Antibacterial properties and toxicity from metallic nanomaterials

Gina V Vimbela[1,*]
Sang M Ngo[2,*]
Carolyn Fraze[3]
Lei Yang[4,5]
David A Stout[5–7]

[1]Department of Chemical Engineering, [2]Department of Electrical Engineering, California State University, Long Beach, CA, [3]Brigham Young University Idaho, Rexburg, ID, USA; [4]Department of Orthopaedics, Orthopaedic Institute, The First Affiliated Hospital, [5]International Research Center for Translational Orthopaedics (IRCTO), Soochow University, Suzhou, Jiangsu, People's Republic of China; [6]Department of Mechanical and Aerospace Engineering, [7]Department of Biomedical Engineering, California State University, Long Beach, CA, USA

*These authors contributed equally to this work

Correspondence: David A Stout
Department of Mechanical and Aerospace Engineering, 1250 Bellflower Blvd, ECS-632 Long Beach, CA 90840, USA
Tel +1 562 985 1502
Email david.stout@csulb.edu

Lei Yang
Orthopaedic Institute of Soochow University, 313 Buidling #1, 708 Renmin Road, Suzhou, Jiangsu 215006, People's Republic of China
Tel +86 512 6778 1540
Email leiy@suda.edu.cn

Abstract: The era of antibiotic resistance is a cause of increasing concern as bacteria continue to develop adaptive countermeasures against current antibiotics at an alarming rate. In recent years, studies have reported nanoparticles as a promising alternative to antibacterial reagents because of their exhibited antibacterial activity in several biomedical applications, including drug and gene delivery, tissue engineering, and imaging. Moreover, nanomaterial research has led to reports of a possible relationship between the morphological characteristics of a nanomaterial and the magnitude of its delivered toxicity. However, conventional synthesis of nanoparticles requires harsh chemicals and costly energy consumption. Additionally, the exact relationship between toxicity and morphology of nanomaterials has not been well established. Here, we review the recent advancements in synthesis techniques for silver, gold, copper, titanium, zinc oxide, and magnesium oxide nanomaterials and composites, with a focus on the toxicity exhibited by nanomaterials of multidimensions. This article highlights the benefits of selecting each material or metal-based composite for certain applications while also addressing possible setbacks and the toxic effects of the nanomaterials on the environment.

Keywords: antibacterial reagents, nanomaterials, synthesis, metals, nanostructure, nanoscale, antibiotic resistance, toxicity, drug delivery

Introduction

Bacteria are serious and potentially life-threatening agents, capable of promoting infectious diseases. The bubonic/pneumonic plague[1] pandemic of the 14th century and the transmission of virulent cholera[2] are both examples of bacteria acting as causative agents for infection. The bubonic/pneumonic plague, or the Black Death, was one of the most devastating disease outbreaks in human history, killing ~50 million people worldwide.[3] Although the etiology of the disease remained highly controversial until recently, the gram-negative *Yersinia pestis* was confirmed to cause the Black Death and later epidemics over the course of four centuries.[4] Pandemics of cholera, a disease caused by *Vibrio cholerae*, have affected millions of people since the early 1800s. The second cholera[5] pandemic (1832) alone claimed the lives of over 15,000 people throughout Asia and Europe. The magnitude and severity of such outbreaks left local governments ill-equipped to supply necessary aid, eventually leading to the establishment of the World Health Organization (WHO) in 1945. The WHO worked to provide local governments with vaccines, laboratory personnel training, and equipment[2] to mitigate the effects of outbreaks. Despite programs from the WHO set in place, outbreaks of diseases such as typhoid and scarlet fever led to drastic fluctuations in morbidity and mortality rates[3] in the 20th century. The discovery of the role of bacteria as infectious agents propelled the scientific community toward the development of synthetic antibacterial agents.

Introduced in 1910, salvarsan was the first antimicrobial agent to be synthesized as a remedy to syphilis. The era of antibiotics soon followed, and antimicrobial agents such as chloramphenicol, nalidixic acid, and macrolides were introduced into clinics.

The introduction of antibiotics in the 20th century provided temporary relief to infectious bacterial pathogens. The emergence of antibiotic-resistant bacteria has been traced to several dynamic mechanisms of action consistent with bacterial roles against antimicrobial host defense.[6] Moreover, bacterial pathogens have also significantly evolved effective countermeasures against antibacterial agents stemming from overexposure to antibiotics, such as efflux pumps that remove the antibacterial agent before it can reach its target site and exert its effect.[7]

Methods for overcoming the emergence of these resistant strains have delved into the development of new antibiotic drugs boasting chemical diversity, such as the antibiotic daptomycin found in soil actinomycetes.[8] Additionally, identification of antibiotic-producing bacteria such as that found on the European beewolf[9] (a hunting wasp) has proven the existence of additional antibiotics from natural, previously unexplored sources. Developments in these areas, however, have not produced sufficient advancement against the rapidly increasing number of resistant bacterial strains.[10,11] Nontraditional antimicrobial agents have now been identified as promising tools against bacteria resistant to traditional antibiotic drugs.[12]

One type of nontraditional antimicrobials recently introduced is nanomaterials. Nanomaterials have proven to demonstrate toxic effects against several bacterial strains during in vitro studies. Because of these results, nanomaterials could be promising in several biomedical applications, including drug and gene delivery, tissue engineering, and imaging techniques. Additionally, these materials could be used as a vehicle for delivering a range of therapeutic agents, including drugs, pharmaceuticals, and antibodies. Paul Ehrlich first sparked the idea of developing drug delivery systems, pioneering the development of drug targeting nanoparticles, and introducing nanomaterials for possible use in the medical field.[13]

Since early 2005, nanomaterials have been widely studied for possible use in both medical and pharmaceutical applications.[13] These materials are characterized by exhibiting at least one dimension measuring 100 nm or less and can be metallic, semiconducting, polymer, or carbon based. Reports of antibacterial activity in nanomaterials have stemmed from observed biophysical interactions occurring between nanoparticles and bacteria, including cellular uptake and nanoparticle aggregation, leading to membrane damage and toxicity.[14] In particular, metallic nanomaterials (such as silver, gold, copper, and titanium) exhibit favorable physicochemical characteristics resulting in significant levels of antibacterial activity. This review focuses on the recent advancements made in metallic nanomaterials as antibacterial agents, with a focus on their toxicity and antibacterial activity based on the structure, dimension, and size of nanomaterials. Furthermore, the benefits of using nanomaterials of silver, gold, copper and titanium metals, as well as zinc and magnesium oxides, will be discussed.

Silver nanomaterials

Silver as an antibacterial reagent can be traced back thousands of years to early civilizations when it was utilized for food preservation and production of potable water.[15] Silver was introduced into the medical field for uses in wound healing and burn treatment when its antibacterial properties were discovered.[16] In the early 1940s, the era of antibiotics emerged, and silver was largely replaced by penicillin.[17] Now as antibiotic-resistant bacterial strains continue to emerge and rise in number, silver has been reintroduced as a promising material in the development of new bactericides.

In recent years, several studies have established the ability of silver nanomaterials to exhibit unique electronic, optical, and chemical properties.[18–23] Studies are exploring the possibility of a relationship between physicochemical characteristics of silver nanomaterials and their delivered magnitude of toxicity. Shapes such as beads,[19] rods,[20] mats,[21] sheets,[22] and nanoprisms[23] are being developed and investigated for their specific antibacterial effects. Recently, studies have reported that size and surface coating of silver nanoparticles (AgNPs) play a large role in antibacterial activity, with smaller nanoparticles observed to deliver a higher magnitude of toxicity.[14,15,19] Furthermore, a study using gene deletion mutants to identify the physiological pathways involved in the antibacterial response of gram-negative bacteria *Escherichia coli* to AgNPs techniques reported from its findings that the mechanism, in addition to the magnitude, of delivered toxicity may be strongly influenced by the physicochemical properties of the nanoparticle.[24]

Many studies have sought to establish a mechanism of action of antibacterial activity exhibited by silver in both its colloidal and ionic form. Two leading theories on the proposed mechanism suggest 1) a disruption of membrane functionality from an interaction between released Ag^+ ions and the cell membrane and 2) extensive cell membrane

damage caused by the formation of reactive oxygen species (ROS) ultimately causing damage to the cell due to oxidative stress (Figure 1).[24–26] In brief, the first mechanism suggests that Ag+ ions released from AgNPs can bind with thiol groups (–SH) of proteins and enzymes found on the cellular surface, causing destabilization of the cellular membrane and a breakdown of the ATP synthesis pathway. AgNPs may then adhere to the membrane wall, causing holes through which they can later penetrate the bacteria and interact with intracellular components or proteins containing sulfur. The second mechanism suggests that ROS, such as singlet oxygen, can be produced at the cell membrane and lead to irreversible damage to DNA replication affecting metabolic processes and cell division.[25] As a better understanding is gained on both mechanisms, several reports suggest the first as causing the most significant damage to bacteria. This section will examine advancements in the antibacterial properties of silver nanomaterials due to different structures and their toxicity activities.

Nanoparticles

Novel techniques for synthesis of silver nanomaterials are being studied to identify green alternatives to conventional synthesis and to explore the properties exhibited by composite and hybrid silver nanomaterials.[27,28] Nanoparticles are categorized as particles that behave as a single unit with respect to transport and reactivity. AgNPs can be immobilized
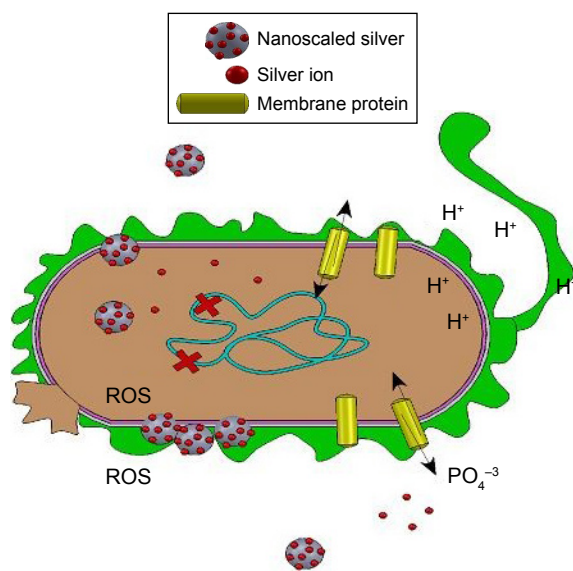

Figure 1 Formation of reactive oxygen species (ROS) and disruption to membrane functionality by nanosilver.
Notes: Antibacterial activities from silver are due to the formation of ROS and disruption of membrane functionality. Formation of ROS causes oxidative stress, which leads to cellular damage. The interaction between the released ions of nanosized silver results in disruption of the membrane functionality.

and coated onto surfaces or may be blended with polymers to form composites for better use of their antimicrobial actions.[29] The excellent bactericidal effects demonstrated by synthesis of such composites suggest high potential of nanoparticles in water treatment, medical devices, burn dressings, and food preservation. Most notably, recent advancements in the synthesis of composites and hybrids suggest potential in drug delivery systems in tissue engineering applications. For this reason, silver nanomaterials have been the source of substantial attention in the medical and technological fields.

Synthesis of AgNPs can be performed through chemical or physical techniques. Conventional synthesis of AgNPs requires an initial reduction of metal salts into pure metals and stabilizing or capping agents to prevent the aggregation of nanoparticles into clusters. They are most commonly synthesized via chemical reduction of silver nitrate or silver tetrachloride resulting in stable colloidal dispersions in solvent.[30] Green alternatives to harsh reducing and stabilizing agents, as well as costly energy consumption, are being investigated and proposed as commercialization of AgNPs continues to rise. Such alternatives include biogenic methods, which use plant extracts, bacteria, fungi, and yeasts as solvents, reducing/capping agents, and/or stabilizing agents.[31]

Eco-friendly, simple synthesis methods utilizing plant and bark extracts are capable of producing polydispersed AgNPs with diameter ranges within 20 nm apart.[32] Sankar et al[33] used *Origanum vulgare*, or oregano, plant leaf extract to synthesize spherical AgNPs displaying cytotoxicity against human lung cancer A549 cell line during in vitro experiments. The study found a direct dose dependence between increased (AgNP) concentration to cytotoxicity against the cancer cell line, with a significant 85% of cell mortality, although anticancer activity mechanism is yet to be understood. Similarly, Nayak et al[34] synthesized crystalline and spherical AgNPs using bark extracts of *Ficus benghalensis* and *Azadirachta indica* and observed antibacterial activity against gram-positive and -negative bacteria, in addition to cytotoxicity against osteosarcoma MG-63 cell line.

Use of cell-free culture supernatants of bacteria for AgNP synthesis is favorable due to its simplicity and ease of genetic modification, although the exact mechanism of this extracellular synthesis is not known. Studies have reported supernatants of *Bacillus cereus*, *Bacillus subtilis*, *Bacillus licheniformis*, *E. coli*, *Enterobacter cloacae*, *Klebsiella pneumonia*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* to induce synthesis of AgNPs.[35] Priyadarshini et al[36] recently reported *Bacillus flexus* S-27 as a potential candidate for extracellular synthesis

of AgNPs that exhibit antibacterial properties against clinically isolated multidrug-resistant bacterial pathogens. Although size of the AgNPs could not be controlled by synthesis factors, AgNPs were determined to be triangular and spherical shaped. Gahlawat et al[37] characterized a novel bacterial strain as *Ochrobactrum rhizosphaerae* and synthesized spherical AgNPs as antimicrobial agents against cholera. The study was able to isolate the biomolecule present in the culture supernatant responsible for synthesis and capping of the AgNPs and identify it as a glycoprotein exopolymer. Observations of antibacterial characteristics demonstrated inhibition of bacterial growth and a reduction in the metabolic activity and cell viability against *V. cholerae*, suggesting a strong potential of the AgNPs for cholera treatment.

Yeast, a single-celled fungus, has recently been identified as a promising candidate for AgNPs synthesis due to its long-term use in industrial scale productions. Eugenio et al[38] report the bioproduction of AgNPs and AgCl-NPs using seven yeast isolates from a termite gut. Two strains of *Candida lusitaniae* (named SNPP1 and SNPP2) of the seven proved to be particularly efficient, with SNPP1 displaying a more uniform size and shape distribution. Agglomerations of AgNPs and AgCl-NPs were observed to be between the outer region of the plasma membrane and the inner cell wall of yeast. No interaction between synthesized AgNPs and AgCl-NPs and yeast intracellular organelles, therefore understanding of whether the synthesized NPs were transferred to their location from within the cell or were deposited there from the cell medium is unknown, requiring further research in the precise mechanism of nanoparticle synthesis.

Other green alternatives for the synthesis of AgNPs include photoinduced or photocatalytic reduction and one-step synthesis of AgNPs using carbon dots as reducing and stabilizing agents. Banasiuk et al[39] reduced the synthesis time by using commercially available LED lights to significantly reduce energy consumption. Furthermore, Jin et al reported a novel green method of stabilizing and reducing AgNPs by fluorescent carbon dots. Previously, carbon dots have been reported to exhibit reducing and oxidizing properties,[40] in addition to predicted stabilizing properties due to various –COOH and –OH groups existing on their surface.[41] With this in mind, Jin et al investigated the synthesis of AgNPs by using carbon dots as reducing and stabilizing agents and the effects of doping the carbon dots in sulfur to modify the size and antibacterial properties of the resulting AgNPs. The group concluded that reduction method was highly dependent upon pH levels, with higher reducing activity correlating with higher pH values. Sulfur doping of the carbon dots fabricated much more stable AgNPs, which may be attributed to high binding energy of sulfur to silver.

The specific physicochemical properties of a nanomaterial play an intrinsic role in its functionality and use. AgNPs may be produced into metallic or composite structures of one or more nanoscale dimensions. Structures such as nanobeads, nanocrystals, and quantum dots are examples of nanostructures with the highest reported antibacterial properties. Because of their uniquely high volume to surface area ratio, they are particularly promising for therapeutic and drug delivery systems. For example, a study on the physiological reactions of *E. coli* to AgNPs of different surface charge and size was found to affect the antibacterial mechanism exhibited by AgNPs.[24] While positively surface charged AgNPs exhibited equal effects to silver nitrate, only the smaller citrate-based NPs caused *E. coli* to initiate a response similar to its response to silver ion. In another study, AgNPs were synthesized during in situ formation of physically cross-linked chitosan (CS) hydrogel beads.[42] The formed AgNPs smoothed the surface morphology of the CH/Ag hydrogel beads, while also decreasing the amount of intact drug, which could be loaded onto the beads. Loading of ibuprofen into resulting nanoparticles showed prolonged release of drug with increased amount of AgNPs in the CH/Ag beads, making the beads particularly favorable for controllable drug release applications.

Nanorods and nanofibers

Silver structures, such as nanorods, nanotubes, and nanofibers, are tubular nanostructures with applications in drug delivery, phototherapy, and water filtration. These nanostructures are versatile because of their potential in controlled release systems. For example, the fibrin morphology of composite Ag/silk fibroin nanofibers is capable of controlling Ag+ ion release, allowing for increased local antibacterial activity at low silver concentrations.[43] Moreover, due to their tubular structures, these nanostructures can be composed of layers in different materials, adding to their versatility and uniqueness. Black et al[44] exploited the photothermal properties of gold and developed gold core-silver shell nanorods as bacterial antibodies. These were used to specifically target bacterial walls and as therapeutic treatment by plasmonic heating and light irradiation. The silver coating over the gold center intensified the heating effects when irradiated by light, with simultaneously increasing antibacterial activity against bacterial walls of both gram-positive and gram-negative bacteria. Similarly, Hassan et al studied bimetallic composites and their capability to improve single-metal properties

by bimetallization. A bimetallic composite demonstrating a spider-net morphology was prepared using zinc oxide, silver, and polyurethane (ZnO:Ag/PU).[45] PU is a biocompatible thermoplastic polymer that has desirable mechanical properties and is water insoluble. Antibacterial activity of ZnO and ZnO:Ag was tested using *E. coli*. ZnO/PU hybrid nanofibers demonstrated significant antibacterial activity, while ZnO:Ag-doped PU showed complete inhibition of *E. coli*. Proliferation of NIH 3T3 mouse fibroblast cells was analyzed in vitro to test bimetallic composite compatibility, results demonstrated noncytotoxic behavior between the cell line and composite. The synergism of the metallic properties in ZnO:Ag composite signals its high potential in water filtration uses.

Nanomats

Structures such as nanofilms, nanosheets, and nanomats are two-dimensional nanostructures with a thickness within the 1–100 nm range size. These structures are particularly useful as nanohybrids and composite materials with biocompatible polymers. For example, nanofiber mats embedded with chitosan-based silver nanoparticles (CS/AgNPs) were blended with polyvinyl alcohol (PVA) and the effects on *E. coli* were studied.[21] In addition to the increased antibacterial efficacy demonstrated by the nanofiber systems, results suggest blending of CS-based systems with PVA produced electrospun fiber mats more easily and with higher mechanical ability making it possible to further explore utilization of promising and biocompatible CS/AgNP-embedded systems in antibacterial applications. Inspired by the adhesive thread found in *Mytilus edulis* foot protein 5 (Mefp-5) of mussels, synthesis of uniform AgNPs on functionalized polydopamine-graphene nanosheets (PDA-GNS) was studied.[22] The authors suggested that utilization of a natural self-polymerizing PDA on functionalized surfaces could provide effective methods for synthesis of highly uniform AgNPs on hybrid materials exhibiting strong biocidal properties with strong potential in clinical and environmental applications.

Complex nanostructures

Nanoprisms, matrices, and dendritic structures are complex nanostructures the potential of which is far from being fully exploited. For example, encapsulated anisotropic AgNPs were synthesized in biocompatible polymer pluronic F-127 into triangular nanoprisms.[46] The resulting nanoprisms had an average edge length between 30 and 50 nm and thickness of 4 and 6 nm. Pluronic F-127 is a synthetic triblock copolymer consisting of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) chains that can self-assemble into micelles to form different close-packed surfaces. Pluronic can bind to the surface of the nanoparticles and form a protective layer by hydrophobic association of the PPO blocks. Surface modifications of synthesized AgNPs were developed to prevent aggregation in biological media, increase stability, and reduce toxicity and probable immune response from living organisms. Antibacterial activity exhibited by the triangular nanoprisms suggests a higher rate of Ag^+ ion release possibly from the crystalline tips and edges, therefore, being ultimately useful for therapeutic applications.

Toxicity

The impact that novel nanoparticles and nanostructures may have on biological organisms and the environment must be understood to minimize potentially detrimental effects of these nanoparticles in any application. Recent studies have investigated the cytotoxic effects of AgNPs on the human body, specifically on the respiratory and cardiovascular systems, osteoblasts and osteoclasts, DNA, and embryo development malformations.[47] Many recent studies aim to counter the lack of consensus in earlier investigations, where large variability between AgNPs synthesis led to widely different conclusions on the toxicological effects of size-varying AgNPs.

Gliga et al[48] investigated physicochemical effects by studying the release of silver ions by AgNPs of 10, 40, 50, and 75 nm and capping agents against healthy human lung cells. Although the capping agent was not found responsible for mediating toxic effects, release of Ag^+ into the cell medium was found to be directly proportional to the total surface of the particle and thus size. Furthermore, investigation of the cellular uptake of Ag^+ showed a higher total cellular content that did not correlate with the most toxic NP size. The authors suggest that the higher Ag^+ content within the cell must be a result of intracellular transformation of AgNPs to Ag^+. The composition of experimental media also plays a role in the release of silver. For information on the differences of toxic effects dependent on media composition, the following reviews on the subject are recommended.[49–52]

Damage to specific cells and cellular structures is also actively studied to determine specific biological responses and possible toxicological mechanisms between AgNPs and the structures. Incurred DNA damage by Ag^+ release is noted to be size independent. One study reported that DNA damage (Figure 2) occurred in all tested samples (regardless of NP size) after a 4-hour incubation period.[48] Although the exact mechanism for this could not be determined, authors suggest that DNA damage could occur due to AgNP interaction with
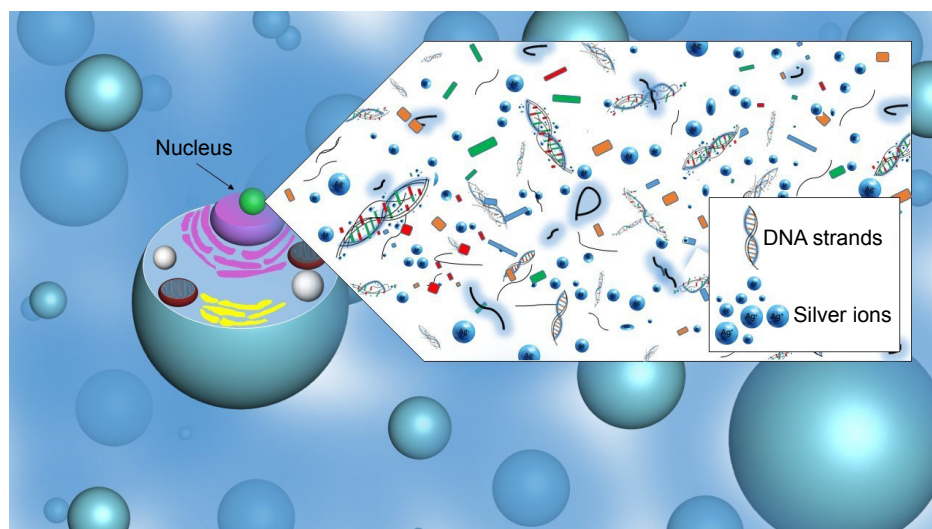
Figure 2 Damaged DNA strands due to silver ions in cell nucleus.
Note: Nanosized silver releases silver ions, which interacts with DNA strands within the cellular nucleus, which results in DNA damage.

DNA repair pathways as previously reported for AgNPs. Damage to mitochondria leading to stimulation of apoptotic pathways has also been observed as a result of AgNP exposure. The study determined cytotoxicity of AgNPs to be mediated by free radical-independent mechanisms since free radicals were determined to play no major role in cytotoxic behavior.[53] Cytotoxicity of AgNPs on red blood cells (RBCs) is of particular interest, as the introduction of AgNPs into the body suggests a high probability of these into the blood. Chen et al[54] found cytotoxicity effects of 15, 50, and 100 nm AgNPs on RBC to be higher when they are a smaller size, while biological-based nanoparticles with larger sizes exhibit higher cytotoxic effects. This points to a possible difference in toxicity mechanism from one type of nanoparticle to the next. The mechanism alludes to higher reactivity between positively charged silver particles and negative surface charge of RBCs, thus more interaction. The study also established an optimal size, ~50 nm, for interaction into the cytoplasm of RBC. Further studies are needed to determine the exact mechanism that gives rises to the selectivity of size and to determine the differences in biological-based nanoparticles vs AgNPs. Furthermore, the possibility of the biocompatibility of orthopedic implants being compromised by the cytotoxicity of AgNPs against osteoblasts and osteoclasts is a major concern. In a study conducted by Pauksch et al,[55] osteoblasts were found to have a higher susceptibility to toxicological effects of less than 5 nm sized AgNPs than osteoclasts. In this study, AgNPs at ~30 nm size released more Ag^+ then the larger AgNPs, alluding to a similar size selectivity as that of the RBC and

AgNP interaction. Antibacterial activities due to AgNPs against bacteria cells are promising from their observation of toxicities in previous studies.

Studies on the environmental toxicity of AgNPs suggest that the impact of nanosilver may be dependent on the different organisms that come into contact with the materials.[56] In one study, 0.1 to 1,000 µM AgNPs in aqueous media were introduced to aquatic organisms and were found to show different accumulation abilities in each organism. Another study suggests that the toxicity of AgNPs on aquatic organisms of different trophic levels might be alleviated by humic substances.[57] The authors suggest that this alteration of toxicity by the humic substance may be through a possible electrostatic barrier limiting the interaction between AgNPs and the aquatic organisms.

Decreasing the size of a nanomaterial leads to a significant increase in surface area relative to volume. Several articles have reported cellular uptake, endocytosis, and the efficiency of particle processing in the endocytic pathway to be dependent on particle size.[58] However, recent studies report agglomeration of AgNPs to occur quickly in protein-stabilized dispersions for particles of sizes between 20 and 50 nm.[59] The formation of agglomerations is reported to decrease hemolytic cytotoxicity by decreasing the effective surface area to volume ratio and, ultimately, the release of Ag^+ and the generation of ROS.[60]

Summary

Advances in synthesis techniques of silver nanomaterials have increased the number of applications, in which silver

nanomaterials may be used as antibacterial reagents. Novel synthesis techniques for silver nanostructures have explored biogenic methods that include plant extracts, bacteria, fungi, and yeasts as solvents, reducing/capping agents, and/or stabilizing agents, providing green alternatives to conventional synthesis. Moreover, novel green synthesis methods for AgNPs production also included photoinduced or photocatalytic reduction and one-step synthesis of AgNPs using carbon dots as reducing and stabilizing agents, reducing synthesis time and high energy consumption compared to classical synthesis methods. Additionally, silver nanomaterials of varying size and structure exhibited varying antibacterial properties. For example, synthesized triangular nanoprisms released higher amounts of silver ions than nanoparticles of the same size and thus demonstrated a higher antibacterial activity. However, nanoparticles of smaller size but same morphological characteristics showed higher antibacterial activity due in large part to ionization rates of the silver ion being higher and also because of the higher chance of the nanoparticle entering into the bacterial cell wall at a smaller size. The potential of nanosized silver as an antibacterial reagent is irrefutable.

Gold nanomaterials

Utilization of colloidal gold as a coloring agent for glass tinting and ceramic adornment can be dated back to as early as the fifth century.[61] Michael Faraday's Bakerian Lecture on the "Experimental Relations of Gold (and other Metals) to Light" in 1857 later reintroduced gold in its nanoparticle dimensions to the scientific community.[62,63] This lecture catalyzed research into the physicochemical properties exhibited by gold nanoparticles (AuNPs) and launched these into tremendous attention for uses in water treatment, catalysis, biosensing, and optics, as well as for therapeutic applications.[62,64] Further research has shown AuNPs as promising bactericidal agents due to their versatile optical and photothermal properties.[65] Advancements in the synthesis and applications of AuNPs as antibacterial reagents are imperative in the fight against antibacterial resistance.[8,66–69] Although not as well studied as the antibacterial mechanism of AgNPs, antibacterial activities of AuNPs are believed to proceed mainly in the two following ways: 1) a change in the membrane potential and prevention of ATPase activities lead to a decline in cellular metabolism (Figure 3A) and 2) the subunit of the ribosome for tRNA binding is inhibited leading to a collapse in biological processes (Figure 3B).[69] In the following sections, advances in the development of gold nanostructures are discussed, including novel synthesis techniques and fabrication of promising nanocomposites. The contribution to their exhibited antibacterial activities stemming from unique morphological features is also discussed.

Nanoparticles

The rapid advancement in nanotechnology and nanomedicine has produced an immense drive to find synthesis methods that are green, cost-effective, and controlled.[70] With this in mind, Ahmed et al synthesized spherical AuNPs with a crystalline pattern using aqueous extracts of *Salicornia brachiata*. The *S. brachiata* served as a necessary catalyst in the synthesis process, with only a small amount of extract necessary for the process to proceed quickly. Additionally, this synthesis process requires no capping or stabilizing agents. Antibacterial
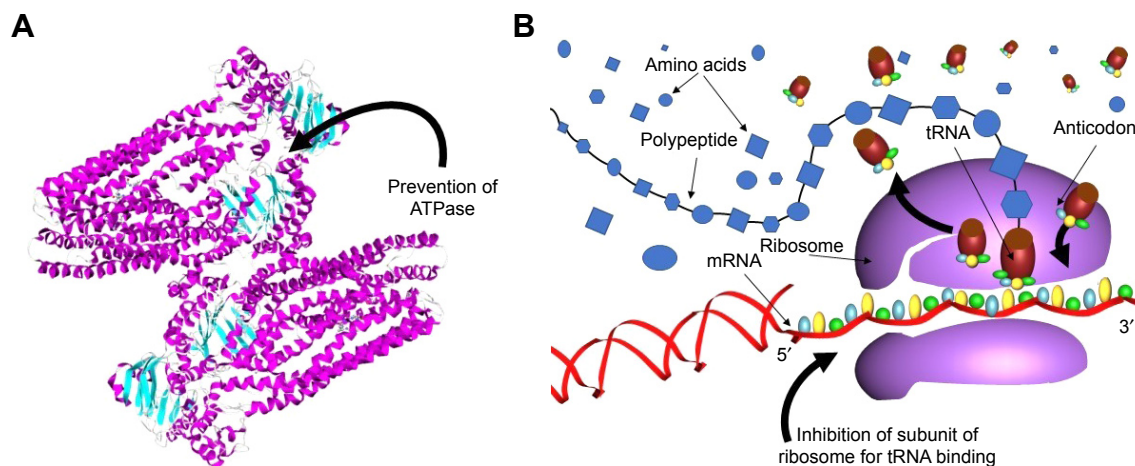

Figure 3 Nanosized gold exhibits antibacterial properties from prevention of ATPase and tRNA binding.
Notes: The antibacterial activities of AuNPs are believed to proceed mainly in the two following ways: (**A**) a change in the membrane potential and a prevention of ATPase activities leading to a decline in cellular metabolism and (**B**) the subunit of the ribosome for tRNA binding is inhibited leading to a collapse in biological processes.

analysis of *S. brachiata*-synthesized AuNPs (*Sb*AuNPs) showed strong antibacterial activity against *Pseudomonas aeruginosa*, *Salmonella typhi*, *E. coli*, and *S. aureus*. Moreover, the maintained clear appearance of *Sb*AuNPs after a 30-day water submersion suggests a minimal aggregation of the *Sb*AuNPs. With similar goals, Annamalai et al[71] fabricated AuNPs using the leaf extract of the medicinal plant *Euphorbia hirta L*. Here, the reduction of gold ions was observed to be catalyzed by the leaf extract and resulted in the formation of stable nanoparticles at a more rapid rate of nanoparticle production when compared to other synthesis methods. Growth inhibition of bacteria cultures was observed to be 88% for *E. coli*, 86% for *P. aeruginosa*, and 94% for *K. pneumonia*. The antibacterial effect of resulting nanoparticles at extremely low concentrations of Au was equal to the effect of large amounts of plant material alone, suggesting that the heightened antibacterial effect of *E. hirta* AuNPs can be attributed to a synergistic effect of the plant extract and gold nanopowder.

Several studies suggest that the inhibition of bacterial growth by AuNPs is highly dependent on the surface area of the nanoparticles. One study suggests that this may be because a high surface area produces Au^{3+} ions and also plays the role of a reservoir for the Au^{3+} ions.[72] In addition to surface area, physicochemical characteristics such as dispersion, shape, and surface capping of the nanoparticles may also affect the antibacterial activity of AuNPs. Zhou et al[14] report that the formation of aggregates of AuNPs reduces the surface area, and subsequently, the interaction between the nanoparticle and bacteria. By using weakly bound capping agents, such as citrate, the group reports bacterial inhibitory interactions. However, a strongly bound capping agent (such as PAH) may be used to reduce aggregation and results in AuNPs with better antibacterial performance.

Nanorods

There is a growing recognition in the engineering of nanomaterials that fabrication of nanostructures, along with their specific physicochemical properties, can regulate the responses of biological entities, such as bacteria.[73] Therefore, fabrication of gold nanorods (AuNRs) where manipulation of size, dimension, and aspect ratio is possible is imperative in the advancement of applications such as drug delivery and water treatment. Zhu et al[74] prepared two- and three-dimensional uniform arrays of AuNRs by confined convective arraying techniques and studied the photoheated bacterial effects on *E. coli*. The resulting AuNRs had an aspect ratio of ~2.5. Examination of antibacterial

activities of 2D and 3D AuNR arrays after their quick and controlled formation was studied on *E. coli* using laser illumination on nanorod-arrayed substrate. Results showed that 98% of *E. coli* was photothermally destroyed indicating that this photothermal treatment using AuNR arrays could be promising in water treatment of pathogenic bacteria in the water. This study developed a targeting agent for selectively killing methicillin-resistant *Staphylococcus aureus* (MRSA) using antiprotein An antibody-conjugated polystyrene sulfonate-coated AuNRs. Results of photothermal therapy resulted in an 82% reduction in MRSA cells. The study compared the results of applying therapy to healthy muscle tissue and infected muscle using a mice model. Results of in vivo testing of infected mice showed a 73% bacterial reduction.[75]

Nanocluster

Gold nanoclusters (AuNCs) possess unique electronic structures with desirable chemical and optical properties. These fluorescent metal nanoclusters are a new class of fluorophore demonstrating promising photostability and biocompatibility.[76] For example, Yang et al[77] developed bovine serum albumin (BSA), histidine (his), and DNA-templated AuNCs for an enhanced fluorescence system based on Eu^+-Tetracycline (EuTC) complex to be utilized as a fluorescent probe. Results demonstrated successful synthesis of BSA/AuNCs, DNA_{c12}/AuNCs, and His/AuNCs, although each displayed different patterns in high-resolution transmission electron microscopic images, with His/AuNCs displaying the smallest size of AuNCs. Results for the analysis of three types of AuNCs synthesized showed remarkably enhanced fluorescent intensity with the introduction of DNA_{c12}/AuNCs to EuTC of ~13-fold compared to EuTC alone. Utilizing this enhanced fluorescence method may significantly broaden the potential ways to detect tetracycline in actual urine samples thus leading to further biological applications.

Composites

Composites are being investigated for the synthesis of biomimetic systems encompassing the favorable photoelectric and thermophysical properties of noble metal nanoparticles, while increasing the stability of nanomaterials.[78] For example, metal oxide nanomaterials have unique capabilities of storing energy by charge separation and creation of electron–hole pairs when exposed to light. He et al constructed ZnO/Au hybrid nanostructures by the photoreduction method resulting in AuNPs of sizes smaller than 3 nm.[79] Size and density of AuNPs were controlled and manipulated by modification

of $HAuCl_4$ concentration during photoreduction; with increasing concentration resulting in an increase in size in lower molar ratios. Enhanced antibacterial activity of ZnO/Au hybrid nanostructures showed distinctive ZnO/Au dependence when introduced to gram-positive *S. aureus* and gram-negative *E. coli* cultures.

Composites made from polymeric nanofibers, such as CS systems, exhibit biocompatible, biodegradable, and favorable antibacterial properties.[80] Yan et al studied the fabrication of AuNRs encapsulated within PVA/CS hybrid nanofibers by electrospinning. The chemical structures of PVA and CS were unchanged after the introduction of AuNRs, signifying viability of the system. The PVA/CS and AuNR/PVA/CS hybrid systems demonstrated low cytotoxicity against human ovarian epithelial cells, indicating biocompatibility of the hybrid system. Researchers have gone further to study systems where utilization of natural cellular membranes in conjunction to gold nanoparticulates has produced nanocomposites with highly tunable physicochemical properties. For example, Gao et al synthesized AuNPs with natural cellular membranes as a new antibacterial biomimetic vaccine strategy. This strategy allows for the manipulation of a desired immune response that may enhance antimicrobial immunity in the host. Bacterial outer membrane vesicles (OMVs) from *E. coli* were collected and coated onto AuNPs. These specialized bacterial–gold nanoparticles (BM-AuNPs) caused rapid maturation and activation of dendritic cells in the lymph nodes after subcutaneous injection into mice. BM-AuNPs elicited specific immunity against the source bacteria in the vaccinated mice. The favorable physicochemical properties of the synthetic AuNPs allow for the manipulation of the size and shape desired for effective antigen presentation to immune cells.[81,82] With similar goals, Ostdiek et al conjugated an active patch of porcine abdominal aortic decellularized tissue to 100 nm AuNPs. The active patch was inserted into the thoracic aorta of six pigs and monitored for 6 months. Gross analysis of the patches within the chest showed minimal scar tissue and lack of aggregation of platelets and a coagulation response. Additionally, constructive remodeling was achieved through elimination of possible immune response elements but maintaining the integrity of the ECM. Histological analysis of images showed a lack of macrophages, signaling no chronic/long-term inflammatory response present 6 months after implantation. Incorporating AuNPs to acellular tissue improves the biocompatibility of the system. In addition to gold being biologically inert and exhibiting antibacterial properties, the presence of these nanoparticles promotes fibroblast cell proliferation and

delayed degradation of acellular tissue. This study showcases a nanocomposite system of decellularized tissue and AuNPs as promising for vascular repair and blood contacting applications. Moreover, the patches were capable of withstanding the aortic pressure within the five surviving pigs while also promoting cell adhesion and proliferation.[83]

Toxicity

Toxic effects of gold nanomaterials from induced production of ROS may lead to a reduction in cell viability. Disruption of the cytoskeleton network may also play a role in the cytotoxicity displayed by AuNPs. Alterations to the actin and tubulin cytoskeleton cause cellular stress, leading to a decrease in cell proliferation and differentiation.[84] Additionally, a comparison between the toxic effects of aggregated and nonaggregated AuNPs suggest that aggregated nanoparticles exhibit less toxic effects.[85] Although AuNPs demonstrate a significant toxic effect against many cell types, effects on dendritic cells were not significant, regardless of increasing gold nanoparticle concentration. Since dendritic cells play an important part in initiating a specific immune response, it is good that AuNPs do not show toxicities among the cells.[86]

The toxicity of gold nanoparticles on the environment, including toxic effects in plants and animals, is currently under investigation. In an in vivo experiment conducted using a mice model, mice were given a weekly injection of 8 mg/kg of AuNPs (ranging in size from 3 to 100 nm). Although nontoxic nanoparticles were administered to the mice, results showed the injections to have lethal effects on the majority of the animals tested. These results are concerning since they are indicative of the lethal effect seemingly nontoxic AuNPs may have on mammals.[87] Another study involving maize elucidates the possible toxic effects gold nanomaterials might impart on feed and food crops. In the study, high concentrations of AuNRs (ie, 350, 5.8, and 14 mg/L) were placed surrounding the roots of maize. The AuNRs were found to accumulate in the roots and leafs of the maize plants and to physically interfere with uptake pathways. The results also showed an inhibition in plant growth and in the nutritional absorption of the plants. Lower concentrations of AuNRs of 4.5×10^{-3}, 0.45, and 2.25 mg/L, during a 10-day exposure, however, showed biocompatibility. The authors suggest that the correlation between the amount of gold nanomaterials with the level of toxicity may be used to increase control over the toxic effects of AuNRs.[88]

Cytotoxic effects of AuNPs against cancer cells are reported to be dependent on the morphology and surface chemistry of the nanoparticles. In a study conducted to

evaluate the anticancer activity of AuNPs, doses of 6.25–100 µg/mL were introduced to human breast cancer cells MCF-7.[89] Results suggest that spherical 25 nm AuNPs show the optimal cytotoxicity to the cancer cells at a concentration of 100 µg/mL. Although the lack of aggregation of the uniformly dispersed, spherical nanoparticles is attributed to the anticancer properties of the synthesized nanoparticles, the exact effect of these morphological properties on cytotoxicity is not understood and should be further investigated.

Summary

Gold nanomaterials display favorable antibacterial properties that can be controlled by selectively fabricating these into the desired size and structural shape. AuNPs are believed to exert antibacterial behavior by causing a change in membrane potential, preventing ATPase, and inhibiting the subunit of ribosome for tRNA. Additionally, increasing the aspect ratio of gold nanostructures allows for utilization of gold's optical properties to work in combination with its bactericidal activity. When fabricated into nanorods, lasers can be used to photothermally reduce the number of viable bacterial cells by ~98%. An increase in the aspect ratio also increases the stability of the produced nanostructure, allowing for proteins or conjugated molecules to attach and suggesting a feasibility for a range of biological applications, including targeted drug delivery and fluorescence identification. For example, when developed into nanoclusters, gold nanostructures can serve as fluorophores due to their photostability and show a largely enhanced fluorescent intensity compared to nongold nanocluster containing controls. Furthermore, these nanoclusters may be synthesized to display different patterns, although further investigations on reliable synthesis techniques are needed. Composites using gold nanomaterials increase the stability of the nanosystems while maintaining the favorable optical and thermal physical properties of the noble metal nanostructures.

Copper nanomaterials

The element copper has been used in ornaments, weaponry, and coinage since early into the 14th century. Copper coinage was originally developed in what is now known as Turkey and Iraq, and soon spread to Spain, Europe, and Sweden. Symbolically, copper was used to mark the beginning of womanhood, consecrate kings, and propitiate ancestors or gods.[90] After the 1850s, copper began its use in electrical wiring. For example, copper was laid across the Atlantic as a telegraph cable in 1866 and used as telephone wire that allowed for communication across larger distances. At the turn of 20th century, copper began to be used in applications within the service activities.[91] Since then, copper has been important in research because of its role in living organisms. A copper deficiency in the human body can lead to anemia and improper fetal development during pregnancy. On a smaller scale, it plays an important role in the transportation of oxygen during the electron transport chain and iron homeostasis. Shortly after reports identified copper as an antibacterial reagent,[92] synthesis techniques for producing nanosized copper were developed.[93] It has been reported that the antibacterial characteristics displayed by copper are a result of cellular damage after contact between released Cu^{2+} ions and bacteria membrane.

This section analyzes different synthesis, antibacterial properties of the structure nanoparticle, comparison of different shapes and sizes, promising composites, and toxicity properties of copper nanomaterial.

Nanoparticles

Copper nanoparticles (CuNPs) are excellent antibacterial reagents due to their heat resistance and chemical stability. This may be attributed to a large surface area to volume ratio, which allows CuNPs to penetrate microbial membranes. CuNPs can be synthesized through several techniques – including chemical reduction, laser ablation, sol–gel processing, and thermal reduction – each resulting in CuNPs displaying varying antibacterial properties.[94]

CuNPs made through different synthesis techniques display varying antibacterial properties. Modifications to classic synthesis techniques, such as the modified polyol method reported by Ramyadevi et al, can favorably affect antibacterial properties of CuNPs. Using the modified polyol method by reduction of copper acetate hydrate showed antimicrobial activity in both bacterial and fungal strains, with different amounts of antibacterial activities observed due to stain.[94] External stimulation, such as exposure to UV radiation during synthesis, may also increase the antibacterial activity of resulting CuNPs. Veerapandian et al[95] synthesized hybrid glucosamine functionalized copper nanoparticles (GlcN-CuNPs) and reported higher in vitro antibacterial activity against two gram-negative and two gram-positive bacteria compared to GlcN-CuNPs not exposed to UV radiation.[95]

Due to copper's antibacterial performances, CuNPs can be used for several applications. As an antibacterial composite in food packaging, CuNPs inhibit bacterial growth. Gram-negative microbial group of *Pseudomonas* spp. is a common strain seen proliferating in processed food and naturally develops a resistance to common antibiotics. CuNPs embedded in a polylactic acid matrix are promising toward

preventing bacteria proliferation of *Pseudomonas* spp.[96] Against urinary tract pathogen, copper oxide nanoparticles (CuONPs) have shown antibacterial performance. *Tabernaemontana divaricata* leaf is an Indian medicinal plant with proven anti-inflammatory and antibacterial properties. Biogenic CuONPs synthesized using *T. divaricata* leaf extract are promising against urinary tract infection.[97]

Investigations into interactions between CuONPs and CuNPs against bacteria suggest that CuNPs inhibit bacterial growth more successfully. Varying concentrations of CuONPs show successful inhibition of bacterial growth, with a greater concentration in CuONPs showing a higher bacterial inhibition. Possible reasons include a more direct interaction between the CuNPs and the bacteria strain, leading to more penetration and rupture of the bacterial membrane. Cellular membrane disruptions can lead to cell enzyme malfunction and eventually cell death.[98] CuNPs display greater antibacterial activity than CuONPs, which may be attributed to a better electron transfer between the bacteria and CuNPs. The slightly negative bacteria and the metallic nanoparticles act as good electron acceptors, both contributing to the electron transfer and rupture of the bacteria membrane. Also, light irradiation can lead to excited electron–holes pairs in CuO, which show that the bacteria's inactivation could be mostly due to a photocatalytic process. Overall, CuNPs still showed a stronger antibacterial performance compared to CuONPs made by sol–gel on thin silica films, which is predicted to be due to a better electron transfer.[99] Poly-L-lysine/reduced graphene oxide/copper nanoparticles (PLL-rGO-CuNPs) hybrid show antibacterial performances for long term. Reduced graphene oxide (rGO) has shown significant antibacterial activity, which may be due to its GNS' sharp edges. The interaction with this material results in cell damage, loss of cell membrane integrity, and RNA leakage.[100]

During copper nanomaterial production, thermal treatment, the size of films, and a number of nanomaterials are factors that may affect antibacterial levels. In regard to CuONPs – silica films, an increase in temperature from 400°C to 600°C and reduction in the thin films reduced the antibacterial activity. The reduction can be due to the thermal reduction of CuONPs to Cu and/or a decrease in copper-based particles on the surface of the films.[99] Applications of nanoparticles include food delivery of drugs, genomics, and cancer cell photothermolysis.[101]

Comparison of shapes and structures

Fabrication of copper nanomaterials of different dimensions demonstrates varying levels of antibacterial performance.

The antibacterial effects exhibited by CuO nanomaterials in the particle (grain like), tube (needle like), and plate structures (Figure 4) were investigated against gram-positive bacteria *Streptococcus iniae* and *Streptococcus parauberis* and gram-negative bacteria *E. coli* and *Vibrio anguillarum*. An increase in the release of copper ions was observed in plate-like CuO nanomaterials compared to the grain-like and needle-like-shaped CuO nanomaterials. Factors that could contribute to the observed antibacterial effects due to dimension and structure of CuO nanomaterials include the amount of surface area for adsorption or desorption and damage leading to tearing of bacterial cells. Such damage occurs by an initial release of copper ions that bind onto the double helix of DNA. The bacterial cell consequently dies, following a biochemical process or from the damage sustained to DNA molecules.[102]

Similarly, the size and dimensions of nanocrystal structures influence the antibacterial effects demonstrated by these nanostructures. Hassan et al observed an increase in bacterial inhibition with an increase in CuO nanocrystal concentration. Factors contributing to the bacterial cell damage include oxidative stress caused by the release of Cu ions and rapid anaerobiosis. The production of ROS from the CuO nanocrystals may begin with the reduction of O_2 and synthesizes of superoxide anion $O_2^{-\bullet}$. These ROS then come into contact with the cellular membrane, generating free radicals that will enter the cell and cause disruption of the cell's internal content. Ultimately, such disruption by ROS causes leakage to the bacterial cell.[103]

Alignment of nanostructures may also affect the antibacterial properties of the resulting nanostructure. For example,
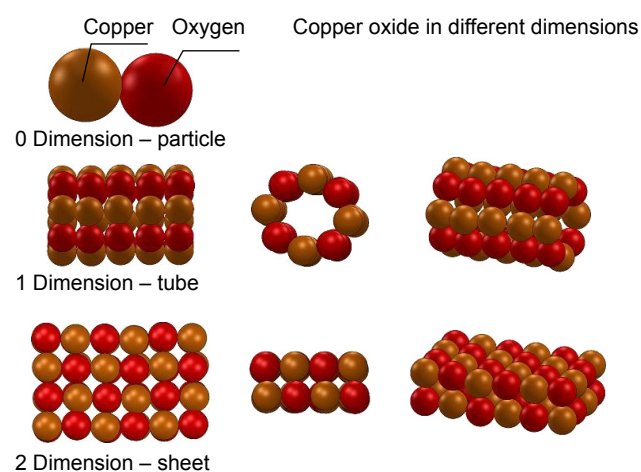

Copper   Oxygen   Copper oxide in different dimensions

0 Dimension – particle

1 Dimension – tube

2 Dimension – sheet

Figure 4 Copper oxide dimensions.
Notes: Different copper oxide dimensions produce different amounts of antibacterial properties due to their ratio of surface area to volume. The simplest structure is a particle, then tube, followed by the sheet.

using the method of template-based electrodeposition, where nanotubular Cu arrays (NT-Cu) are vertically aligned, levels of antibacterial activities are different compared to unaligned NT-Cu. This is attributed to the structure of vertically aligned NT-Cu possessing a high ratio aspect and ultrafine pores in comparison to other structures.[104]

Promising composites

Dissolution of CuNPs from copper nanocomposites results in favorable high antibacterial behavior. For example, TaN-Cu nanocomposite thin films where CuNPs lay on the surface of TaN resulted in a galvanic effect. Because of the galvanic effect, when the CuNPs came into contact with TaN films, there was a higher rate of dissolution. This implies that the particle size and density affect the antibacterial activities. TaN-Cu nanocomposite thin films showed antibacterial properties due to the copper ions that will dissolute. Positively charged copper ions come into contact with negatively charged cell walls of the bacteria, which leads to damage of the cell walls and cell death.[105] Aside from the dissolution of copper particles, production of ROS also causes antibacterial activities. For example, copper iodide nanoparticles can act as an antibacterial agent against gram-negative and gram-positive bacteria, due to ROS production. ROS normally leads to DNA damage, as observed when CuNPs were introduced to bacterial strains of *DH5(alpha)* and *B. subtilis*.[101]

Toxicity

In regard to certain metals such as copper, the toxicity of nanometal oxides is attributable to the soluble metal ions from the material. The solubility of nanometals is an important factor for its toxicity. In comparison of CuONPs that are more soluble than bulk CuO, CuONPs are more toxic. Against algae, CuONPs are toxic at sub milligram/liter concentration, due to the release of copper ions.[106] However, the number of copper ions released by the CuONPs was independent of nanoparticle size. Comparison of bulk and CuNPs has shown a greater toxicity for CuNPs due to a larger surface area-to-volume and increased reactivity, with smaller CuNPs displaying higher toxicity compared to their larger counterparts.[107] Size-dependent toxic effects of CuNPs allow for the control of toxicity. Copper nanoparticles are seen to be more toxic than larger copper nanoparticles in zebra fish. However, copper submicron-sized particles of 400 nm were more toxic than copper nanoparticles.[108]

There is evidence of the toxic effects of CuNPs in the environment. The activity of the dissolved copper ions in the presence of micrometer-sized CuO (Figure 5A) was less than in the presence of CuONP (Figure 5B).[109] Additional studies suggest evidence of concentrations of Cu^{2+} in soil organisms, such as earthworms[110] and white worms[111] from CuNPs. Even among plants, a greater root toxicity is present with small CuNPs compared to larger sizes. Metal oxide particles show
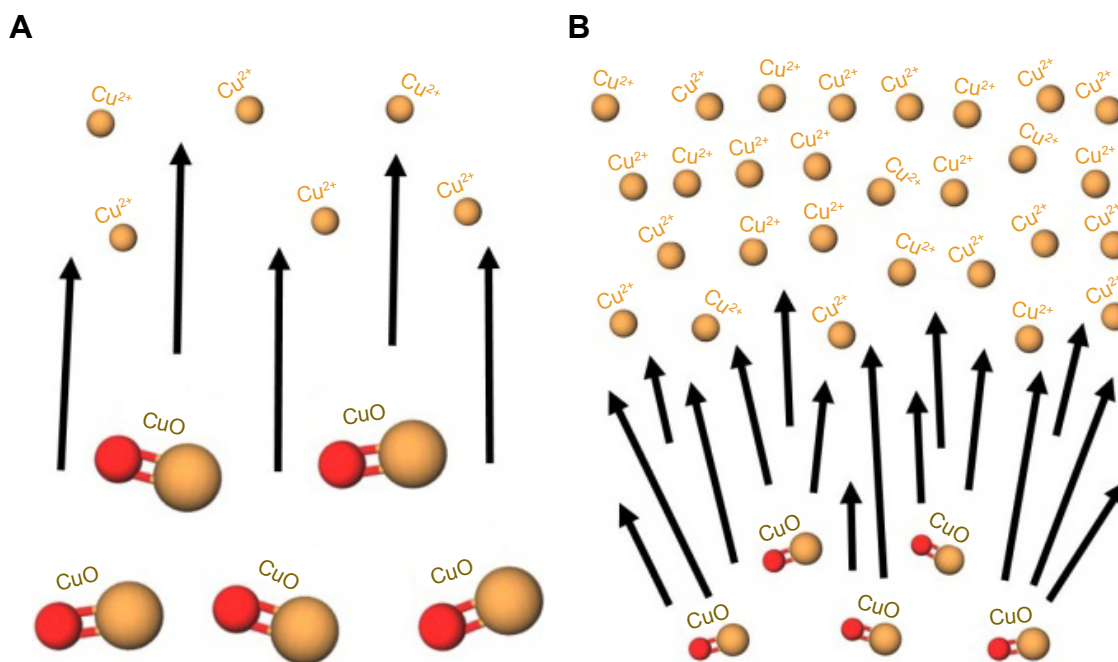

Figure 5 Comparison of released toxicity from microsized vs nanosized copper oxide.
Notes: Due to the differences in the ratio of surface area to volume, toxicity levels are dependent on the size of copper oxide. Smaller structures such as (**B**) nanosized copper oxide compared to (**A**) microsized produce larger amounts of copper ions. The amount of toxicity is dependent on a number of copper ions released.

increased toxicity correlating with smaller sized particles.[112] Among cells, high concentrations of CuNPs have been shown to lower cell mortality. However, the mechanism of action for the toxic effects exhibited by CuNPs can occur through alternative methods, including the dissolution of ions from copper.[113] Against algae cells, neither chemical analysis nor biological testing has confirmed cupric ions in toxic amount. Since death of algal cells is observed with low amounts of Cu^{2+}, the dissolution of ions is believed to be a partial factor in CuNP toxicity.[114]

Summary

Copper on a nanoscale has been observed to be a promising antibacterial agent. Based on the dimension of the nanomaterial, different levels of antibacterial performances will be exhibited. In a comparison of grain-like, needle-like, and plate-like shapes, the structure of plate-like copper nanomaterials showed the most promising antibacterial activities due to factors such as a large surface area and release of copper ions. Certain composite with copper also shows a significant amount of antibacterial activities such as CuO nanomaterial and CuNPs embedded in polylactic acid. Methods for composing the nanomaterials include using laser ablation, sol–gel processing, coprecipitation, chemical reduction, microwave irradiation, and others. Antibacterial studies show that copper nanoparticles produce ROS against bacteria, leading to DNA damage. Because of the significant antibacterial performances of copper, the element has been used for applications, including drug delivery, antibacterial composite food packaging, and water filter treatment.

Titanium nanomaterials

Application of titanium in the biomedical field started in the early 1940s when laboratory animals were used to test titanium implants. In 1959, two American researchers explored the idea of using cast titanium to make implant posts.[115] Soon after, titanium became popular in the field of dentistry, which led to the first titanium cast for dental prostheses in 1977.[115] Since then, use of orthopedic implants has steadily increased for both internal and external fixation and prostheses. However, despite sterilization techniques, infections remain the main cause of implant failure. Because of the success of elemental titanium in the dentistry and orthopedic fields, titanium nanomaterials are now being investigated as antibacterial reagents. This section will examine the effect of different synthesis techniques of titanium nanomaterials on their exhibited antibacterial properties. We will also discuss the unique crystal structures of titanium and their antibacterial properties due to photocatalytic activities.

Synthesis

Synthesis of TiO_2 nanostructured layers in organic viscous electrolytes has shown antibacterial behaviors. Electrochemical anodization method obtains metal oxides at a nanoscale, while also being easy and cost-effective. Control of electrochemical conditions affects morphology, making it easy to make nanoporous self-organized nanotubular TiO_2.[116] Other methods have been seen promising such as nano-TiO_2 synthesized by liquid hydrolysis under light which has shown promising antibacterial activities. The method of liquid hydrolysis is cheap and simple compared to the sol–gel method, which is expensive, and precipitation method, which is cheap, but is time-consuming and produces low purity products.[117]

Control of titanium surfaces such as crystallinity, nanotube size, and chemistry affects responses toward bacteria strains, such as *S. epidermidis* and *S. aureus*. Additionally, levels of antibacterial performances are influenced by the diameter of titanium nanotubes, with TiNTs of an 80 nm diameter showing the most antibacterial activity. Therefore, controlled anodized Ti nanotube diameter size formation is important toward promising amounts of antibacterial performances, which can be applied to improved antimicrobial behavior and tissue growth properties.[118] Aside from diameters, under a solar-simulated light, double-doped TiO_2 nanoparticles (TiO_2NPs) have acted as a visible light-active antibacterial agent. Using sol–gel method, double-doped TiO_2NPs exhibited the most antibacterial activity in comparison to single-doped TiO_2NPs under visible light irradiation. This can be due to the attribution of two different electronic states that act as electron traps in the TiO_2, which is responsible for a narrower band gap and therefore shifts the optical response from the region of UV into visible light. Due to this significant antibacterial performance of double-doped instead of single-doped TiO_2NPs, this extra step can lead to further biomedical application for a decrease in infection and bacterial count.[119]

Crystal structures

The three crystal structures of nano-TiO_2 include anatase (Figure 6A), brukide (Figure 6B), and rutile (Figure 6C). Among the three crystal structures, anatase has the most antibacterial activity. Under temperatures of 600°C–700°C, anatase structures produce •OH in a photocatalytic reaction, puncturing the bacterial wall and killing the bacteria.[117]
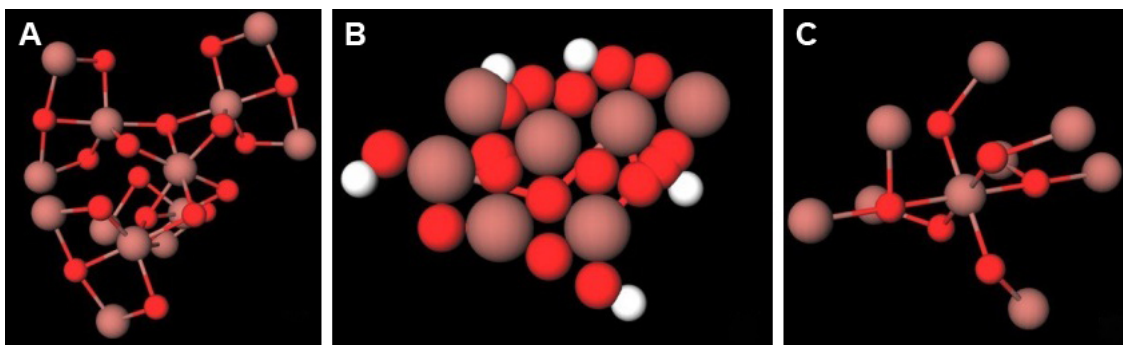
Figure 6 Titanium dioxide crystal structures.
Notes: The three crystal structures of titanium dioxide show different amounts of antibacterial properties due to its production of •OH in its photocatalytic reaction, which causes cellular damage. (**A**) Anatase compared to other crystal structures such as (**B**) Brukide and (**C**) Rutile shows the most antibacterial activity.

The presence of light is important to the antibacterial properties exhibited by titanium. To examine the effects of light on the antibacterial properties of TiO_2 nanomaterials, antibacterial activity was compared at varying concentrations. Although an increase in nano-TiO_2 concentration may lead to an increase in bactericidal performance, antibacterial activity was reported to decrease at a concentration of 0.8 g/L. This observation suggests that with an increase in nano-TiO_2 concentration, there is also an increase of contact with the bacteria. When the concentration is larger than 0.8 g/L, however, the nano-TiO_2 has less contact with light, resulting in a decrease in photocatalytic activity, as well as its antibacterial effects. It is important to note that an increase in temperature changes the anatase structure into rutile, deteriorating the photocatalytic activity.

Photocatalytic effects

The antibacterial properties of nanoscaled titanium are due to its photocatalytic properties. These properties completely decompose contaminants and kill microbes under UV light irradiation. The positively charged nanomaterials affect bacterial growth and viability by changing the permeability when the nanomaterial interacts with negatively charged lipid membranes of bacteria, blocking nutrient intake. Bacteria is also killed by high ROS produced by the photocatalytic process of TiO_2, such as hydroxyl radical, superoxide, or hydrogen peroxide.[120] As a photocatalyst, titanium dioxide both oxidizes organic contaminants and damages microbial cells. Significant antibacterial performances are light activated from the nanomaterial of TiO_2 nanoparticle-based film.[121] Another example of its photocatalytic properties is from nanostructured titania (nTiO_2). Its antibacterial activities are due to the photocatalytic effect of nTiO_2/PU nanocomposite film when irradiated under solar illumination (Figure 7A). The nTiO_2/PU composite produces OH•

radicals that damage bacterial cells because of a decrease in coenzyme A (CoA), a mediator of electron transport between the cell and nTiO_2. Without solar illumination, neither pure PU film nor nTiO_2/PU nanocomposite film demonstrates any antibacterial activities against the *E. coli* (Figure 7B). A control experiment using the pure PU film under solar illumination for 50 minutes showed only a slight activity in antibacterial performances. Using the "grafting from" polymerization method to make nTiO_2/PU composite coatings, under solar irradiation almost all the bacteria were killed within 50 minutes due to nTiO_2's photocatalytic effect. This antibacterial observation of nTiO_2/PU composite coatings has significance for outdoor application to prevent undesired bacteria from growing.[122] Factors that contributed to the differences in amount of antibacterial activities include small average particle size, surface chemical state, high surface area, large band gap, structure and size distribution of pore.[123]

Toxicity

Titanium nanomaterials exhibit lower toxicity than other nanometal oxides. As nanoparticles, nano-ZnO was found to be the most toxic at sub milligram/liter concentrations, followed by nano-CuO and finally nano-TiO_2. The toxic effects of nanosized TiO_2 are reported to result from the entrapment of cells, rather than from the dissolution of metal ions, as is reported in a study involving algae *Pseudokirchneriella subcapitata*.[106] Even though toxicity of nanotitanium can be useful against unwanted bacterial growth, toxicity level can be lowered to use the material for implants within the body without compromising the viability of cells. Manipulation of shape and length changes toxicity levels, making the material a better bactericidal reagent that will not harm the body. The composite nanotrititanate has the same bactericidal effects, despite changes in shape and length when compared
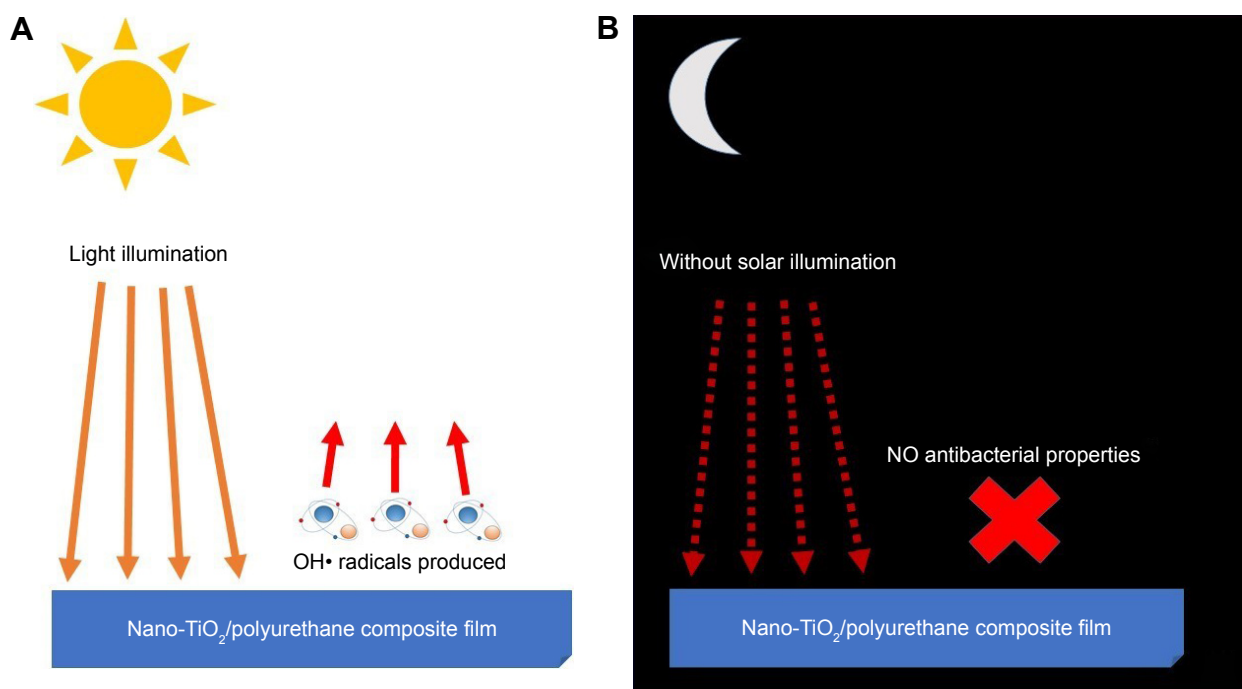
Figure 7 Antibacterial activities due to titanium dioxide illumination. Nanotitanium dioxide produces antibacterial properties when (**A**) light illumination is present due to released hydroxyl radicals which cause cellular damage. (**B**) Without illumination of any kind, no antibacterial properties are produced.

with unchanged dimensions. However, changes in dimensions from nanotubes to nanoplates were able to lower the toxicity, and study found that titanium nanoplates showed the lowest toxicity to HEp-2 cells.[124]

Nano-TiO_2 is reported to be toxic to many aquatic organisms such as fish, cyanobacteria, and algae. However, in relevance to benthic microbial communities which are key components of freshwater habitats, not much is known about the results of added nano-TiO_2. Benthic bacterial communities contribute to the ecosystem process such as with their primary production, decomposition of organic material, nutrient cycling, and pollutants bioremediation. In a study of its effects on artificial stream communities, a single addition of nano-TiO_2 with a concentration of 1 mg/L demonstrates a rapid decrease in bacteria activity, with a return of bacterial abundance at control level within a period of 3 weeks. The significant decrease in bacterial cell count due to an addition of nano-TiO_2 in artificial streams for a short period of time demonstrates the antibacterial performance of nano-TiO_2. This study is a good model of the effects nano-TiO_2 could have on bacterial cells in wastewater.[125]

Toxicity observations due to nanotitanium have been tested on live animals. Single low doses of nanoanatase TiO_2 particles in ICR mice's abdominal cavity, once a day for 14 days, have shown little differences in comparison to control mice. Higher doses, however, showed damages seen in the kidney, liver, myocardium, blood sugar level, and lipids level.[126] However, in regard to rat's tissues, single injections of 10% TiO_2 concentration in distilled water or silicone demonstrate no significant changes or toxic effects. Health and behavior of the rats were normal while no detectable levels of TiO_2 were found in the blood cells, lymph nodes, plasma, or brain. These low doses of TiO_2NPs are safe due to no detectable organ toxicity, long-term changes within the rat's bodies, and no changes in inflammatory response.[127] In other animals, low toxicity exposures of 10–40 μg/mL for less than 60 days have also been studied, such as the Chinese hamster. Ovary cells of Chinese hamster have no cyto- or genotoxic effects, adaptable to chronic exposures, and detoxify excess ROS.[128] Among other animals such as the zebra fish, there have been seen the amount of toxicity from nano-TiO_2, which are due to its generated •OH radicals. In comparison to bulk particles suspensions of 128–949 nm diameters, nanoparticles suspensions of 20–70 nm diameters showed higher levels of toxicity even with similar size distributions. Toxicity mechanism comes from oxidative effects or lipid peroxidation from generated •OH radicals.[129] Depending on the location of injection, doses, and type of animal, nanotitanium shows different toxicity levels.

An increase in the usage of titanium in everyday environments has led to pressure on legislators to have tighter

environmental standards among industries to help inform the public on its safety.[130,131]

Summary

Due to the antibacterial activities of titanium as a nanomaterial, it is a promising material in the industries for a reduction in bacteria growth and medical field for the decrease in infections. Antibacterial activities by titanium are due to the photocatalytic reaction when under solar illumination or exposure to light, which produces •OH that would puncture the bacterial cell, leading to the death of bacteria. In the medical field, titanium has been used because of its mechanical properties and biocompatibility. The combination of antibiotics and nanotitanium is promising materials in regard to decreasing infections and bacterial growth in orthopedic implants and other medical applications.[131] In industries, titanium on a nanoscale is used for its antibacterial activities such as long storage of wool which would have bacterial growth due to moist environment.[132] Titanium as a nanomaterial is promising in both the medical field and industries due to its antibacterial performances. Toxicity studies are important to daily life such as sunscreen and cosmetics products, toxicity studies are important to prevent biological harms to the body. Different levels of toxicities are seen due to certain locations of injections or exposures, different amounts, and type of animal tested on.

Zinc nanomaterials

Zinc (Zn) and its alloys have been used extensively in biomedical applications due to their abundance and low toxicity.[133] These materials have shown promise in biosensing, imaging, and drug delivery due to their electrical, optical, and photocatalytic properties. Recently, the incorporation of Zn into biomedical devices and bioceramics has increased. One reason for the addition of zinc into biomedical devices is its importance in several biological functions including DNA synthesis and nucleic acid metabolism and because it is known to be an important trace element in human bone.[134] Additionally, Zn-containing nanomaterials have been reported to exhibit excellent antibacterial qualities. Although zinc has been successfully incorporated into materials using techniques, such as plasma electrolytic oxidation, the most commonly studied zinc-containing nanomaterial is zinc oxide nanoparticles.

Zinc oxide (ZnO) nanomaterials are unique in possessing semiconducting and piezoelectric properties, in addition to exhibiting biocompatible and biodegradable features. Although the mechanism through which more complex

Zn-containing nanomaterials produce antibacterial effects remains largely unknown, the most supported toxicity mechanisms of zinc oxide nanoparticles (ZnONPs) are thought to result from ROS generation and Zn^{2+} release. ROS, such as superoxide anion, hydrogen peroxide, and hydroxide, can damage lipids and proteins once inside the bacterial cell membrane, while the release of Zn^{2+} from ZnONPs can disrupt important metabolic pathways.[135] Because synthesis techniques and resulting morphology of ZnO nanomaterials can impact their antibacterial properties, current research efforts are focused equally on developing novel synthesis methods and application.

Advancements in synthesizing techniques of ZnONPs include using precursors (coordination polymers and biological extracts). By using ultrasound radiation, Fard et al[136] synthesized ZnONPs from two zinc(II) coordination polymers. The simple and controlled process resulted in ZnONPs without significant impurities and good crystallinity, but with nonhomogeneity in nanoparticle size. Meanwhile, Thatoi et al synthesized ZnONPs using mangrove plant extracts.[137] The nanoparticles produced from the *Sonneratia apetala* mangrove plant exhibited relatively higher antibacterial capabilities, as well as anti-inflammatory properties.

Investigations into discovering additional applications for ZnONPs have progressed significantly in dental prosthetics and anticancer treatments. Ciereh et al suggest ZnONP-modified acrylic glass as a promising material for dentures. By combining ZnONPs and acrylic glass, or polymethylmethacrylate (PMMA), the group observed inhibition of fungal biofilm formation that could treat conditions such as denture stomatitis.[138] After several reports of ZnONPs exhibiting selective toxicity toward cancer cells surfaced, Akhtar et al[139] report the selective killing of cancer cells to induce apoptosis after exposure to ZnONPs. The group suggests induced apoptosis of human cancer cells to result from the production of tumor suppressor protein p53.

Magnesium nanomaterials

Magnesium (Mg), similarly to Zn, is an abundant metal that is biocompatible, biodegradable, and capable of inhibiting bacterial growth.[140] Due to its significant presence in bone, and its suspected role in increasing osteoblast activity, it is now being investigated for orthopedic implants.[141] Nano-sized Mg and its alloys have been reported to exhibit anti-inflammatory and antioxidant activity.[142] A few common mechanisms, including ROS generation, have been proposed

to explain the antibacterial mechanism of magnesium oxide nanoparticles (MgONPs). However, the exact mechanisms remain unknown. Recent studies into Mg-containing nanomaterials include MgONPs for orthopedic implant coating and fabrication of MgO-containing nanocomposites as scaffolds for bone tissue regeneration. Wetteland et al[143] report possible guidelines for MgONP-coated biomaterial design. The study showed a significant increase in bone marrow-derived mesenchymal stem cells and antibacterial activity when exposed to 200 µg/mL of MgONPs. By adding MgONPs to polycaprolactone hydroxyapatite (PCL/Hap) composites, Roh et al reports enhanced adhesion, proliferation, and differentiation of preosteoblasts.[144] These results propose possible improvements to 3D-scaffolds for encouraging bone tissue regeneration.

Conclusion and future directions

Reports of antibacterial activity in nanomaterials have stemmed from observed biophysical interactions occurring between nanoparticles and bacteria, including cellular uptake and nanoparticle aggregation, leading to membrane damage and toxicity.[14] In particular, metallic nanomaterials (such as silver, gold, copper, and titanium) exhibit favorable physicochemical characteristics resulting in significant levels of antibacterial activity. This review has focused on the recent advancements made in metallic nanomaterials as antibacterial agents, with a focus on their toxicity and antibacterial activity based on the structure, dimension, and size of nanomaterial. Furthermore, it has also discussed the benefits of using nanomaterials of silver, gold, copper, titanium, zinc, and magnesium metals in biomedical applications. A summary of the antibacterial properties exhibited by each nanomaterial is shown in Table 1.

However, with such advances comes new needed regulations and restrictions. Although most manufactured nanomaterials are dispersed readily when in contact with water,[144] there are some exceptions. For example, metallic nanotubes are not dispersed in water, even with the use of physical methods. Due to the nondispersing properties, potential harmful nanomaterials will contaminate resources that could interact with humans, animals, and the environment. Therefore, future prospects should focus toward establishing new regulations that should result from thorough chemical and toxicological characterization of fabricated nanomaterials. Furthermore, ecotoxicity tests should be set into place when considering any applications directly involving humans and other living organisms. As studies move from in vitro to in vivo experiments, regulations need to be set on

Table 1 Antibacterial properties of nanomaterial

	Nanostructure	Material	Dimension	Synthesis	Results	References
1	Nanoparticles	Silver	100 nm	Biosynthesis using *Ceropegia thwaitesii*	Silver nanoparticles were synthesized from *C. thwaitesii* leaf extract which exhibited significant antibacterial activity against bacterial strains	32
2	Nanoparticles	Silver	136±10.09 nm	*Origanum vulgare*-mediated biosynthesis	Dose-dependent response against human lung cancer cells and inhibition of human pathogens	33
3	Nanoparticles	Silver	40 and 50 nm	Green synthesis using bark extract	Antibacterial properties against human nosocomial strains and dose-dependent toxicity against osteosarcoma MG-63 cell line	34
4	Nanoparticles	Silver	6 to 13 nm	Extracellular synthesis using psychrophilic bacteria	Synthesis of antibacterial AgNPs from psychrophilic bacteria is dependent on temperature, pH, and species of the bacteria	35
5	Nanoparticles	Silver	12 and 65 nm	Synthesis using *Bacillus flexus* strain	Antibacterial properties against multidrug-resistant microorganisms	36
6	Nanoparticles	Silver	10 nm	Green synthesis using *Ochrobactrum rhizosphaerae*	Antibacterial properties against *V. cholera*	37
7	Nanoparticles	Silver, silver chloride	2 to 10 nm	Yeast-derived biosynthesis	Strong growth inhibition against gram-positive and gram-negative bacteria	38
8	Nanoparticles	Silver	–	Photomediated reaction in an aqueous environment	Strong antibacterial properties and concentration-dependent cytotoxicity levels against human keratinocyte cell line and human peripheral blood mononuclear cells	39
9	Nanoparticles	Silver	7±1 and 6±1 nm	Precipitation	Sulfur-containing carbon dots produced AgNPs with better stability and excellent antibacterial activity	41

(*Continued*)

Table 1 (*Continued*)

	Nanostructure	Material	Dimension	Synthesis	Results	References
10	Nanocomposite	Silver	<25 nm	One-pot synthesis	Good antibacterial effects against *E. coli* and *S. aureus*	42
11	Nanofibers	Silver	200 to 600 nm	Electrospinning	Random coil silk structure demonstrated a high release of silver ions and good inhibition against bacterial strains	43
12	Nanofibers	Silver, zinc oxide	200 to 300 nm	Electrospinning	Nanofibers of large surface to volume ratio, with potent bactericidal effects and noncytotoxic behavior	45
13	Nanofibers mats	Silver	150 nm	Electrospinning	Release of silver lead to the reduction and slowing of growth in aerobic bacteria	21
14	Nanoprisms	Silver	30 to 50 nm	Two-step capping	Crystalline facets of higher reactivity that release a higher rate of ions from their tips and edges	46
15	Nanoparticles	Silver	50 nm	Purchased	Size-dependent cytotoxicity levels against osteoblasts and osteoclasts by releasing silver ions	47
16	Nanoparticles	Silver	10, 40, 50, 75 nm	Purchased	Cytotoxicity and DNA damage from AgNPs may occur through independent mechanisms	48
17	Nanoparticles	Silver	15, 50, and 100 nm	–	15 nm silver nanoparticles exhibited the highest induced hemolysis and membrane damage (compared to 50 and 100 nm) from direct interaction with RBCs, which caused oxidative stress and membrane injury	54
18	Nanoparticles	Silver	<5 nm	Purchased	Interactions of AgNPs against human mesenchymal stem cells and osteoblasts showed an increase in cellular stress, high cytotoxicity, and dose- and time-dependent inhibition of proliferation	55
19	Nanoparticles	Gold	–	Reduction of tetrachloroauric acid by sodium borohydride	Antibacterial properties by collapsing the membrane potential (inhibiting ATPase activity) and inhibiting ribosomal subunits from binding tRNA	69
20	Nanoparticles	Gold	27 nm	Standard wet chemical procedure	Laser photoexcitation of AuNPs lowered the concentration of nanoparticles needed to increase necrotic rates against MRSA	67
21	Nanoparticles	Gold	22 to 35 nm	Green synthesis using *Salicornia brachiata* plant extract	Superior antibacterial activity against pathogenic bacteria when used in combination with the antibiotic ofloxacin	70
22	Nanoparticles	Gold	6 to 71 nm	Green synthesis from *Euphorbia hirta* leaf extract	Low concentrations showed significant antibacterial properties	71
23	Nanorods	Gold	19±1×49±2 nm	Seeding method	High concentrations of laser-irradiated AuNRs exhibited rapid photoheating and subsequent antibacterial activity	74
24	Nanorods	Gold	15±50 nm	Purchased	Anti-protein A antibody and AuNRs conjugates exhibited an 82% reduction in MRSA cell viability	75
25	Nanoclusters	Gold	<3 nm	–	DNA-templated AuNCs can be used as a fluorescence-enhanced reagent used to detect tetracycline in milk samples and human urine	77
26	Nanocomposite	Gold, zinc oxide	<3 nm	Photoreduction	AuNPs deposited on ZnO nanoparticles greatly enhance the photocatalytic and antibacterial activity of ZnO	79
27	Nanorods	Gold	<100 nm	Electrospinning	AuNRs containing PVA/CS hybrid nanofibers inhibit proliferation of ovary cancer cells	80
28	Nanoparticles	Gold	30 nm	–	Bacterial outer membrane vesicles coated on AuNPs generated antibody response and maturation of dendritic cells in an in vivo mice model	82
29	Nanoparticles	Gold	2 nm	Two phase liquid–liquid system	AuNPs functionalized to selectively target MRSA, which displayed low toxicity against mammalian cells	81

#	Type	Material	Synthesis	Size	Description	Ref
30	Nanocomposite	Gold	—	100 nm	A patch of AuNPs embedded in decellularized tissue showed a minimal inflammatory response and smooth muscle and endothelial cell regeneration	83
31	Nanoparticles	Gold, silver	Green synthesis using resveratrol as a reducing agent	8 to 21 nm	Resveratrol nanocarriers with AuNPs and AgNPs were observed to have a higher antibacterial activity than resveratrol alone	78
32	Nanoparticles	Gold	Synthesis in organic solvent	4 nm	High concentrations caused a reduction in cell viability, proliferation, and differentiation from production of ROS	84
33	Nanoparticles	Gold	Synthesis using hydroquinone-mediated growth of 16 nm seeds	30, 45, and 100 nm	Aggregated nanoparticles exhibited a 25% decrease in cellular uptake compared to single or monodispersed nanoparticles	85
34	Nanoparticles	Gold	Synthesis in aqueous medium	10 nm	AuNPs accumulated inside dendritic cell compartments dedicated to antigen processing	86
35	Nanoparticles	Gold	Seeding method	3, 5, 8, 12, 17, 37, 50, and 100 nm	AuNPs between 8 and 36 nm caused an increase in liver Kupffer cells, loss of lung structural integrity, diffusion of white pulp in the spleen, and severe sickness in mice	87
36	Nanorods, nanowires	Gold	—	10±34, 20±75, and 40±96 nm	High concentrations caused low water uptake, slow growth, and inhibition of nutritional uptakes in maize plants	88
37	Nanosheets, nanoparticles	Silver	Individually purchased materials mixed and centrifuged together	15 nm	Ag-polydopamine-graphene nanosheets showed high antibacterial properties against gram-negative and gram-positive bacteria	22
38	Nanoparticles	Silver	Purchased	10, 20, and 40 nm	Antibacterial properties are very dependent on physicochemical properties of the nanoparticles, especially surface characteristics	24
39	Nanoparticles	Silver, gold, nickel, silicon	—	20 to 30 nm	ROS generation and metal ion release significantly enhance the antibacterial activity of uncoated silver, gold, nickel, and silicon nanoparticles	25
40	Nanoparticles	Silver	Precipitation	8.6 to 1.2 nm	AgNPs on glass surfaces produced high antimicrobial behavior within 2 hours	26
41	Nanoparticles	Copper	Purchased	93±8 nm	UV irradiation of glucosamine functionalized CuNPs enhanced the nanoparticles' antibacterial properties	95
42	Nanoparticles	Copper	Modified polyol method	–	CuNPs exhibit more inhibitory properties against bacterial strains than fungus	94
43	Nanotubes	Copper	Template-based electrodeposition	20 to 250 nm	Vertically aligned nanotubular copper arrays reduced 99.99% of S. aureus population after 6 hours but showed less reduction of other bacteria	104
44	Nanoparticles	Copper, copper oxide	Sol-gel	20 nm	CuNPs accumulated on silica thin films were more toxic to bacteria than CuONPs when prepared in dark conditions	99
45	Nanocomposite	Copper	Laser ablation	36±9 nm	Nanosized copper embedded in polylactic acid showed ion release data matching bioactivity tests against Pseudomonas spp	96
46	Nanoparticles	Copper oxide	Green chemistry using Tabernaemontana divaricata leaf extract	48±4 nm	CuONPs synthesized from Tabernaemontana divaricata leaves exhibited inhibition of urinary tract pathogen	97
47	Nanoparticles	Copper oxide	Thermal decomposition	15 to 30 nm	Produced free radicals leading to antibacterial activity against E. coli and P. aeruginosa	98
48	Nanoparticles	Copper	Purchased	10 to 50 nm	CuNPs in poly-L-lysine-modified reduced graphene oxide produced long-term antibacterial activities and high water solubility	100

(Continued)

Table I (*Continued*)

	Nanostructure	Material	Dimension	Synthesis	Results	References
49	Nanoparticles, nanotubes, nanoplates	Copper oxide	–	Wet chemical and hydrothermal	Nanoplates showed the most antibacterial activity when compared to nanoparticles and nanotubes, suggesting that antibacterial activities are shape dependent	102
50	Nanocrystals	Copper oxide	6 nm	Wet chemical	CuO nanocrystals led to bactericidal effects by causing irreversible damage to the cell envelope	103
51	Nanocomposite	Copper	50 nm	Cosputtering and rapid thermal annealing	CuNPs showed a higher dissolving rate into TaN films due to galvanic effects, leading to faster antibacterial performance	105
52	Nanoparticles	Copper iodide	8 nm	Reduction step of the coprecipitation	Copper iodide nanoparticles exhibited antibacterial activity due to production of ROS, which led to DNA damage against gram-negative and gram-positive bacteria	101
53	Nanoparticles	Copper, silver	<50 and <100 nm	Coprecipitation	Phytotoxicity of nanosized material is higher when compared to bulk material	107
54	Nanoparticles	Copper	25, 50, and 100 nm	Purchased	Toxic effects on zebra fish embryos are size dependent, with a smaller size showing increased toxicity	108
55	Nanoparticles	Copper oxide, copper (II) nitrate	25 to 50 nm	Purchased	Nanosized CuO exhibited more Cu^{2+} activity than microsized CuO, but less activity than CuO salt	109
56	Nanoparticles	Copper oxide, Cu^{2+}	–	–	Cu^{2+} causes the most toxicity by oxidative stress on *Chlorella* sp, followed by nano- and micro-CuO	114
57	Nanotubes	Titanium dioxide	2 nm	Anodization in electrolytes	TiO_2 nanotubes layered on a titanium substrate exhibited increased corrosion stability and antibacterial activities against *E. coli*	116
58	Nanoparticles	Titanium dioxide	–	Liquid hydrolysis	Nano-TiO_2 exhibits the most antibacterial activity under 600°C–700°C when it contains the highest amount of anatase	117
59	Nanoparticles	Titanium dioxide	21 nm	Purchased	Wool treated with tetracarboxylic acid and nano-TiO_2 displayed higher antibacterial activity	132
60	Nanoparticles	Titanium dioxide	–	Donated	Single additions of nano-TiO_2 in artificial streams showed short-term toxicity effects in sediment-associated aquatic bacterial communities	125
61	Nanohybrid	Titanium dioxide	–	Chemical precipitation	100% antibacterial activity against *E. coli* within 24 hours and high photocatalytic activity	120
62	Nanoparticles	Titanium dioxide	<10 nm	Sol–gel	Exposure to material led to cellular membrane damage due to decomposition of membrane fatty acids from photocatalysis	121
63	Nanospheres	Titanium dioxide	25 nm	Inert atmosphere (argon gas)	Within 1 hour, solar-irradiated composite coatings killed 99% of gram-negative bacteria *E. coli*	122
64	Nanoparticles	Silver, nitrogen	–	Sol–gel	Silver and nitrogen doping of nanoparticles enhanced the photocatalytic and antibacterial activity of the nanoparticles when exposed to visible light irradiation	119
65	Nanotubes	Titanium	20, 40, 60, or 80 nm	Convectional titanium	Titanium nanotubes of 80 nm diameter produced the most antibacterial activity compared to 20, 40, and 60 nm	118
66	Nanoparticles	Copper oxide, titanium dioxide, zinc oxide	25 to 70 nm	Purchased	ZnO nanoparticles were observed to be the most toxic, followed by CuO nanoparticles and TiO_2NPs	106
67	Nanotubes, nanoplate	Titanium dioxide	250 to 300 nm	Hydrothermal	Nanotrititanate showed higher bactericidal capabilities compared to nano-TiO_2	124

68	Nanoparticles	Titanium dioxide	5 nm	Hydrolysis of titanium tetrabutoxide	In mice, high amounts caused damaged liver, myocardium, and kidney and disturbed blood sugar and lipids	126
69	Nanoparticles	Titanium dioxide	<100 nm	Ultrasonication and vigorous mixing with a blender	Low doses could be safely used due to no changes in enzymes and cytokines and no signs of organ toxicity and inflammatory response	127
70	Nanoparticles	Titanium dioxide, zinc oxide	1 to 100 nm	Purchased	Titanium dioxide nanoparticles produced •OH radicals that caused oxidative damage on cell membranes while zinc oxide released metal ions	129
71	Nanoparticles	Titanium dioxide	<25 nm	Purchased	Nanomaterials did not cause genetic mutation nor exhibited cytotoxic or genotoxic to long-term exposed cells	128
72	Nanoparticles	Zinc oxide	25 to 40 nm	Green synthesis using plant extract	Size controlled, morphology controlled, inexpensive, and toxic contaminant-free nanoparticles	133
73	Nanoparticles	Zinc oxide	20 nm	–	Six weeks of exposure to zinc oxide nanoparticles showed homeostasis disturbance in fish due to affected immune and coagulation systems	135
74	Nanoparticles	Zinc(II) oxide	–	Sonochemical	Successful synthesization of zinc(II) oxide from two new zinc(II) coordination polymers compounds	136
75	Nanoparticles	Silver, zinc oxide	–	Photomediated green synthesis	Silver and zinc oxide nanoparticles produce enhanced biological properties such as antibacterial, antioxidant, and anti-inflammatory properties	137
76	Nanoparticles	Zinc oxide	30 nm	Microwave-assisted hydrothermal synthesis	Increasing concentration of nanosized zinc oxide showed increased antifungal activity due to increased anti-oxidative stress among microorganism cells	138
77	Nanoparticles	Zinc oxide	–	–	Among human cancer cells, zinc oxide nanoparticles causes selectively induced apoptosis from produced ROS	139
78	Nanoparticles	Magnesium fluoride	30 nm	Microwave-based synthesis method	Magnesium fluoride penetrates into bacterial pathogens resulting in antibiofilm activity such as membrane potential disruption, DNA binding, and increased lipid peroxidation	141
79	Nanoparticles	Magnesium oxide	44 nm	Plant-mediated synthesis using calcination	Magnesium oxide nanoparticles exhibit 65% inhibition activity and potential antioxidant activity due to bioactive components from plant extract	142
80	Nanoparticles	Magnesium oxide	23±5 nm	–	Magnesium oxide nanoparticles caused dose-dependent bacterial adhesion, due to direct contact with cell wall and membrane damage	143
81	Nanoparticles	Magnesium oxide	<50 nm	Purchased	Addition of magnesium nanoparticles and hydroxyapatite in three-dimensional composite scaffolds of polycaprolactone caused initial adhesion, differentiation, and proliferation of MC3T3-E1 cells	144

Abbreviations: *V. cholera*, *Vibrio cholerae*; AgNP, silver nanoparticle; *E. coli*, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; RBC, red blood cell; AuNP, gold nanoparticle; MRSA, methicillin-resistant *Staphylococcus aureus*; PVA/CS, polyvinyl alcohol/chitosan; ROS, reactive oxygen species; *P. aeruginosa*, *Pseudomonas aeruginosa*.

the dosage of nanomaterials inserted into living organisms to mitigate extreme toxicity effects on the body leading to biological damages. Many studies on the antibacterial effects of the aforementioned nanomaterials suggest that manipulating the physicochemical properties of the nanomaterials affects their resulting antibacterial activity. However, there is a large gap on the information on the exact mechanisms leading to these changes in antibacterial activity. Future studies should focus on investigating the effects of morphology and surface chemistry, especially for materials other than nanosilver.

Acknowledgments

Disclosure

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