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Antimicrobial Activity of Metal and Metal-Oxide Based Nanoparticles

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With an increase in antibiotic resistance, a growing interest in developing new antimicrobial agents has gained popularity. Metal- and metal-oxide-based nanoparticles, surface-to-volume is able to distinguish bacterial cells from mammalian cells and can provide long-term antibacterial and biofilm prevention. These nanoparticles elicit bactericidal properties through the generation of reactive oxygen species (ROS) that are able to target physical structures, metabolic pathways, and DNA synthesis of prokaryotic cells leading to cell death. In this progress report, a critical analysis of current literature on antimicrobial effect of metal and metal-oxide nanoparticles are examined. Specifically, the antimicrobial mechanisms of metal ions and metal nanomaterials are discussed. Antimicrobial efficiency of nanomaterials is correlated with the structural and physical properties, such as size, shape, and/or zeta potential. A critical analysis of the current state of metal and metal-oxide nanomaterial research advances our understanding to overcome antibiotic resistance and provide alternatives to combat bacterial infections. Finally, emerging approaches to identify and minimize metallic poisoning, specifically for biomedical applications, are examined.

1. Introduction

Metals have been used as an antimicrobial agent for thousands of years, dating back to 1500 BP where Egyptians first recorded the use of copper salts as an astringent.[1] Indians, Egyptians, Persian kings, Phoenicians, Greeks, and Romans have also used copper and silver to preserve food and disinfect water.[2,3] More recently, silver has been used as sutures and infection preventatives.[4] The antimicrobial properties of metals have been utilized in a variety of applications throughout history, however its use in medical applications has rapidly diminished upon the discovery of antibiotics in 1920.[1]

Antibiotics are the current standard to kill bacteria-induced infections by altering prokaryotic cell components not present in eukaryotic cells.[5,6] Generally, antibiotics affect bacterial cell wall synthesis, translational machinery, and DNA replication, therefore preventing or eliminating biofilm production.[7] However, bacterial microorganisms continuously mutate causing resistance to antibiotics. This resistance arises from multiple mechanisms. The primary mechanism includes bacterial production of enzymes that can modify, degrade, or inactivate the antibiotic.[7] An example of this is shown through the ability of bacteria to adapt and produce the β-lactamase enzyme, cleaving the β-lactam ring, and neutralizing penicillin.[5] Multi-drug resistance (MDR) against numerous antibiotics can also develop from bacterial modifications and alterations of efflux pumps, binding target sites, and drug entry ports which alter the medications entry or clearance from the cell.[7]

Antimicrobial resistance is projected to reach epidemic proportions on a global scale by 2050, accounting for 10 million deaths (**Figure 1**a).[8,9] Antibiotics contain significant deficits including weak antimicrobial activity, major risk to healthy bacteria, and difficulty monitoring and extending function. This permits bacterial microorganisms to agglomerate, irreversibly adhere to a substrate, and proliferate into colonies, known as biofilms (Figure 1b).[10,11] Bacteria within a biofilm enclose themselves within a matrix of polysaccharides and proteins, forming a slimy layer. This slimy matrix inhibits antibiotic infiltration through the film, causing slow movement into the biofilm. This retarded penetration causes antibiotic deactivation before the agent is able to diffuse.[10] Multicellular bacteria are also able to differentiate into a protective phenotype due to the anaerobic environment it is exposed to. This compromised environment disables antibiotics from disrupting biofilm formation and eliminating bacteria. In addition to the anaerobic environment, biofilms contain enzymes that disrupt nutrients and accumulate waste products, altering antibiotics and antagonizing the antimicrobial response.[10,12]

With growing concern of resistant bacterial strains and biofilm-associated infections, there is a clinical need for an effective, long-term antibacterial and biofilm preventative. Given the use of metals throughout history, these materials have been

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extensively studied as antimicrobial agents. Metals are able to selectively inhibit metabolic pathways,[13] interact with bactericidal activity,[14] and kill multi-drug resistant bacteria.[15,16] Just like antibiotics, metal compounds are able to discern between bacterial and mammalian targets, however this is due to the cells deviating metal transport systems and metalloproteins.[1] This difference allows for the use of metals as an effective, long-term antibacterial and biofilm preventing material. To understand the use of metals as an antimicrobial agent, a recent literature search from ISI Web of Science (February 2018) indicated the growing interest in studying antimicrobial agents, specifically the use of metals, drugs, and their combinations (Figure 1c).

Metal can be synthesized into nanomaterials (NMs), ranging from 1 to 100 nm. Nanoparticles (NPs) provide strong, targeted, and extended antimicrobial activity at smaller dosages.[17] Due to dimensions smaller than bacteria and the large surface area to volume ratio, metallic nanomaterials allow for strong antimicrobial interaction with bacteria and biofilms. In this progress report, the potential of metal-based NPs as antimicrobial agents is critically evaluated. Specifically, a detail literature assessment of bacterial interactions with metal and metal-oxide NPs is performed to evaluate its potential as antimicrobial agents. To provide a wholistic viewpoint, the physical properties of metal-based NMs in relation to its bactericidal effects are also evaluated.

2. Mode of Antimicrobial Action by Metal Ions and Metal Nanoparticles

Similar to antibiotics, metal-based NPs are able to differentiate prokaryotic (bacterial cells) from eukaryotic (mammalian cells) through bacteria's metal transport system and metalloproteins. However, unlike antibiotics, metal-based NPs prompt bactericidal efficiency via multiple mechanisms. Given this distinction, multiple gene mutations within the same bacterial cell are needed to elicit any form of resistance. Metal NPs physically interact with bacterial cells through three major pathways (**Figure 2**) as discussed below. **Table 1** summarizes the antimicrobial activity of metal-based nanoparticles, specifically highlighting bacterial strains tested, mode of action, and fabrication techniques used.

2.1. Interactions with Phospholipid Bilayer

Metal-based NPs can disrupt the cell membrane potential and integrity by binding electrostatically to the bacterial cell wall and/or releasing metallic ions.[18] Given the positive charge of the NPs and the negative charge of cellular components, the two interact at the surface through electrostatic communication. These interactions disrupt the membrane and produce increased oxidative stress that damages bacterial proteins. Due to breaking of cell barrier, abundant amount of water from the cytosol is released. Cells try to compensate for this loss through the bacteria's proton efflux pumps and electron transport. However, the high demand of these ions causes severe damage to these transmembrane systems.[19] Overall, this imbalance of ions and membrane stability results in impaired respiration, interruption of energy transduction, and eventually cell death.[20] This effect has been demonstrated through the interaction of silver, gold, zinc oxide, magnesium oxide, and titanium oxide NPs. Silver NPs specifically interact with sulfur-containing constituents within the cell membrane and the ions produced impede cell wall synthesis.[21]

2.2. Binding to Cytosolic Proteins

The main mechanism in which metallic-based NPs induce an antimicrobial response is through binding to cytosolic proteins, such as enzymes and DNA. This interaction leads to decreased function, inhibiting respiratory and metabolic pathways and ATP production. For example, silver binds to enzymes within the respiratory chain and DNA, inhibiting replication and division.[22,23] Gold, on the other hand, interacts with DNA by upregulating genes within the cell.[24] This results in decreased membrane integrity and a buildup of ROS within the cytosol of the cell.

2.3. Formation of Reactive Oxygen Species

An alternative mechanism by which NPs kill bacteria is through the production of reactive oxygen species (ROS) or oxygen free radicals, such as hydrogen peroxide (H_2O_2) or superoxide anions.[25] The production of ROS is indirectly induced by the NPs themselves. ROS lead to severe oxidative stress and damage to the cell's macromolecules which overall cause lipid

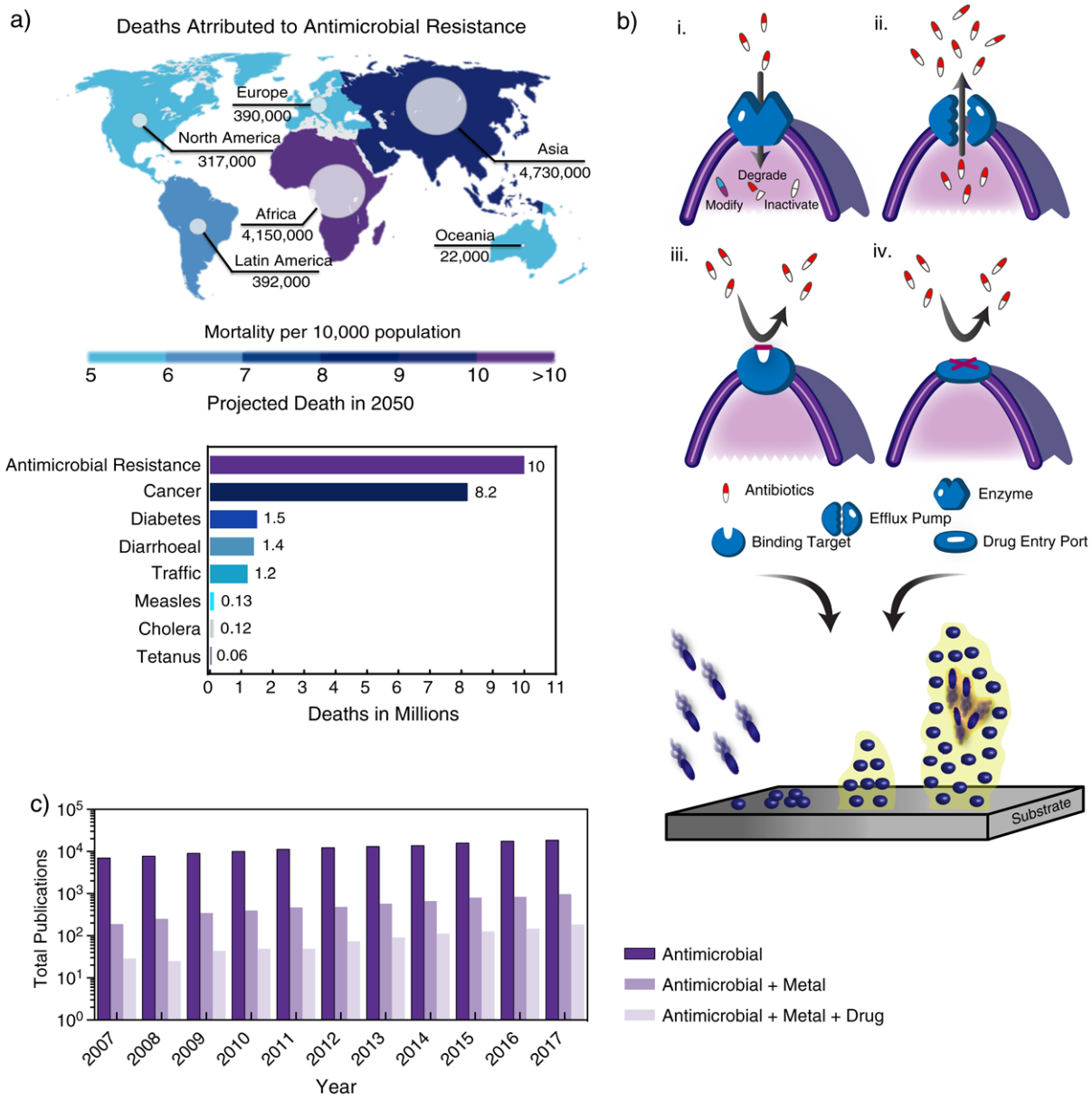

Figure 1. Trend in antimicrobial resistance. a) Antimicrobial resistance is a global epidemic that is projected to result in greater mortality than both cancer and diabetes. Adapted under the terms of the CC-BY license.[9] Copyright 2014. b) Bacterial cells continuously mutate to resist treatment with antibiotics through four different mechanisms: i) Production of enzymes that modify, degrade, or inactivate medication; ii) Modifications of cells efflux pump clearing medication from cells; iii) Altering binding target to prevent entrance into the cell; iv) Blocking of drug entry port to eliminate influx into the cell. This resistance of antibiotics causes an accumulation of bacterial cells that agglomerate and proliferate into a biofilm. c) Number of publications related to "antimicrobial" over the past 10 years, specifically looking at "antimicrobial," "antimicrobial + metal," and "antimicrobial + metal + drug" according to ISI Web of Science (Data obtained in February 2018).

peroxidation, alteration of proteins, inhibition of enzymes, and RNA/DNA damage.[19,26] This severe oxidative stress can also form holes or pits within the bacterial membrane, causing cell lysis.[22] Hydroxyl radical (OH) formation has been observed with silver.[21,27,28] Gold, zinc oxide, and magnesium oxide demonstrate ROS formation through increased catalytic activity generating H_2O_2 from glucose oxidase.[24,29,30] Titanium dioxide, upon exposure to light, has elicited ROS formation from both OH and H_2O_2.[31] Gallium, a unique case, has induced ROS production when mistaken for iron.[32]

3. Fabrication Techniques

Metal-based nanoparticles can be easily synthesized and/or chemically modified for an intended application. The methods used to fabricate various nanoparticles is categorized into two different categories, termed top-down and bottom-up.[33] The top-down approach, as implied by its name, is starting with a bulk material, or the top, that is broken down to be within the nanoscale, such as ball milling or attrition. Although this is simple to do for nanocomposites and nanograined bulk materials,

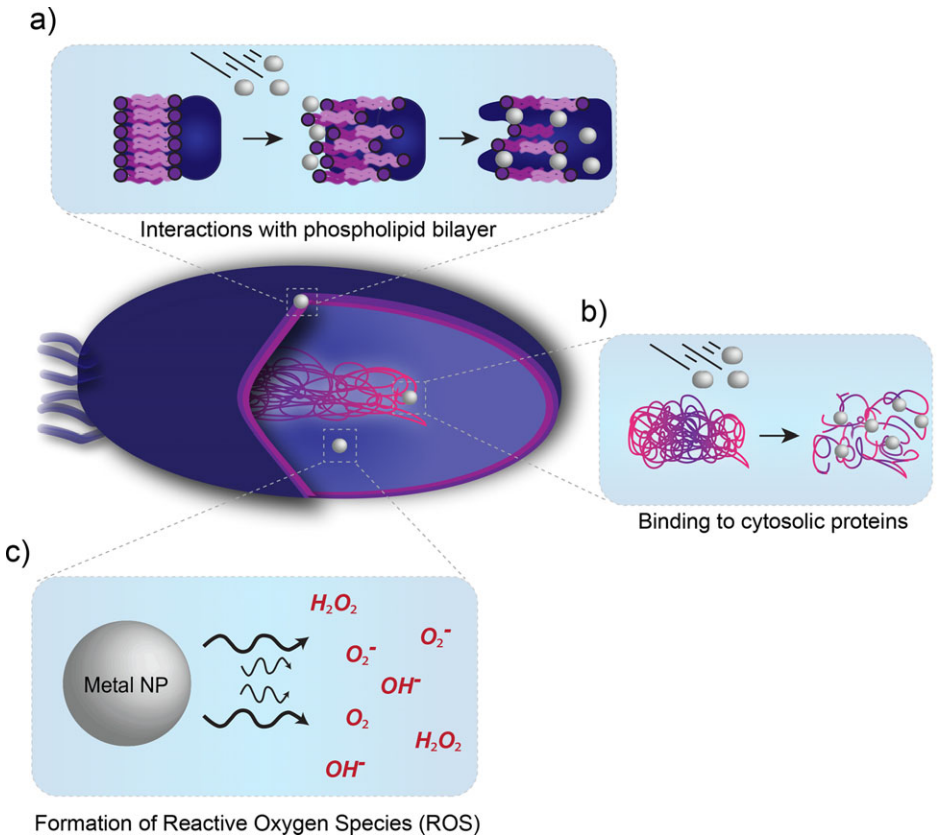

Figure 2. Metal nanomaterials are able to physically interact with prokaryotic cells compromising cellular functions. a) Metal nanomaterials are able to destabilize the phospholipid bilayer of the cell, causing cell lysis. b) Metal nanoparticles are able to bind to cytosolic proteins, such as DNA, triggering cell death. c) Metal nanomaterials produce ROS, leading to increased oxidative stress and cell instability.

this technique results in a broad size distribution, nonuniform particle geometries, and contains increased impurities. On the contrary, bottom-up approaches utilize diverse techniques to build a nanoparticle from raw chemicals and physical environments to a completed, finished product. This approach can be time consuming; however, permits for precise control over the chemical output and produces consistent particle shapes, sizes, and geometries with little defects. Examples of this fabrication technique include colloidal methods, atomic layer deposition, or sol-gel nanofabrication. Several comprehensive reviews are available,[33,34] providing a thorough discussion of the basic understanding and properties in nanoparticle synthesis.

4. Antimicrobial Effect of Metal Ions and Metal Nanoparticles

Although not understood, bacteria precipitate metal compounds as oxides, sulfides, protein aggregates, or elemental crystals.[35] These precipitates form particulates that meticulously interact with the membrane, sequestering these materials into the cell. Metal compounds have been shown to disrupt biofilm production and synergistically exert antimicrobial effects by inhibiting enzyme activity, altering membrane stability and function, damaging DNA, and overall inhibiting planktonic growth.[1,14] Due to the mutating nature of bacteria, studying the antimicrobial effects of metal and metal ions has been slow and difficult.[12] However, metal NPs, specifically silver, gold, and gallium, have demonstrated unique antimicrobial effects that have been extensively investigated (see Table 1).

4.1. Antibacterial Effect of Metallic and Ionic Silver

Silver (Ag), a corrosion resistant noble metal, releases ions via retarded oxidation.[36] These biochemically active ions interact with bacterial cell membranes, eliciting antimicrobial properties. The use of Ag NPs has demonstrated effective antimicrobial properties; however, the exact mechanism is still a topic of debate. Overall, the antibacterial properties can be divided into three forms: metallic, ionic, and salts.

Ag NPs, when in the metallic form, are not attacked by acidic and neutral fluids; however, Ag NPs are able to anchor to the membrane of certain bacteria and continuously release small amounts of Ag^+ ions.[36] Once ions are released from the surface, the metallic Ag causes "pit" formation on the bacterial cell wall, leading to increased membrane permeability and causing the cell to no longer undergo vital transport processes.[22] The mechanism by which Ag NPs anchor to the membrane of bacteria is not fully understood, however multiple theories exist. The concept of electrostatic interactions causing cell membrane communications was eliminated due to interactions occurring

Table 1. Assessment of metal-based nanoparticles for antimicrobial activity.

Nanoparticle	Size [nm]	Bacteria	Mode of action	Synthesis	Reference
Ag	4–24 (mode = 12)	*E. coli*	Attach to building elements on bacterial membrane (sulfur-containing proteins); causes pits in membrane, leading to cell lysis	Colloidal chemical method	Sondi et al.[22]
Ag	21.22 ± 5.17	*E. coli, P. domonas, S. aureus, Salmonella typhi , K. pneumoniae, Shigella*	Attach to membrane and inhibits cell wall synthesis; cannot maintain metabolic activity and cellular upkeep	Chemical reduction (colloidal chemical method)	Prema et al.[38]
Ag	13.4 ± 2.6	Yeast, *E. coli, St. aureus*	Interact with phospholipid bilayer, destroying and/or penetrating the membrane; destroys intracellular organelles and cell lysis	Colloidal chemical method	Kim et al.[21]
Ag	16 ± 8	*E. coli*	Attach to cysteine residues of NADH dehydrogenases; inactivates enzymes, preventing respiratory chain processes	Synthesized by Nanotechnologies Inc.	Morones et al.[23]
Ag	7–30	Bacteriophage ΦX174 and murine norovirus	Production of ROS and complexes formed between Ag ions and thiol groups of the viral proteins	Colloidal chemical method	Park et al.[42]
Ag	16.19 ± 6.86 (foamy) 6.53 ± 2.41 (PVP-coated) 3.12 ± 1.00 (BSA surface)	HIV-1	Binds to proteins responsible for virus–host cell interactions (disulfide groups); prevents infection of host cells	Synthesized by Nanotechnologies Inc., polyol method, and/or silver nitrate reduction	Elechiguerra et al.[43]
Au	≈ 6.0 ± 3.0	*P. aeruginosa, B. subtilis, S. epidermidis, E. coli,* and *S. aureus*	Causes a metabolic imbalance, upregulating oxidative enzymes and downregulating reductive enzymes (accumulating ROS intracellularly)	Colloidal chemical method	Zheng et al.[24]
Au	1.8 ± 0.5		MPA ligand induces ROS production	CO reduction method	Tay et al.[25]
Au	2	*E. coli, Enterobacter cloacae, P. aeruginosa,* and *S. aureus*	Electrostatic interactions and hydrophobicity promotes for NP and bacterial interactions, leading to destabilization of the phospholipid bilayer	Brust–Schiffrin two-phase method	Li et al.[51]
Au–Ag	209 ± 0.7	*E. coli, E. faecalis, P. aeruginosa, S. aureus,* and their combinations	NP-induced depressions in the cell walls, permitting for penetration of NPs into cytoplasm of cell and promoting Ag leaching and interaction with sulfur and phosphorous cellular components	Bacteriogenic synthesis (metal-reducing bacteria)	Ramasamy et al.[53]
Def and Ga-protoporphyrin encapsulated in hydrogel network	NA	*S. aureus,* MRSA, *S. epidermis, P. aeruginosa,* and *Androstachys johnsonii*	Internalized, but cannot be reduced by bacteria; disrupts cellular pathways, limits respiration through production of ROS, damages DNA	NA	Richter et al.[32]
ClGaTCPP-Grafted onto Platinum NP	28 ± 6	*S. aureus*	Increased surface area or small NP have larger number of atoms on the surface which influence the stability of the cell wall	Oxygen reduction reaction; Ga(III) TCPP conjugated on surface	Managa et al.[58]
Ga	305 ± 0.29	HIV, *M. tuberculosis,* and coinfection	Inhibited the release of specific cytokines, binds to cytokines and removes from system	Double emulsification; colloidal chemical method	Narayanasamy et al.[65]
ZnO	2000–12	*E. coli* and *S. aureus*	Larger surface area, increased production of ROS	Sol-gel (chemical solution deposition)	Ohira et al.[76]

(*Continued*)

Table 1. Continued.

Nanoparticle	Size [nm]	Bacteria	Mode of action	Synthesis	Reference
ZnO	100–800	*E. coli* and *S. aureus*	Larger surface area, increased production of ROS	Planetary ball mill	Yamamoto et al.[77]
ZnO	12, 25, 88, 142, and 212	*S. aureus*, *Proteus vulgaris*, *Salmonella typhimurium*, *Shigella flexneri* , and *Bacillus cereus*	Increased crystallinity leads to decreased ROS formation and ion release	Room temperature and solvo thermal	Raghupathi et al.[78]
ZnO	18–28	*E. coli* and *S. aureus*	Difference in bacteria membrane thickness and membrane ROS sensitivity affects NP interactions with bilayer	Sol-gel combustion	Azam et al.[81]
MgO	≈ 20	*E. coli*	Formation of ROS (UV-illuminated group); attachment to bacterial membranes by interacting with phosphate groups, causing an increase in membrane permeability (no light exposure)	Purchased from Nanostructured & Amorphous Materials Inc. or MK Impex Corp.	Leung et al.[86]
MgO	30–50 or 70–130	*E. coli* and *S. aureus*	Electrochemical interactions between NP and cell walls cause disruption and penetration into cell, causing leakage of metabolites, prevents cellular function, and obstructs reproduction	Wet chemical method	Sundrarajan et al.[88]
MgO	11 (MgO-1) 25 (MgO-2)	*E. coli* and *S. aureus*	Production of ROS directly oxidizes proteins and DNA; increased surface area of smaller NP permits for increased ROS production	Commercial supplier (not named)	Sellik et al.[89]
MgO–ZnO vs. MgO–nisin	20	*E. coli* and *S. typhimurium*	MgO–nisin forms pores on bacteria surface, releasing ions, amino acids, and ATP; nisin causes pores and MgO releases ROS	Purchased from Nanostructured & Amorphous Materials Inc.	Jin et al.[90]
TiO_2	20	*S. aureus, Enterococcus hirae, P. aeruginosa, E. coli*, and *Bacteroides fragilis*	Surface coatings prevent bacterial adhesion; light exposure causes ROS formation	Aqueous sol-gel preparation (chemical solution deposition)	Daoud et al.[98]

between negatively charged Ag NPs and the negative bacterial membranes. Instead, it was suggested that the Ag NPs (diameter = 4–24 nm; mode = 12 nm) attach to building elements on the bacterial membrane, such as sulfur-containing proteins.[22,23] Cellular contents, such as lipopolysaccharide molecules and membrane proteins, can leak out of pits formed by the attachment of Ag NPs, resulting in lysis and eventually cell death.[22] This "pitting" has been observed in *Escherichia coli*, a Gram-negative bacterium. However, Gram-positive bacteria, such as *Staphylococcus aureus*, have not been shown to undergo "pitting" due to a thicker peptidoglycan layer.[37] Instead, Ag NPs (21.22 ± 5.17 nm) attached themselves to the membrane and inhibited cell wall synthesis. In other words, the bacterial cell was not able to maintain homeostasis and metabolic activity. Thus, the antibacterial mechanism of metallic Ag NPs can vary for different types of bacteria, generally Gram-positive bacteria require a higher lethal dosage of NPs compared to Gram-negative bacteria.[38]

Due to the inherent instability of metallic Ag NPs, the material tends to ionize when in solution. Once ionized, Ag's ions are able to penetrate bacterial cell walls, cause DNA molecules to condense, and inhibit replication or biofilm development. Since bacterial cells no longer reproduce, the cell undergoes necrosis.[39] The ions leached from the metallic form of Ag have been shown to bind to the thiol groups located on the cysteine amino acid, rather than sulfur-containing proteins. The protein conformation changes upon ion interactions, resulting in a large modification of active sites and contributes to cell death.[40] A special case where the Ag ion–thiol group attachment is especially deleterious to the cell is when ions interfere with an enzyme involved in the respiratory chain. In *E. coli*, it was speculated that Ag ions attached to the cysteine residues of NADH dehydrogenases. Once attached, the Ag ions inactivate the enzymes, preventing the normal processes vital in the bacterial respiratory chain.[23]

Metallic Ag relies on oxidation of the NP to release Ag^+ ions. This process is retarded under physiological conditions, leading to insufficient silver concentrations to elicit an antimicrobial effect. To overcome this time constraint, Ag salts (e.g., Ag nitrate, Ag halides, and Ag sulfides) are used, leading to an increased, localized delivery of Ag^+ ions. Ag salts are commonly used for topical applications, such as wound healing.[2,41] Upon exposure to

physiological conditions, Ag salts dissociate and release Ag^+ ions and a complexing anion (e.g., chloride, phosphate, sulfide).[27] This dissociation has the potential to inactivate silver through protein binding wound exudate release, therefore increased antimicrobial activity is increased with sustained release of silver.[16] However, silver salts contain poor antibacterial effects due to the inactivation of silver, therefore need repeated application for sustained antimicrobial effects.[16]

A recent development in the study of antimicrobial action of metallic Ag NPs is the evidence of ROS formation. Electron spin resonance spectroscopy has revealed the formation of ROS with Ag NPs (13.4 ± 2.6 nm). Once formed, the reactive molecules further destroy and even penetrate the membrane, abolishing various organelles.[21] In one study, Ag NPs were shown to be effective in inactivating bacteriophage ΦX174 and murine norovirus.[42] Different sizes were tested (ranging from 7–30 nm), and it was shown that the smallest sizes of the Ag NPs were the most successful in inactivating the viruses. The mechanisms by which the inactivation occurred were suspected to be the production of ROS and the complexes formed between Ag ions and thiol groups of the viral proteins. Ag NPs (1–10 nm) have also demonstrated antimicrobial properties when exposed to HIV-1.[43] It was found that the Ag NPs attached to special surface proteins on the HIV-1 virus that are responsible for binding to host cells. When these surface proteins were covered with Ag NPs, the virus was not able to infect host cells. This is due to Ag NPs being attracted to the disulfide groups in these surface proteins.

Overall, Ag has demonstrated antibacterial properties for Gram-positive, Gram-negative, and viruses. Given the multiple mechanisms of action, Ag has been used in a variety of applications for over 7000 years.[36] The different forms of Ag, permit for use as a textiles or sprays,[44] food preservation additive,[45] dental resin composites,[46] cosmetics,[47] medical device coatings,[48] implants,[49] and instruments.[49]

4.2. Antimicrobial Effect of Gold

Unlike Ag, gold (Au) is an inert and highly stable metal that cannot be easily dissociated. This characteristic contributes to Au's cytotoxic and genotoxic effects in mammalian systems and is further observed when size is reduced to the nanocluster (NC) range (core size less than 2 nm).[24,25] Although compatible with eukaryotic cell lines, Au's inert properties does not lead to highly active antimicrobial properties unless used in very high concentrations or ionic complexes. Instead, Au NP-based antimicrobial systems rely on using the material as a passive drug carrier, grafting antimicrobial compounds such as peptides, zwitterionic ligands, or antibiotics.[50,51] Based on the properties, Au is able to exhibit antimicrobial activity by both disrupting the bacterial membrane as well as generating ROS.

Many studies have been conducted on Au NC to support its use in biological applications, specifically NC effects on cellular uptake.[24,25,52] The surface to volume ratio of Au NC is significantly higher compared to Au NP, therefore driving enhanced interactions between Au NCs and biological systems. Studies have supported the antimicrobial activity of Au NC through the increased generation of ROS due to their catalytic activity, analogous to glucose oxidase generating H_2O_2.[25] Zheng et al. compared the antimicrobial activity of Au NC (<2 nm) and Au NP ($\approx 6 \pm 3$ nm) in order to determine the possible mechanism that drives the broad activity.[24] In this study, Au NCs was able to kill ≈96% of Gram-positive *S. aureus* and Gram-negative *E. coli* while Au NPs was only able to kill ≈3% of the *S. aureus* and ≈2% of the *E. coli* populations when administered at the same particle count.[24] Overall, Au NCs were able to efficiently kill *Staphylococcus epidermidis, Bacillus subtilis, E. coli,* and *Pseudomonas aeruginosa*, however elicited a stronger response to Gram-positive bacteria.[25] Using microarray gene expression profiling, Au NC treatment created a metabolic imbalance, upregulating oxidative enzymes and downregulating reductive enzymes, leading to an accumulation of intracellular ROS.[24] The Au NP treatment expressed a decreased metabolic pathway activity. Overall, this suggests the internalization of Au NCs into bacterial cells to induce ROS production and subsequent killing. In addition to bacterial metabolism, Au NCs have also been found to upregulate genes related to membrane integrity and downregulate genes related to cell wall surface anchor protein.[24] This indicates damage to the bacterial membrane that cannot be repaired. Au NCs have also been shown to promote downregulation of transcription and translation, therefore preventing resistance of antibiotics.[24]

Excess ROS generation results in cellular stress and impairs basic cell function. However, ROS production could be altered based on the surface chemistry. Tay et al. studied glutathione (GHS) and mercaptopropionic acid (MPA) ligands on the surface of Au NC (<2 nm) to determine its effect on cellular uptake and ROS generation.[25] MPA ligands on Au NCs demonstrated a tenfold increased uptake compared to GSH ligands.[25] MPA Au NC internalization was also able to be modulated by altering the number of surface-bound ligands. MPA Au NCs also generated high levels of ROS compared to GSH Au NPs which did not express ROS.[25]

Alterations to Au NP's surface chemistry and landscape properties can alter Au's antimicrobial activity. Li et al. investigated the structure–activity relationship between a library of ligands functionalized to Au NP (2 nm core).[51] Through this study, it was evident that surface hydrophobicity can modulate Au NP antimicrobial effect. Specifically, more hydrophobic functionalities correlated with an increased antimicrobial effect due to the hydrophobic nature of bacteria (like dissolves like). In addition, there was a strong correlation between bacterial interactions and cationic functionalities. Together, this demonstrated that cationic and hydrophobic functionalized Au NPs are able to inhibit bacterial growth of both Gram-positive and Gram-negative bacteria.[51]

Combining Au and Ag into bimetallic NPs enables for an additive antimicrobial effect. Coprecipitating Au and Ag simultaneously produces NPs (209 ± 0.7 nm) with Ag domain surrounded by Au shells. This permits for immediate and precise leaching of antimicrobial Ag ions into cellular environments[53]. These particles' bactericidal activity was tested against *E. coli*, *Enterococcus faecalis*, *P. aeruginosa*, *S. aureus*, and their combinations. Au–Ag NPs were able to inhibit and disrupt all strain's biofilm formation in a dose-dependent manner, ranging from 10 to 250 μM (**Figure 3**a). Low magnified images present irregular morphology and nonuniform distribution. The high magnified images portray the compromised membrane integrity through the bacteria's wrinkling, rupturing, and completed disintegrating

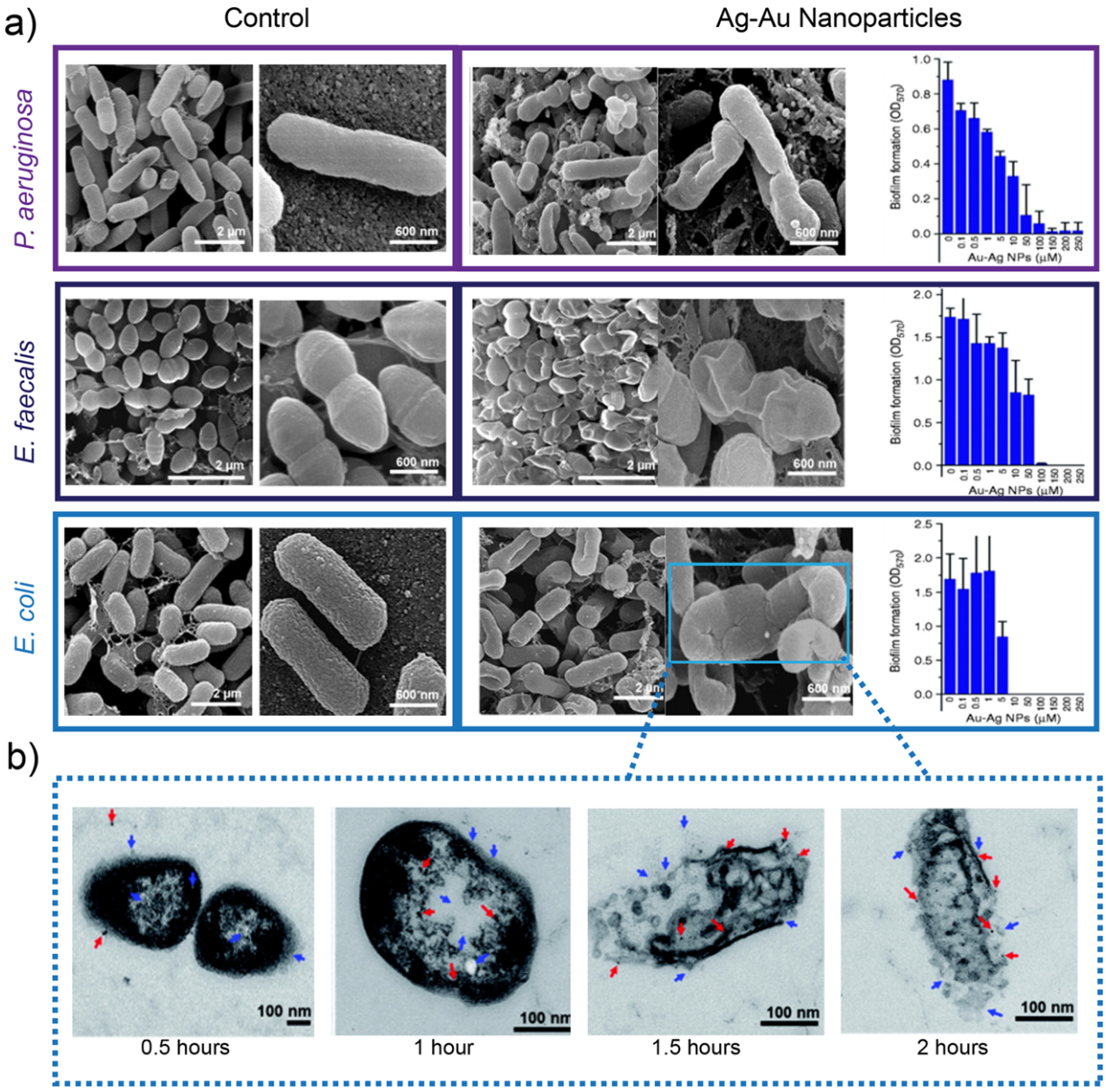

Figure 3. Antimicrobial activity of gold–silver (Au–Ag) nanoparticles. a) The additive effects of Au–Ag NP on different bacterial strains (*P. aeruginosa*, *E. faecalis*, and *E coli*) and their biofilm production. b) Isolating *E. coli* over a variation of time permits for the tracking of NP internalization and membrane interactions that lead to overall cell death. Reproduced with permission.[53] Copyright 2016, SAGE Publishing.

appearance. Together, these images suggest the severity of cellular damage through NP-induced depressions in the cell walls. Such depressions permit for the penetration of NPs into the cytoplasm of the cell, promoting Ag leaching and possible interaction with sulfur and phosphorous cellular components. To investigate Au–Ag NPs interactions with *E. coli*, cross-sectional TEM images were conducted at different time points (Figure 3b). At 0.5 h, cell maintained a healthy structure, however Au–Ag NPs began to initiate cellular damage through structural damage. After exposure up to 1 h, Au–Ag NPs are internalized, as depicted by arrows, and cells begin to undergo lysis. The internalization of the Ag–Au NPs is hypothesized to be due to the organic molecules surrounding the particle. This organic surrounding causes NP attachment to the bacterial membrane. The attached NP begins to associate itself with the fatty segments of the phospholipid bilayer, causing internalization into the cytoplasm. Incubating further, *E. coli* experiences complete disintegration. This is assumed to be due to the high affinity of Ag to molecules inside the bacteria, causing protein and enzyme inactivation and inhibition of ATP production.[53]

Au provides a tunable system based on the size (NP vs NC) and surface chemistry (e.g., conjugation of ligand, antibiotics), therefore provides a method to govern fundamental antibacterial parameters like cell death, metabolism, and ROS generation. This permits for modulation of Au NM to be used in a variety of applications.

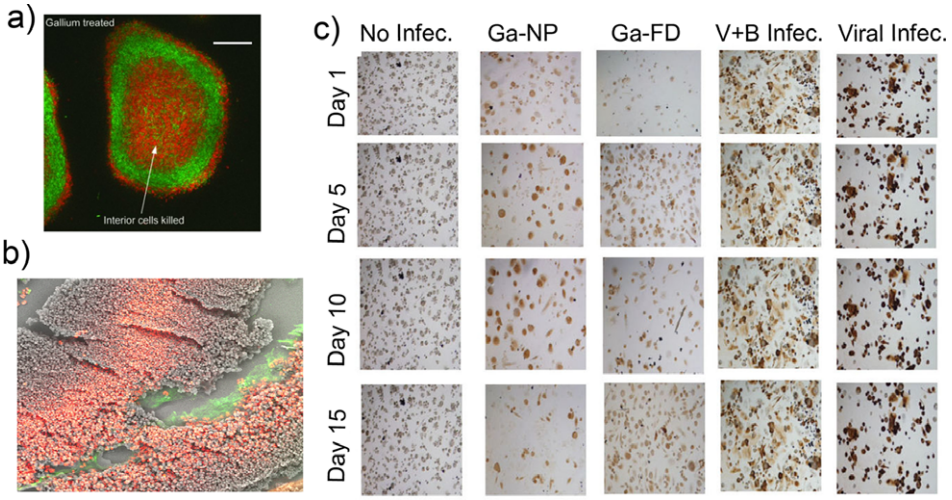

Figure 4. Antimicrobial activity of gallium (Ga) nanoparticles. a) High magnified image of GFR-labeled *P. aeruginosa* biofilm elimination by treating with Ga NP for 48 h. Image is stained with propidium iodide (dead cells—red), showing Ga NP preferentially kills biofilm in the central regions. Reproduced with permission.[57] Copyright 2018, American Society for Clinical Investigation. b) Live–dead staining of a biofilm wound healing model exposed to hydrogels containing both Def and Ga NP, showing Ga NP ingestion into the cells induces bacterial death and biofilm reduction. Reproduced with permission[32] © 2017, American Society for Microbiology. c) p24 staining over the course of 15 days exhibiting the ability of Ga NP to inhibit both viral (HIV) and bacterial (*M. tuberculosis*) formation and reproduction. Reproduced with permission.[65] Copyright 2015, Nature Publishing Group.

4.3. Antimicrobial Effect of Gallium

Gallium (Ga) antimicrobial properties stem from its ferro-mimicking properties, meaning bacteria cannot differentiate between Ga^{3+} and iron (Fe). Fe is essential to the reproductive, metabolic, and growth processes.[54,55] Specifically, bacteria utilize the reduction of Fe^{3+} to Fe^{2+} to support enzymes that protect the bacteria from ROS and support DNA synthesis.[54] Although Ga has no natural function in the body, bacteria cannot distinguish Ga^{3+} from Fe^{3+}.[56,57] This enables Ga^{3+} to enter cells utilizing Fe^{3+} uptake mechanisms. However, unlike Fe^{3+}, Ga^{3+} cannot be reduced, therefore halting oxidation–reduction (redox) reactions. The prevention of redox reactions causes inhibition of planktonic growth and DNA synthesis, therefore leading to cell death, preferentially killing bacteria from the inside, outward (**Figure 4**a).[57] Overall, Ga can be used as an antimicrobial agent in itself or can be grafted or combined with other materials, therefore providing an antimicrobial platform to be used for a variety of applications.

Ga can be used in several forms, such as Ga-protoporphyrin or Ga(III) tetra-(4-carboxypenyl) porphyrin (ClGaTCPP), for its antimicrobial activity.[32,58] Richter et al. utilized deferiprone (Def), an iron chelator that induces starvation and upregulation of iron systems, to isolate all bacterial growth and survival through its iron metabolism.[59] Due to Ga's similarity to Fe^{3+}, Ga-protoporphyrin is recognized by the cell as iron, therefore is metabolized via the same mechanism. This inhibits the essential pathways in bacterial cells, disrupts cellular respiration, and induces ROS production. To exploit these properties, Richter et al. combined Def and Ga-protoporphyrin into a hydrogel, controlling the kinetics to promote antimicrobial activity of both Gram-positive and Gram-negative biofilms.[32] When used in wound model, this hydrogel demonstrated the ability of Ga to be taken up as a favorable iron source. Once digested, Ga disrupts vital cellular pathways (prevents electron transfer for ATP production by respiratory pathways, enzymes are inhibited to breakdown Ga, obstructing nutrient/iron release and promoting starvation, Ga's inability to be reduced like iron blocks efflux pumps).[60] Together, this limits cellular respiration through the production of ROS, therefore damaging cell DNA, prompting cell death, and destruction of biofilm formation (Figure 4b).[32]

In a similar study, Managa et al. conjugated ClGaTCPP to platinum NP (28 ± 6 nm) of various shapes and sizes and evaluated its antimicrobial properties.[58] Platinum permits for the inactivation of microbes through enzyme, protein, and DNA interactions to inhibit cell growth.[61] By grafting of ClGaTCPP onto the surface of the platinum nanoparticles, antimicrobial activity can be targeted with photodynamic activation.[62] Specifically, when in a dark environment, these particles demonstrate increased toxicity effects. ClGaTCPP-Cubic platinum NPs showed the best photodynamic activity, with only 11% of *S. aureus* survival (0.2 mg mL^{-1}, 4.64 log reduction).[58] These NP were incorporated into electrospun fibers to demonstrate its potential use in tissue engineered constructs.[58]

In another study, the ability of Ga NP (305 ± 0.29 nm) versus Ga free drug (FD) to inhibit the growth of HIV, *Mycobacterium tuberculosis,* and their coinfection was tested over the course of 15 days using p24 staining.[63] This staining showed complete inhibition of HIV development as well as coinfection of the virus with bacterial infection (V + B) via GA NPs within 5 days (Figure 4c). An additional way in which Ga inhibits growth of the HIV cultures is by stopping the release of specific cytokines. IL-6 and IL-8 are cytokines released by macrophages that propagate the HIV virus.[54,64,65] Ga NPs bind to these cytokines and remove them from the system, therefore having antimicrobial effects on HIV.[66] Overall, Ga has been shown to be the first single drug to successfully inhibit both a virus and bacteria coinfection of HIV–*M. tuberculosis*.[65]

The unique ability of Ga to be disguised as Fe allows for cellular uptake via similar pathways to elicit a bactericidal effect. Due to increased stability compared to Fe, Ga causes inhibition of planktonic growth and DNA synthesis. This antimicrobial agent serves as a platform that can be combined with other materials (e.g., Ga-protoporphyrin, maltol, conjugated to platinum) to vary the antimicrobial response. For example, Ga maltolate (GaM) is a coordination complex between both Ga and maltol that is soluble in both water and lipids.[67] This material is commonly used to supply Ga ions into a system and has been shown to significantly reduce the number of colony-forming units of various bacteria families, preventing biofilm formation.[68] Ga's ability to mimic the ferric ion allows for the material to take advantage of the microorganism's iron-dependent growth, therefore preventing any form of resistance to the antimicrobial material. GaM has also demonstrated pain relief with topical applications at lower dosages, reducing inflammation.[69]

5. Antimicrobial Effect of Metal-Oxide Nanoparticles

Metallic elements can be combine with oxygen to form a metal oxide (MeO). Based on the different Lewis-dot structures, MeOs elicit diverse physiochemical and functional properties, such as magnetic, mechanical, electrical, and optical characteristics.[70] MeOs have been shown to interact with bacteria through electrostatic interactions that alter the prokaryotic cell wall and enzyme or DNA pathways through ROS production.[71] MeOs have gained attention as an NM that can be formed into specific size and shape. Considering these unique properties, MeO NPs are being used as antimicrobial agents (see Table 1), such as MeOs of zinc, magnesium, and titanium.

5.1. Antimicrobial Effect of Zinc Oxide

Zinc is a vital nutrient that plays a crucial role in growth, development, and well-being for mammals.[72] For example, zinc oxide (ZnO) is safely acknowledged by the U.S. food and drug administration (21CFR182.8991). On the nanoscale, ZnO has shown antimicrobial effects and is commonly used for food preservation, manufacturing stability, and increasing the shelf life of products.[73] Although commercially used, the exact mechanism of antimicrobial action is unknown and attributed to be from electrostatic interaction with the membrane, formation of ROS, and/or release of ions.

The attachment of the NPs to the bacterial membrane is a vital first step for ZnO's antibacterial mechanisms. However, the method of attachment for the ZnO NPs to the bacterial membrane is not completely understood. Currently, the consensus of interaction is a result of electrostatic forces between the ZnO NPs and the bacterial membrane. Once the ZnO NPs attach to the bacterial membrane, "pitting" occurs in the membrane due to ROS formation, which fatally damages the cell.[29,74] ROS has been correlated to particle size, surface area, and crystallinity.[75,76] Specifically, the antimicrobial properties of ZnO increases as the surface area increases and particle size and crystallinity decreases.[76–78] Padmavathy et al. studied the effect of ZnO NP particle size

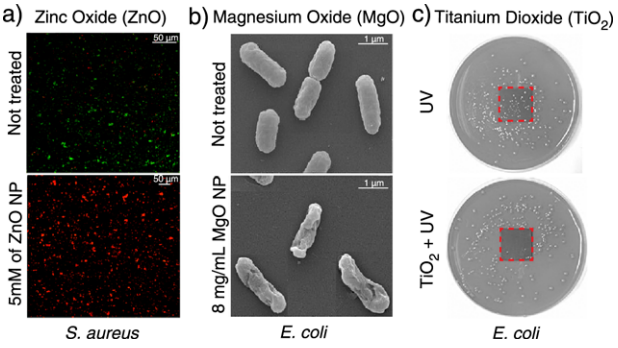

Figure 5. Metal oxides (e.g., ZnO, MgO, TiO_2) exhibit antimicrobial activity. a) Confocal micrographs of fluorescein isothiocyanate (FITC) and propidium iodine (PI) stained *S. aureus* not treated (top) and treated (bottom). The green fluorescein represents live cells, whereas the red represents dead cells. Reproduced with permission.[78] Copyright 2011, American Chemical Society. b) SEM electron micrographs of *E. coli* with and without MgO treatment. The MgO treatment portrays a compromised bacterial membrane and irregular cell surface. Reproduced with permission.[90] Copyright 2011, Springer Nature. c) Bactericidal effect, looking at *E. coli*, of metal plate exposed to UV compared to a TiO_2 NP coated metal plate exposed to UV. The red, dotted box represents the location of the metal sheet, depicting that TiO_2 coated metal plate contained significantly decreased bacterial colonies. Reproduced with permission.[96] Copyright 2008, John Wiley and Sons.

(2 μm–12 nm) on antimicrobial properties. They showed enhanced biocidal activity in smaller ZnO NPs (12 nm) compared to larger ZnO NPs (2 μm).[75] Similarly, Raghupathi et al. demonstrated the increased bacterial growth inhibition with smaller ZnO NP (12 nm compared to 25, 88, 142, and 212 nm particles).[78] This phenomenon is speculated to be a result of the greater surface area of ZnO NPs. Due to the large surface area, increased production of ROS from the ZnO NP resulted in bacterial cell damage (**Figure 5**a). However, with increasing crystallinity, ROS formation and ion release from the NP is decreased, thereby inhibiting antimicrobial activity.

The structural properties discussed relate to ZnO optical characteristics. ZnO is a semiconductor with a wide bandgap (3.37 eV), causing sensitivity to short wavelengths.[79] Upon light exposure, electron–hole pairs are created on the surface of ZnO. These holes split water molecules and cause ROS formation, specifically OH^-, H_2O_2, and O_2^{2-}.[80] Relating this to ZnO's structural properties, NPs with more surface defects cause a decrease in crystallinity and increase in surface area. Therefore when exposed to short wavelengths (such as light), these defects cause faster and more effective ROS generation.[75] Once formed, hydrogen peroxide can enter the prokaryotic cell and destroy various organelles. In addition, lipid peroxidation can occur on the bacterial membrane, weakening membrane integrity and promoting cell lysis.

ZnO NP's antimicrobial activity is also dependent on the bacterial species. Although ZnO has been used as an antimicrobial agent against both Gram-positive (*B. subtilis, S. aureus)* and Gram-negative bacteria (*P. aeruginosa, Campylobacter jejuni, E. coli)*, this material shows higher susceptibility and increased sensitivity to Gram-positive bacteria compared to Gram-negative (specifically comparing *S. aureus* to *E. coli)*.[81] This has been attributed from several factors, the main factor being the difference in the membrane thickness and membrane ROS

sensitivity.[82] Gram-positive bacteria have a membrane and cell wall composed of peptidoglycans, teichoic acid, and lipoteichoic acid that is easier to penetrate compared to the complex cell wall of Gram-negative bacteria, containing an outer membrane of lipopolysaccharides and a peptidoglycan layer.[83] This prevents the absorption of ROS and ions through the membrane and into the cell. The difference between bacterial types has also been suggested to be due to the increased affinity of ZnO to *S. aureus*, sensitivity to stress, and differences between intracellular content, specifically carotenoid pigments.[84] These carotenoid pigments promote ROS resistance in the presence of catalase.

5.2. Antimicrobial Effect of Magnesium Oxide

Magnesium is an essential nutrient for the body, promoting cardiorespiratory function and metabolism regulation.[85] With this in mind, nanotechnology has inspired the use of this material as an antimicrobial agent. One of the primary antibacterial mechanism of magnesium oxide (MgO) NPs is the production of ROS under light exposure.[30] Once produced, the ROS (primarily H_2O_2) can enter the bacterial membrane and induce oxidative stress on bacterial organelles and induce lipid peroxidation. Lipid peroxidation refers to the oxidative degradation of lipid molecules of cellular membranes. This form of oxidative stress weakens the integrity of the bacterial membrane and can ultimately lead to cytoplasm leakage.[30]

Aside from ROS production, MgO NPs are also able to attach themselves to bacterial membranes. This attachment causes an increase in membrane permeability, thus making it difficult for the bacteria to maintain its vital transport processes. Leung et al. conducted a study exposing MgO NPs (≈20 nm) to *E. coli* in the absence and presence of UV illumination.[86] The UV-illuminated MgO NPs were more deleterious to the *E. coli* than the MgO NPs in darkness due to the formation of ROS. However, MgO NPs in darkness still exhibited substantial antibacterial activity. It was speculated that the MgO NPs attached to the bacterial membranes by interacting with phosphate groups, causing an increase in membrane permeability.

Currently, MgO is used in magnesium dietary supplements and many different types of medications. At relatively low concentrations, MgO was not observed to be toxic to human cells.[87] The lack of toxicity and the precedence of ingestion via dietary supplements reinforce the concept of MgO NPs in food packaging. MgO NPs were proven to be particularly potent in killing and preventing the growth of several foodborne bacterial pathogens such as *C. jejuni*, *E. coli*, and *Salmonella enteritidis* by the aforementioned mechanisms.[30] Since microbes can be involved in the food spoiling process, MgO NPs incorporated into packaging would slow or even prevent food from spoiling.

The bactericidal degree of MgO NPs was found to be dependent upon the size of the nanoparticles. In a study conducted by Sundrarajan et al., $Mg(OH)_2$ NP was synthesized via wet chemical routes and the annealing temperature was changed to obtained different sizes of NPs.[88] The effect of size on the materials' antimicrobial properties was tested using Gram-positive *S. aureus* and Gram-negative *E. coli* bacteria. Smaller MgO NPs (30–50 nm), showed larger inhibition zones for both Gram-positive and Gram-negative bacteria. On the other hand, larger NPs (70–130 nm), expressed antimicrobial effects on only Gram-negative bacteria. The difference in antimicrobial efficiency based on size of the NP is due to an electrochemical interaction between the NP and the cell walls. This interaction causes disruption and penetration into the cell, leading to a leakage of the metabolites, prevents cellular function, and obstructs reproduction.

Similarly, Sellik et al. demonstrated the effect of MgO NP size, structure, and arrangement on neutralizing hazardous materials and its antimicrobial productivity by comparing MgO-325 mesh (MgO-1) and MgO nanopowders (MgO-2).[89] MgO-1 NP (11 nm) are arranged into sheets while the MgO-2 NP (25 nm) are elongated. MgO-1 exhibited an increased activity against *E. coli* and *S. aureus*, whereas MgO-2 had no action toward the two strains. This is assumed to be the production of ROS that directly oxidizes proteins and DNA. Smaller NPs have an increased surface area, permitting for increased production of ROS. The MgO-1 NPs are smaller and arranged into sheets, leading to increased antimicrobial activity compared to the MgO-2 NPs. The MgO-1 NPs arrangement into a sheet permits for larger surface area and therefore an enhanced catalytic effect, compared to the elongated MgO-2 NPs.

Aside from its physical properties, MgO NP can be combined with other materials to provide an additive antimicrobial affect. Jin et al. compared the antimicrobial effect of MgO NPs (average size: 20 nm) alone and in combination with either ZnO (average size: 20 nm) or nisin (a polycyclic antibacterial peptide) against *E. coli* and *Salmonella*.[90] Upon administering MgO, the bacterial cells exhibit a compromised bacterial membrane and irregular cell surface (Figure 5b). When combined MgO and ZnO were administered to both bacteria, there was no significant difference in bactericidal effects compared to just administering MgO. However, when combined with nisin, a synergistic effect was observed and explained to be from divergent antimicrobial mechanisms. Nisin causes bacterial cell death, in both Gram-positive and Gram-negative species, due to the formation of pores within the membrane.[91] This leads to a release of ions, amino acids, and ATP.[91] This arises from an interaction between nisin with the phospholipid components of the cytoplasmic membrane.[92] With this in mind, when the two materials were combined *E. coli* cell morphology transformed. Specifically, cells administered with just MgO NPs appeared to have compromised surface and membrane integrity. Administering just nisin resulted in the formation of donut shapes to the cells, yet no significant alterations of the cell's surface or membrane. When combining these materials, cells shrunk, forming small and round shapes. The mechanisms of this combination are not fully understood; however, it is assumed that nisin causes pores within the cell membrane, permitting MgO to penetrate into the cell and elicit antimicrobial activity through the release of ROS. Overall, the combination of these materials and potentially other metal oxides permits for increased antimicrobial effects in both Gram-positive and Gram-negative bacteria, regardless of the properties and composition of the bacteria's phospholipid layer.[90]

5.3. Antimicrobial Effect of Titanium Dioxide

Titanium dioxide (TiO_2) is a strong photocatalytic material with high oxidizing powers and long-term stability. The ability of TiO_2

to generate ROS with wavelengths less than 385 nm permits for its use as an antimicrobial agent.[93] TiO_2 NPs have been used as antimicrobial agents for a broad spectrum of bacteria, including both Gram-positive and Gram-negative bacteria.[94] The antimicrobial properties of titanium dioxide (TiO_2) NPs can be photodependent. A photocatalytic reaction causes generation of free radicals from the TiO_2 NP. When a light source strikes the excited NP, the valence band releases an electron from the surrounding water or hydroxyl ions to become more stable. This produces a hydroxyl radical (OH) that can be used to reduce oxygen into a superoxide anion (O_2^-). Oxygen can also be directly reduced from the TiO_2 NP or indirectly reduced from the superoxide to produce O_2. This mechanism produces three different types of ROS (OH, O_2^-, and O_2^-) that are capable of disrupting the bacterial cell membrane and lead to cell death (Figure 5c). The free and surface-bound OH·is the main ROS that contributes the TiO_2 NP's antibacterial and antiviral properties.[31] Due to peroxidation, these free radicals affect the lipopolysaccharide, peptidoglycan, and phospholipid bilayers.[31,95–97] Given TiO_2's photo-dependent properties, it is commonly used for surface coatings due to its large effective surface area, or surface area to volume ratio, which enhances surface reactions of the material.[98–100] In addition, the smaller the NPs used for the surface coatings, the higher the photocatalytic and photoelectrical chemical conversion.[99,101] This is due to an increased surface area for light to interact with, therefore increasing the formation of ROS.

Daoud et al. developed TiO_2 NP to provide a surface coating to cellulose fibers.[97] This was completed by submerging cellulose fibers into a nano-solution of titanium tetraisopropoxide and a nitric acid–water solution. Once taken out of this solution, the fibers were pressed with an automatic press under high pressure. The cellulose fiber surface treatment with TiO_2 suggested a direct chemical bond between the two materials.[97] These coatings were exposed to different intensities of light and the cell density was calculated over time. This study showed that with increasing light intensities, in this case ultraviolet light, the cell density drastically decreases at a faster rate than other intensities used.

TiO_2 can be used for a variety of applications, however where it is used depends on its crystal structure. In the rutile form, TiO_2 is able to alter a material's opacity and resistance to discoloration (oxidation), such as with paints, paper, inks, plastics, cosmetics, and pharmaceuticals.[102,103] This form of TiO_2 is also commonly found in chewing gums, candies, and sweets.[103] However, when in the anatase crystal structure, TiO_2 is 100 times more toxic, thus used to elicit antimicrobial properties.[104] Specifically, exposing the anatase form of TiO_2 to a broad spectrum of wavelengths permits for control over the production of ROS, therefore regulating the bactericidal effect. This permits for use in sterilization of medical devices, household cleaning products, air-conditioning surfaces, water treatment facilities, and textiles.[105]

6. Physical Properties of Nanomaterials on Antimicrobial Activity

The mechanism of NMs as antibacterial agents is dependent not only on material chemistry, but is strongly correlated to the NM's physical properties, such as its shape, size, solubility, agglomeration, and surface charge. For example, morphology of NPs has been demonstrated to play a role in the efficacy of NPs antiseptic activities. A study conducted by Raza et al. exposed spherical and triangular NPs of varying sizes to *P. aeruginosa* and *E. coli*.[106] In both cases, the smallest, spherical particles were the most effective in destroying the bacteria. Small NPs (less than 30 nm) are more likely to penetrate bacterial cell walls due to the increased surface area to volume ratio.[101] The higher efficacy of smaller NPs was confirmed in a separate study, showing that spherical nanoparticles of 5 nm in diameter had the highest probability to interact with *E. coli*.[23] The [1 1 1] facets on the spherical NPs contributed to the high reactivity with the bacterial membrane.

Although the physical size of NPs strongly influences the ability of NMs to enter or penetrate bacterial cell walls, nanoparticle solubility and agglomeration also play an essential role in the cytotoxic response. Agglomeration dictates the behavior and genotoxicity of the NP within a system. Specifically, particle solubility assesses NPs' intrinsic and extrinsic properties, governing the bioavailability within a living system.[107] Poor solubility has been shown to elicit a decreased cytotoxic response.[107] This is attributed from two different actions, chemical composition (release of ions, ROS, or surface chemistry) and/or stimuli induced by the surface, size, or shape of the NPs.[107] Particle solubility also influences the ability of a particle to agglomerate within a system. Agglomeration has been shown to control interactions of NPs with cells.[108] Specifically, highly agglomerated NPs do not have the capability to enter a cell or produce a significant amount of ROS.[109] This can be due to an overall size increase and decrease in surface area exposed within the overall system which corresponds with particle agglomeration. However, when particles do not agglomerate, they are able to distribute themselves, thereby increasing interactions and ROS production. Agglomeration is highly dependent on the materials' hydrophobicity, interactions in dispersed medium (e.g., pH, protein content), and surface charge.[108]

Morones et al. theorized that the increased bactericidal activity was also due to "electronic effects," or zeta potential, of the NPs.[23] NPs with positive zeta potentials permit for electrostatic interactions with bacteria's negatively charged surface. This pulls the NM into the bacteria, penetrating the cell membrane. Strong zeta potentials promote a strong interaction, causing membrane disruption, bacteria flocculation, and a reduction of viability.

While physical attraction of metal-based NP to bacteria is a desirable property, the opposite holds true for nanosurfaces. Nanosurfaces are commonly used on prosthetic or graft implants, to prevent bacteria adhesion and biofilm formation.[110] For this NM, a negative zeta potential or surface charge is vital to minimize the interactions with the negatively charged bacteria. With this in mind, nanosurfaces of comparable chemistry to NPs may not contain antimicrobial properties. The roughness provided by nanosurfaces prevents contact of bacteria with material surfaces due to relative rigidity of the cell.[101] Considering these properties, by controlling the particle's zeta potential and diameter, the antimicrobial effects can be modulated.

The multifaceted associations between NM physical properties (e.g., structure, shape, and zeta potential) and antimicrobial efficiency have emerged as a developing field to investigate novel solutions across a broad scientific community. NMs have yet to be fully understood and characterized, thereby limiting its

translational potential. However, this lack of knowledge may lead to novel antimicrobial breakthroughs. Specifically, given the increased surface area of 2D NMs, such as graphene, black phosphorous, or transition metal dichalcogenides, have the potential for increased interactions with the bacteria and increased production of ROS, therefore it can elicit an increased antimicrobial response. While graphene has currently been studied as antimicrobial agents,[111] these studies can be extended to materials such as black phosphorous or molybdenum disulfide (MoS_2), which has currently demonstrated ROS production with photo-thermal stimuli.[112]

7. Conclusions

With an increase in antibiotic resistance, metal-based NPs provide a novel antimicrobial therapy alternative. These NMs provide a solution for an effective, long-term antibacterial and biofilm preventing material by exhibiting bactericidal properties through ROS generation, protein adhesion, and membrane instability. In the future, metal-based NMs are foreseen to be combined with antibiotics for optimal antimicrobial activity due to its additive nature. Metals are able to discriminate between prokaryotic and eukaryotic cell types, however they are limited due to their potential toxicity to human cells. Eukaryotic cell injuries associated with metal poisoning needs to be clearly identified in order to apply such antimicrobial agents in medical applications. In order for these materials to be used in modern day health care, the key challenge will be to ensure human toxicity is prevented and/or minimized. New strategies to target metal toxicity can aid in overcoming the concerns associated with utilizing metal-based nanoparticles as an antimicrobial agent in the clinical setting.

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Conflict of Interest

The authors declare no conflict of interest.

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antimicrobial, metal nanoparticles, metal-oxide nanoparticles, multi-drug resistant bacteria, nanomaterials

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