.; Schito, A.M. From Nanobiotechnology, Positively Charged Biomimetic Dendrimers as Novel Antibacterial Agents: A Review. *Nanomaterials* **2020**, *10*, 2022.
(65) Abd-El-Aziz, A. S.; Agatemor, C. Emerging Opportunities in the Biomedical Applications of Dendrimers. *J. Inorganic Organometallic Polymers Materials* **2018**, *28* (2), 369−382.
(66) Killops, K. L.; Campos, L. M.; Hawker, C. J. Robust, Efficient, and Orthogonal Synthesis of Dendrimers via Thiol-ene "Click" Chemistry. *J. Am. Chem. Soc.* **2008**, *130* (15), 5062−5064.
(67) Chen, C. Z.; Cooper, S. L. Recent Advances in Antimicrobial Dendrimers. *Adv. Mater.* **2000**, *12* (11), 843−846.
(68) Uhrich, K. Hyperbranched polymers for drug delivery. *Trends Polym. Sci.* **1997**, *12* (5), 388−393.
(69) Ndayishimiye, J.; et al. Understanding the relationship between solubility and permeability of γ-cyclodextrin-based systems embedded with poorly aqueous soluble benznidazole. *Int. J. Pharm.* **2022**, *616*, 121487.
(70) Sánchez-López, E.; et al. Metal-Based Nanoparticles as Antimicrobial Agents: An Overview. *Nanomaterials* **2020**, *10* (2), 292.
(71) Maleki Dizaj, S.; et al. Antimicrobial activity of carbon-based nanoparticles. *Adv. Pharmaceutical Bull.* **2015**, *5* (1), 19−23.
(72) Guerrero Correa, M.; et al. Antimicrobial metal-based nanoparticles: a review on their synthesis, types and antimicrobial action. *Beilstein J. Nanotechnol.* **2020**, *11*, 1450−1469.
(73) López de Dicastillo, C.; et al. Antimicrobial Bilayer Nanocomposites Based on the Incorporation of As-Synthetized Hollow Zinc Oxide Nanotubes. *Nanomaterials* **2020**, *10* (3), 503.
(74) de Dicastillo, C. L.; et al. Novel hollow titanium dioxide nanospheres with antimicrobial activity against resistant bacteria. *Beilstein J. Nanotechnol.* **2019**, *10*, 1716−1725.
(75) Wang, H.; et al. Preparation of silver nanoparticles by chemical reduction method. *Colloids Surf., A* **2005**, *256* (2), 111−115.
(76) Sergeev, G. B. Cryochemistry of Metal Nanoparticles. *J. Nanopart. Res.* **2003**, *5* (5), 529−537.
(77) Thakkar, K. N.; Mhatre, S. S.; Parikh, R. Y. Biological synthesis of metallic nanoparticles. *Nanomedicine* **2010**, *6* (2), 257−62.
(78) Shah, M.; et al. Green Synthesis of Metallic Nanoparticles via Biological Entities. *Materials* **2015**, *8* (11), 7278−7308.
(79) Chandrasekaran, R.; et al. Formulation of Carica papaya latex-functionalized silver nanoparticles for its improved antibacterial and anticancer applications. *J. Mol. Liq.* **2016**, *219*, 232−238.
(80) Agarwal, H.; Venkat Kumar, S.; Rajeshkumar, S. A review on green synthesis of zinc oxide nanoparticles − An eco-friendly approach. *Resource-Efficient Technologies* **2017**, *3* (4), 406−413.

https://doi.org/10.1021/acsinfecdis.1c00660
ACS Infect. Dis. 2022, 8, 693−712

(81) Hameed, A. S. H.; et al. In vitro antibacterial activity of ZnO and Nd doped ZnO nanoparticles against ESBL producing Escherichia coli and Klebsiella pneumoniae. *Sci. Rep.* **2016**, *6* (1), 24312.
(82) Das, P.; Karankar, V. S. New avenues of controlling microbial infections through anti-microbial and anti-biofilm potentials of green mono-and multi-metallic nanoparticles: A review. *J. Microbiol. Methods* **2019**, *167*, 105766.
(83) Soenen, S. J.; et al. Cellular toxicity of inorganic nanoparticles: Common aspects guidelines for improved nanotoxicity evaluation. *Nano Today* **2011**, *6* (5), 446−465.
(84) Gupta, A.; et al. Combatting antibiotic-resistant bacteria using nanomaterials. *Chem. Soc. Rev.* **2019**, *48* (2), 415−427.
(85) Matsuzaki, K. Control of cell selectivity of antimicrobial peptides. *Biochim. Biophys. Acta* **2009**, *1788* (8), 1687−92.
(86) Nederberg, F.; et al. Biodegradable nanostructures with selective lysis of microbial membranes. *Nat. Chem.* **2011**, *3* (5), 409−414.
(87) Hou, Z.; et al. Nanoparticles of Short Cationic Peptidopolysaccharide Self-Assembled by Hydrogen Bonding with Antibacterial Effect against Multidrug-Resistant Bacteria. *ACS Appl. Mater. Interfaces* **2017**, *9* (44), 38288−38303.
(88) Zhang, W.; et al. The membrane-targeting mechanism of host defense peptides inspiring the design of polypeptide-conjugated gold nanoparticles exhibiting effective antibacterial activity against methicillin-resistant Staphylococcus aureus. *J. Mater. Chem. B* **2021**, *9* (25), 5092−5101.
(89) Wu, Y.; et al. Superfast Water-Insensitive Polymerization on α-Amino Acid N-Carboxyanhydrides to Prepare Polypeptides Using Tetraalkylammonium Carboxylate as the Initiator. *Angew. Chem., Int. Ed.* **2021**, *60* (50), 26063−26071.
(90) Zhang, D.; et al. Alpha-beta chimeric polypeptide molecular brushes display potent activity against superbugs-methicillin resistant Staphylococcus aureus. *Science China Materials* **2019**, *62* (4), 604−610.
(91) Palermo, E. F.; Kuroda, K. Structural determinants of antimicrobial activity in polymers which mimic host defense peptides. *Appl. Microbiol. BioTechnol.* **2010**, *87* (5), 1605−15.
(92) Huo, S.; et al. Fully Zwitterionic Nanoparticle Antimicrobial Agents through Tuning of Core Size and Ligand Structure. *ACS Nano* **2016**, *10* (9), 8732−7.
(93) Huo, S.; et al. Fully Zwitterionic Nanoparticle Antimicrobial Agents through Tuning of Core Size and Ligand Structure. *ACS Nano* **2016**, *10* (9), 8732−8737.
(94) Dallas, P.; Sharma, V. K.; Zboril, R. Silver polymeric nanocomposites as advanced antimicrobial agents: classification, synthetic paths, applications, and perspectives. *Adv. Colloid Interface Sci.* **2011**, *166* (1−2), 119−35.
(95) El Badawy, A. M.; et al. Surface charge-dependent toxicity of silver nanoparticles. *Environ. Sci. Technol.* **2011**, *45* (1), 283−7.
(96) Malek, I.; et al. Vertically aligned multi walled carbon nanotubes prevent biofilm formation of medically relevant bacteria. *J. Mater. Chem. B* **2016**, *4* (31), 5228−5235.
(97) Hurdle, J. G.; et al. Targeting bacterial membrane function: an underexploited mechanism for treating persistent infections. *Nat. Rev. Microbiol.* **2011**, *9* (1), 62−75.
(98) Landis, R. F.; et al. Biodegradable Nanocomposite Antimicrobials for the Eradication of Multidrug-Resistant Bacterial Biofilms without Accumulated Resistance. *J. Am. Chem. Soc.* **2018**, *140* (19), 6176−6182.
(99) He, G. U. I.; et al. Impacts of Silver Nanoparticles on Bacterial Multidrug Efflux Pump. *FASEB J.* **2013**, *27* (S1), 575.12−575.12.
(100) Christena, L. R.; et al. Copper nanoparticles as an efflux pump inhibitor to tackle drug resistant bacteria. *RSC Adv.* **2015**, *5* (17), 12899−12909.
(101) Dey, N.; et al. Role of nanomaterials in deactivating multiple drug resistance efflux pumps − A review. *Environ. Research* **2022**, *204*, 111968.
(102) Wang, L.; Hu, C.; Shao, L. The antimicrobial activity of nanoparticles: present situation and prospects for the future. *Int. J. Nanomed.* **2017**, *12*, 1227−1249.
(103) Ren, E.; et al. Leveraging metal oxide nanoparticles for bacteria tracing and eradicating. *VIEW* **2020**, *1* (3), 20200052.
(104) Zhou, S.; et al. TiO2 Nanoparticle-Induced Nanowire Formation Facilitates Extracellular Electron Transfer. *Environ. Sci. Technol. Lett.* **2018**, *5* (9), 564−570.
(105) Lemire, J. A.; Harrison, J. J.; Turner, R. J. Antimicrobial activity of metals: mechanisms, molecular targets and applications. *Nat. Rev. Microbiol.* **2013**, *11* (6), 371−84.
(106) Memar, M. Y.; et al. Antimicrobial use of reactive oxygen therapy: current insights. *Infection Drug Resistance* **2018**, *11*, 567−576.
(107) Zhao, X.; Drlica, K. Reactive oxygen species and the bacterial response to lethal stress. *Curr. Opin. Microbiol.* **2014**, *21*, 1−6.
(108) Fang, F. C. Antimicrobial actions of reactive oxygen species. *mBio* **2011**, *2* (5), 11.
(109) Kohanski, M. A.; et al. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* **2007**, *130* (5), 797−810.
(110) Liu, Y.; Imlay, J. A. Cell death from antibiotics without the involvement of reactive oxygen species. *Science* **2013**, *339* (6124), 1210−3.
(111) Keren, I.; et al. Killing by bactericidal antibiotics does not depend on reactive oxygen species. *Science* **2013**, *339* (6124), 1213−6.
(112) Miller, K. P.; et al. Inorganic nanoparticles engineered to attack bacteria. *Chem. Soc. Rev.* **2015**, *44* (21), 7787−807.
(113) Pramanik, A.; et al. A novel study of antibacterial activity of copper iodide nanoparticle mediated by DNA and membrane damage. *Colloids Surf., B* **2012**, *96*, 50−5.
(114) Matai, I.; et al. Antibacterial activity and mechanism of Ag-ZnO nanocomposite on S. aureus and GFP-expressing antibiotic resistant E. coli. *Colloids Surf., B* **2014**, *115*, 359−67.
(115) Bernardos, A.; et al. Mesoporous Silica-Based Materials with Bactericidal Properties. *Small* **2019**, *15* (24), e1900669.
(116) Tao, Y.; et al. Bifunctionalized mesoporous silica-supported gold nanoparticles: intrinsic oxidase and peroxidase catalytic activities for antibacterial applications. *Adv. Mater.* **2015**, *27* (6), 1097−104.
(117) Shamaila, S.; et al. Gold Nanoparticles: An Efficient Antimicrobial Agent against Enteric Bacterial Human Pathogen. *Nanomaterials* **2016**, *6* (4), 71.
(118) Chatterjee, A. K.; Chakraborty, R.; Basu, T. Mechanism of antibacterial activity of copper nanoparticles. *Nanotechnology* **2014**, *25* (13), 135101.
(119) Zhao, Y.; et al. Small molecule-capped gold nanoparticles as potent antibacterial agents that target Gram-negative bacteria. *J. Am. Chem. Soc.* **2010**, *132* (35), 12349−56.
(120) Ashmore, D.; et al. Evaluation of E. coli inhibition by plain and polymer-coated silver nanoparticles. *Rev. Inst. Med. Tropical Sao Paulo* **2018**, *60*, e18.
(121) Onyeji, C.; Nightingale, C.; Marangos, M. Enhanced killing of methicillin-resistantStaphylococcus aureus in human macrophages by liposome-entrapped vancomycin and teicoplanin. *Infection* **1994**, *22* (5), 338−342.
(122) Pumerantz, A.; et al. Preparation of liposomal vancomycin and intracellular killing of meticillin-resistant Staphylococcus aureus (MRSA). *Int. J. Antimicrob. Agents* **2011**, *37* (2), 140−144.
(123) Sande, L.; et al. Liposomal encapsulation of vancomycin improves killing of methicillin-resistant Staphylococcus aureus in a murine infection model. *J. Antimicrob. Chemother.* **2012**, *67* (9), 2191−2194.
(124) Kilian, G.; et al. Antimicrobial activity of liposome encapsulated cyclo(L-tyrosyl-L-prolyl). *Pharmazie* **2011**, *66* (6), 421−423.
(125) Yang, K.; et al. Antimicrobial peptide-modified liposomes for bacteria targeted delivery of temoporfin in photodynamic antimicrobial chemotherapy. *Photochem. Photobiol. Sci.* **2011**, *10* (10), 1593−601.

https://doi.org/10.1021/acsinfecdis.1c00660
ACS Infect. Dis. 2022, 8, 693−712

(126) Yang, K.; et al. Wheat germ agglutinin modified liposomes for the photodynamic inactivation of bacteria. *Photochem. Photobiol.* **2012**, *88* (3), 548−56.
(127) Pornpattananangkul, D.; et al. Bacterial Toxin-Triggered Drug Release from Gold Nanoparticle-Stabilized Liposomes for the Treatment of Bacterial Infection. *J. Am. Chem. Soc.* **2011**, *133* (11), 4132−4139.
(128) Monck, J. R.; Fernandez, J. M. The fusion pore and mechanisms of biological membrane fusion. *Curr. Opin. Cell Biol.* **1996**, *8* (4), 524−533.
(129) Düzgüneş, N. Molecular control of vesicle aggregation and membrane fusion: hypotheses on bile vesicles. *Hepatology* **1990**, *12* (3), 67S−72S.
(130) MacDonald, R. C. Surface chemistry of phospholipid vesicles relevant to their aggregation and fusion. *Hepatology* **1990**, *12* (3), 56s−60s.
(131) Cevc, G.; Richardsen, H. Lipid vesicles and membrane fusion. *Adv. Drug Delivery Rev.* **1999**, *38* (3), 207−232.
(132) Esmaeili, F.; et al. Preparation and antibacterial activity evaluation of rifampicin-loaded poly lactide-co-glycolide nanoparticles. *Nanomedicine: Nanotechnol. Biology Medicine* **2007**, *3* (2), 161−167.
(133) Martins, D.; et al. Antitumoral activity of L-ascorbic acid-poly-D,L-(lactide-co-glycolide) nanoparticles containing violacein. *Int. J. Nanomed.* **2010**, *5*, 77−85.
(134) Sun, Y.; et al. Chitosan microparticles ionically cross-linked with poly(γ-glutamic acid) as antimicrobial peptides and nitric oxide delivery systems. *Biochemical Engineering Journal* **2015**, *95*, 78−85.
(135) Dugal, S.; Mamajiwala, N. A novel strategy to control emerging drug resistant infections. *J. Chem. Pharmaceutical Res.* **2011**, *3*, 584−589.
(136) Huang, L.; et al. Synergistic Combination of Chitosan Acetate with Nanoparticle Silver as a Topical Antimicrobial: Efficacy against Bacterial Burn Infections. *J. Antimicrobial Agents Chemotherapy* **2011**, *55* (7), 3432−3438.
(137) Sahariah, P.; et al. Synthesis of guanidinylated chitosan with the aid of multiple protecting groups and investigation of antibacterial activity. *Carbohydr. Polym.* **2015**, *127*, 407−17.
(138) Cavassin, E. D.; et al. Comparison of methods to detect the in vitro activity of silver nanoparticles (AgNP) against multidrug resistant bacteria. *J. Nanobiotechnol.* **2015**, *13*, 64.
(139) Son, G.-H.; Lee, B.-J.; Cho, C.-W. Mechanisms of drug release from advanced drug formulations such as polymeric-based drug-delivery systems and lipid nanoparticles. *J. Pharmaceutical Investigation* **2017**, *47* (4), 287−296.
(140) Langer, R.; Peppas, N. Chemical and Physical Structure of Polymers as Carriers for Controlled Release of Bioactive Agents: A Review. *J. Macromol. Sci., Part C* **1983**, *23* (1), 61−126.
(141) Alexis, F.; et al. Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Mol. Pharmaceutics* **2008**, *5* (4), 505−515.
(142) Zielińska, A.; et al. Polymeric nanoparticles: production, characterization, toxicology and ecotoxicology. *Molecules* **2020**, *25* (16), 3731.
(143) Pedraza, D.; et al. Amine-Functionalized Mesoporous Silica Nanoparticles: A New Nanoantibiotic for Bone Infection Treatment %J Biomedical Glasses. *Biomed. Glasses* **2018**, *4* (1), 1−12.
(144) Braun, K.; et al. Membrane interactions of mesoporous silica nanoparticles as carriers of antimicrobial peptides. *J. Colloid Interface Sci.* **2016**, *475*, 161−170.
(145) Seleem, M. N.; et al. Silica-Antibiotic Hybrid Nanoparticles for Targeting Intracellular Pathogens. *Antimicrob. Agents Chemother.* **2009**, *53* (10), 4270−4274.
(146) Braun, K.; et al. Influence of mesopore size and peptide aggregation on the adsorption and release of a model antimicrobial peptide onto/from mesoporous silica nanoparticles in vitro. *Mol. Syst. Des. Eng.* **2017**, *2* (4), 393−400.
(147) Gounani, Z.; et al. Loading of polymyxin B onto anionic mesoporous silica nanoparticles retains antibacterial activity and enhances biocompatibility. *Int. J. Pharm.* **2018**, *537* (1), 148−161.
(148) Tenland, E.; et al. Effective delivery of the anti-mycobacterial peptide NZX in mesoporous silica nanoparticles. *PLoS One* **2019**, *14* (2), No. e0212858.
(149) Makarovsky, I.; et al. Novel Triclosan-Bound Hybrid-Silica Nanoparticles and their Enhanced Antimicrobial Properties. *Adv. Funct. Mater.* **2011**, *21* (22), 4295−4304.
(150) Guo, Y.; Rogelj, S.; Zhang, P. Rose Bengal-decorated silica nanoparticles as photosensitizers for inactivation of gram-positive bacteria. *Nanotechnology* **2010**, *21* (6), No. 065102.
(151) Gounani, Z.; et al. Mesoporous silica nanoparticles carrying multiple antibiotics provide enhanced synergistic effect and improved biocompatibility. *Colloids Surf. B Biointerfaces* **2019**, *175*, 498−508.
(152) Rudramurthy, G. R. Nanoparticles: Alternatives Against Drug-Resistant Pathogenic Microbes. *Molecules* **2016**, *21* (7), 836.
(153) Hemeg, H. A. Nanomaterials for alternative antibacterial therapy. *Int. J. Nanomed.* **2017**, *12*, 8211.
(154) Dizaj, S. M.; et al. Antimicrobial activity of the metals and metal oxide nanoparticles. *Mater. Sci. Eng.: C Mater. Biol. Appl.* **2014**, *44*, 278−84.
(155) Hao, N.; Li, L.; Tang, F. Roles of particle size, shape and surface chemistry of mesoporous silica nanomaterials on biological systems. *Int. Mater. Rev.* **2017**, *62* (2), 57−77.
(156) Liau, S.; et al. Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterialaction of silver ions. *Lett. Appl. Microbiol.* **1997**, *25* (4), 279−283.
(157) Feng, Q. L.; et al. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. *J. Biomed. Mater. Res.* **2000**, *52* (4), 662−8.
(158) Golubeva, O. Y.; et al. Synthesis and study of antimicrobial activity of bioconjugates of silver nanoparticles and endogenous antibiotics. *Glass Phys. Chem.* **2011**, *37* (1), 78−84.
(159) Brown, A. N.; et al. Nanoparticles functionalized with ampicillin destroy multiple-antibiotic-resistant isolates of Pseudomonas aeruginosa and Enterobacter aerogenes and methicillin-resistant Staphylococcus aureus. *Appl. Environ. Microbiol.* **2012**, *78* (8), 2768−74.
(160) Ren, G.; et al. Characterisation of copper oxide nanoparticles for antimicrobial applications. *Int. J. Antimicrob. Agents* **2009**, *33* (6), 587−590.
(161) Kruk, T.; et al. Synthesis and antimicrobial activity of monodisperse copper nanoparticles. *Colloids Surf., B* **2015**, *128*, 17−22.
(162) Jayaramudu, T.; et al. Chitosan capped copper oxide/copper nanoparticles encapsulated microbial resistant nanocomposite films. *Int. J. Biol. Macromol.* **2019**, *128*, 499−508.
(163) Huang, L.; et al. Exploration of Copper-Cysteamine Nanoparticles as a New Type of Agents for Antimicrobial Photodynamic Inactivation. *J. Biomed. Nanotechnol.* **2019**, *15* (10), 2142−2148.
(164) Jones, N.; et al. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. *FEMS Microbiol Lett.* **2008**, *279* (1), 71−6.
(165) Azizi-Lalabadi, M.; et al. Antimicrobial activity of Titanium dioxide and Zinc oxide nanoparticles supported in 4A zeolite and evaluation the morphological characteristic. *Sci. Rep.* **2019**, *9* (1), 17439.
(166) Xie, Y.; et al. Antibacterial activity and mechanism of action of zinc oxide nanoparticles against Campylobacter jejuni. *Appl. Environ. Microbiol.* **2011**, *77* (7), 2325−31.
(167) Abdolmajid, E.; et al. Titanium oxide nanoparticles fabrication, hemoglobin interaction, white blood cells cytotoxicity, and antibacterial studies. *J. Biomol. Struct. Dyn.* **2019**, *37* (11), 3007−3017.
(168) Sundrarajan, M.; et al. Obtaining titanium dioxide nanoparticles with spherical shape and antimicrobial properties using M.

https://doi.org/10.1021/acsinfecdis.1c00660
ACS Infect. Dis. 2022, 8, 693−712

citrifolia leaves extract by hydrothermal method. *J. Photochem. Photobiol. B* **2017**, *171*, 117−124.
(169) Hou, J.; et al. Toxicity and mechanisms of action of titanium dioxide nanoparticles in living organisms. *J. Environ. Sci. (China)* **2019**, *75*, 40−53.
(170) Zheng, K.; et al. Antimicrobial Gold Nanoclusters. *ACS Nano* **2017**, *11* (7), 6904−6910.
(171) Li, X.; et al. Functional gold nanoparticles as potent antimicrobial agents against multi-drug-resistant bacteria. *ACS Nano* **2014**, *8* (10), 10682−6.
(172) Bresee, J.; et al. Growth inhibition of Staphylococcus aureus by mixed monolayer gold nanoparticles. *Small* **2011**, *7* (14), 2027−31.
(173) Fakhri, A.; Tahami, S.; Naji, M. Synthesis and characterization of core-shell bimetallic nanoparticles for synergistic antimicrobial effect studies in combination with doxycycline on burn specific pathogens. *J. Photochem. Photobiol. B* **2017**, *169*, 21−26.
(174) Chen, W.-Y.; et al. Self-Assembly of Antimicrobial Peptides on Gold Nanodots: Against Multidrug-Resistant Bacteria and Wound-Healing Application. *Adv. Funct. Mater.* **2015**, *25* (46), 7189−7199.
(175) Labruère, R.; et al. Poly (vinyl benzoate) nanoparticles for molecular delivery: studies on their preparation and in vitro properties. *J. Controlled Release* **2010**, *148* (2), 234−240.
(176) Satishkumar, R.; Vertegel, A. Charge-directed targeting of antimicrobial protein-nanoparticle conjugates. *Biotechnol. Bioeng.* **2008**, *100* (3), 403−412.
(177) Song, H.; et al. Silica Nanopollens Enhance Adhesion for Long-Term Bacterial Inhibition. *J. Am. Chem. Soc.* **2016**, *138* (20), 6455−6462.
(178) Ang, C. W. Mesoporous Silica Nanoparticles Improve Oral Delivery of Antitubercular Bicyclic Nitroimidazoles. *ACS Biomater. Sci. Eng.* **2021**, DOI: 10.1021/acsbiomaterials.1c00807.
(179) Pumerantz, A.; et al. Preparation of liposomal vancomycin and intracellular killing of meticillin-resistant Staphylococcus aureus (MRSA). *Int. J. Antimicrob. Agents* **2011**, *37* (2), 140−4.
(180) Sande, L.; et al. Liposomal encapsulation of vancomycin improves killing of methicillin-resistant Staphylococcus aureus in a murine infection model. *J. Antimicrob. Chemother.* **2012**, *67* (9), 2191−4.
(181) Labruere, R.; Sona, A. J.; Turos, E. Anti-methicillin resistant Staphylococcus aureus (MRSA) nanoantibiotics. *Front. Pharmacology* **2019**, *10*, 1121.
(182) Xue, Y.; Xiao, H.; Zhang, Y. Antimicrobial polymeric materials with quaternary ammonium and phosphonium salts. *Int. J. Mol. Sci.* **2015**, *16* (2), 3626−3655.
(183) Cheng, Y.; et al. External electrostatic interaction versus internal encapsulation between cationic dendrimers and negatively charged drugs: which contributes more to solubility enhancement of the drugs? *J. Phys. Chem. B* **2008**, *112* (30), 8884−8890.
(184) Möhler, J. S.; et al. Silver bullets: A new lustre on an old antimicrobial agent. *Biotechnol. Advances* **2018**, *36* (5), 1391−1411.
(185) Ayala-Núñez, N. V.; et al. Silver Nanoparticles Toxicity and Bactericidal Effect Against Methicillin-Resistant Staphylococcus aureus: Nanoscale Does Matter. *NanoBiotechnology* **2009**, *5* (1), 2−9.
(186) Muller, M. Bacterial Silver Resistance Gained by Cooperative Interspecies Redox Behavior. *Antimicrob. Agents Chemother.* **2018**, *62* (8), e00672-18.
(187) Elkrewi, E.; et al. Cryptic silver resistance is prevalent and readily activated in certain Gram-negative pathogens. *J. Antimicrob. Chemother.* **2017**, *72* (11), 3043−3046.
(188) Ruden, S.; et al. Synergistic Interaction between Silver Nanoparticles and Membrane-Permeabilizing Antimicrobial Peptides. *Antimicrob. Agents Chemother.* **2009**, *53* (8), 3538.
(189) Bankier, C.; et al. Synergistic Antibacterial Effects of Metallic Nanoparticle Combinations. *Sci. Rep.* **2019**, *9* (1), 16074.
(190) Baker, S.; Pasha, A.; Satish, S. Biogenic nanoparticles bearing antibacterial activity and their synergistic effect with broad spectrum antibiotics: Emerging strategy to combat drug resistant pathogens. *Saudi Pharmaceutical J.* **2017**, *25* (1), 44−51.
(191) He, S.; et al. Antibiotic-decorated titanium with enhanced antibacterial activity through adhesive polydopamine for dental/bone implant. *J. .Royal Society, Interface* **2014**, *11* (95), 20140169−20140169.
(192) Qayyum, S.; Khan, A. U. Nanoparticles vs. biofilms: a battle against another paradigm of antibiotic resistance. *MedChemComm* **2016**, *7* (8), 1479−1498.
(193) Monds, R. D.; O'Toole, G. A. The developmental model of microbial biofilms: ten years of a paradigm up for review. *Trends Microbiol.* **2009**, *17* (2), 73−87.
(194) Branda, S. S.; et al. Biofilms: the matrix revisited. *Trends Microbiol.* **2005**, *13* (1), 20−6.
(195) Flemming, H. C.; Wingender, J. The biofilm matrix. *Nat. Rev. Microbiol.* **2010**, *8* (9), 623−33.
(196) Li, Z.; et al. Design and Application of Conjugated Polymer NanoMater. for Detection and Inactivation of Pathogenic Microbes. *ACS Appl. Bio Materials* **2021**, *4* (1), 370−386.
(197) Zhang, H.; et al. Dual-Mode Antibacterial Conjugated Polymer Nanoparticles for Photothermal and Photodynamic Therapy. *Macromol. Biosci.* **2020**, *20* (2), 1900301.
(198) Tian, S.; et al. Water-Soluble Organic Nanoparticles with Programable Intermolecular Charge Transfer for NIR-II Photothermal Anti-Bacterial Therapy. *Angew. Chem., Int. Ed.* **2021**, *60* (21), 11758−11762.
(199) Wang, L.-S.; Gupta, A.; Rotello, V. M. Nanomaterials for the Treatment of Bacterial Biofilms. *ACS Infectious Diseases* **2016**, *2* (1), 3−4.
(200) Malaekeh-Nikouei, B.; et al. The role of nanoTechnol. in combating biofilm-based antibiotic resistance. *J. Drug Delivery Sci. Technol.* **2020**, *60*, 101880.
(201) Han, C.; et al. Recent developments in the use of nanoparticles for treatment of biofilms. *Nanotechnol. Rev.* **2017**, *6* (5), 383−404.
(202) Oliver, S.; et al. Enhancing the antimicrobial and antibiofilm effectiveness of silver nanoparticles prepared by green synthesis. *J. Mater. Chem. B* **2018**, *6* (24), 4124−4138.
(203) Wolska, K. I.; et al. The Potential of Metal Nanoparticles for Inhibition of Bacterial Biofilms. In *Nanotechnology in Diagnosis, Treatment and Prophylaxis of Infectious Diseases*; Rai, M., Kon, K., Eds.; Academic Press: Boston, 2015; Chap. 8, pp 119−132.
(204) Taglietti, A.; et al. Antibiofilm activity of a monolayer of silver nanoparticles anchored to an amino-silanized glass surface. *Biomaterials* **2014**, *35* (6), 1779−88.
(205) Makabenta, J. M. V.; et al. Polymeric Nanoparticles Active against Dual-Species Bacterial Biofilms. *Molecules* **2021**, *26* (16), 4958.
(206) Nguyen, T.-K.; et al. Rational Design of Single-Chain Polymeric Nanoparticles That Kill Planktonic and Biofilm Bacteria. *ACS Infectious Diseases* **2017**, *3* (3), 237−248.
(207) Fang, F. C. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. *Nat. Rev. Microbiol.* **2004**, *2* (10), 820−32.
(208) Nguyen, T.-K.; et al. Co-delivery of nitric oxide and antibiotic using polymeric nanoparticles. *Chem. Sci.* **2016**, *7* (2), 1016−1027.
(209) Slomberg, D. L.; et al. Role of size and shape on biofilm eradication for nitric oxide-releasing silica nanoparticles. *ACS Appl. Materials Interfaces* **2013**, *5* (19), 9322−9.
(210) Khan, S. T.; Musarrat, J.; Al-Khedhairy, A. A. Countering drug resistance, infectious diseases, and sepsis using metal and metal oxides nanoparticles: current status. *Colloids Surf., B* **2016**, *146*, 70−83.
(211) Carlson, C.; et al. Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. *J. Phys. Chem. B* **2008**, *112* (43), 13608−19.
(212) Ahamed, M.; et al. DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. *Toxicol. Appl. Pharmacol.* **2008**, *233* (3), 404−410.
(213) Sung, J. H.; et al. Subchronic Inhalation Toxicity of Silver Nanoparticles. *Toxicol. Sci.* **2009**, *108* (2), 452−461.

(214) Wang, J.; et al. Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. *Toxicol. Lett.* **2007**, *168* (2), 176−185.
(215) Azim, S. A.; et al. Amelioration of titanium dioxide nanoparticles-induced liver injury in mice: possible role of some antioxidants. *Exp. Toxicol. Pathol.* **2015**, *67* (4), 305−14.
(216) Hong, F.; et al. Suppression of neurite outgrowth of primary cultured hippocampal neurons is involved in impairment of glutamate metabolism and NMDA receptor function caused by nanoparticulate TiO2. *Biomaterials* **2015**, *53*, 76−85.
(217) Hu, Y. L.; et al. Toxicity evaluation of biodegradable chitosan nanoparticles using a zebrafish embryo model. *Int. J. Nanomed.* **2011**, *6*, 3351−9.
(218) Park, M.-R. Chitosan Nanoparticles Cause Pre- and Postimplantation Embryo Complications in Mice1. *Biol. Reprod.* **2013**, *88* (4), 1−13.
(219) Murugadoss, S.; et al. Toxicology of silica nanoparticles: an update. *Arch. Toxicol.* **2017**, *91* (9), 2967−3010.
(220) Cho, Y. M.; et al. Size-dependent acute toxicity of silver nanoparticles in mice. *J. Toxicologic Pathology* **2018**, *31* (1), 73−80.
(221) Recordati, C.; et al. Tissue distribution and acute toxicity of silver after single intravenous administration in mice: nano-specific and size-dependent effects. *Particle Fibre Toxicol.* **2015**, *13*, 12.
(222) Bahamonde, J.; et al. Gold Nanoparticle Toxicity in Mice and Rats: Species Differences. *Toxicol. Pathol.* **2018**, *46* (4), 431−443.
(223) Chen, Z.; et al. Acute toxicological effects of copper nanoparticles in vivo. *Toxicol. Lett.* **2006**, *163* (2), 109−20.
(224) Feng, Q.; et al. Uptake, distribution, clearance, and toxicity of iron oxide nanoparticles with different sizes and coatings. *Sci. Rep.* **2018**, *8* (1), 2082.
(225) Jia, X.; et al. The Potential Liver, Brain, and Embryo Toxicity of Titanium Dioxide Nanoparticles on Mice. *Nanoscale Res. Lett.* **2017**, *12* (1), 478.
(226) Gao, L.; et al. Acute toxicity of zinc oxide nanoparticles to the rat olfactory system after intranasal instillation. *J. Appl. Toxicol.* **2013**, *33* (10), 1079−1088.
(227) Wang, D.; et al. Acute toxicological effects of zinc oxide nanoparticles in mice after intratracheal instillation. *Int. J. Occupational Environ. Health* **2017**, *23* (1), 11−19.

https://doi.org/10.1021/acsinfecdis.1c00660
ACS Infect. Dis. 2022, 8, 693−712