Leveraging metal oxide nanoparticles for bacteria tracing and eradicating

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Abstract
Rapid emergence of antibiotic resistance facilitates the development of a number of novel-acting alternatives. Among these emerging approaches, metal oxide nanoparticles receive great attention due to their distinctive performance in antimicrobial stewardship. These nanoparticles can not only target the cell wall, membrane, and cytoplasmic contents to disrupt cellular homeostasis, but can also generate reactive oxygen species highly cytotoxic for virtually all microorganisms without resistance concern. By taking advantage of inherent imaging characteristics and facile surface functionalization with specific imaging moieties, the metal oxide nanoparticles show great promise in the bacterial tracing and eradicating. In this review, we examine a critical analysis of antimicrobial mechanisms, physicochemical characteristics, and modification strategies for metal oxide nanoparticles. The diagnosis of metal oxide nanoparticles for bacterial infections, coupled with their potential for bacterial theranostics, has been highlighted. We anticipate that this review will provide new insights on design and development of advanced metal oxide nanoparticles to manage bacterial infections, particularly those caused by multidrug-resistant species.

KEYWORDS
antibiotic resistance, metal oxide nanoparticles, reactive oxygen species, theranostics

INTRODUCTION
Pathogenic bacterial infections possess mounting public health concerns and cause an enormous medical or financial burden due to the rapid emergence of antibiotic resistance.1,2 Generally, the main reason for antibiotic resistance is the production of variant enzymes that can modify, degrade, or inactivate the antibiotic in bacteria.3 For example, the bacteria can adapt and produce the β-lactamase enzyme for cleaving the β-lactam ring and neutralizing penicillin.4 Besides, the bacterial alterations of efflux pumps, binding target sites, and drug entry ports can change the medications entry or clearance from bacterial cell, which will accelerate the emergence of
antibiotic resistance. To catch more attention of this issue, the World Health Organization declared that we have entered the “postantibiotic” era in which minor wound and infections can induce deaths easily. Even worse, the discovery of novel-acting antibiotics is essentially frozen. In the quest for novel alternatives, the development of new multi-mechanism drugs against bacteria, especially the multidrug-resistant (MDR) species, is extremely urgent.

In addition to multidrug resistance, another major obstacle for antimicrobial stewardship is the relatively delayed diagnostic technologies. Current detections of bacteria mainly rely on conventional tissue biopsies and microbiological testing, which are time-consuming and highly inefficient. In clinical cases, many infectious diseases are still diagnosed by their clinical presentation. However, in the early stage of the disease, clinical symptoms and signs of clinical manifestations are often unspecific, resulting in continuous increasing of misdiagnosis and subsequent mismanagement. On the contrary, fast diagnosis of bacterial infections can guarantee the implementation of correct therapeutic regimen, avoid the inordinate prescription of antibiotics and, in turn, reduce the emergence of antibiotic resistance. Therefore, management of bacterial infections, especially those caused by MDR bacteria, requires a multipronged approach that contains not only the development of novel-acting antibacterial agents, but also fast diagnosis for early-stage infection, so as to ensure implementation of targeted and rational infection control principles.

With the blooming interface of nanotechnology and biotechnology, nanomedicine is becoming a significantly attractive strategy for bacteria theranostics and has substantially altered the concepts of conventional antimicrobial stewardship. Nanomaterials possess great ability for improving the pharmacokinetics and effectiveness of therapeutic or imaging agent because their high accumulation at disease site, long-term blood circulation, and great flexibility for functionalization due to larger surface area. Besides, the potent capability of nanomaterials for drug loading also provides an efficient platform for integrating collaborative treatments. For instance, the succeed application of genetically engineered cell membrane-derived nanovesicles effectively combined antibacterial sonodynamic therapy with antivirulence immunotherapy, resulting in a complete eradication of MDR bacterial infections. Among all these newly discovered nanomaterials, the metal oxide nanoparticles play a crucial role in antimicrobial domains before the discovery of antibiotics in 1920. These oxide nanoparticles possess greater durability, lower toxicity, and higher stability. Just like antibiotics, metal compounds are able to discern between bacterial and mammalian targets due to the cell-deviated metal transport systems and the specific functions of metalloproteins. Till now, a diversity of metal oxide nanoparticles, such as Ag₂O, CuO, MgO, TiO₂, Fe₃O₄, ZnO, and Co₃O₄ have been proved to exhibit selective toxicity on bacteria, while showing a minimal impact on mammalian cells. More strikingly, it is great bonus that some metal oxide nanoparticles also possess inherent imaging characteristics and therefore can be used as imaging probe to diagnose bacterial infections. For example, Fe₃O₄ nanoparticles, by taking advantage of superior superparamagnetic qualities, have the potential to be manipulated by the external magnetic field and developed as magnetic resonance imaging (MRI) agents. From this perspective, metal oxide nanoparticles as a promising theranostic platform provide golden opportunities for enhancing therapeutic responses and improving our ability to implement and optimize current antimicrobial stewardship.

Although currently developed metal oxide nanoparticles have been elaborated with specific characteristics, great improvements are also available because the performance of metal oxide nanoparticles can be affected by a variety of factors, such as synthesis, shape, size, composition, and the addition of stabilizer. As an alternative medicine for bacterial theranostics, the biosafety of metal oxide nanoparticles plays a significant role governing their potential clinical translation. However, it is inevitable for metal oxide nanoparticles to remain some poison components. Moreover, their physicochemical characteristics can not only influence the antibacterial activity, but determine the potential cytotoxicity of nanoparticles on normal cells. Therefore, elaborately regulating the physicochemical properties of metal oxide nanoparticles is of great pivotal for bacterial theranostic application.

In this review, we focus on the antibacterial activity of metal oxide nanoparticles with detailed introduction on the mechanisms of antibacterial activity and physicochemical characteristics that may influence their bioactivity and toxicity. The proper modification strategies that can reduce their toxicity and improve therapeutic effect are discussed either. Furthermore, the diagnosis of metal oxide nanoparticles for bacterial infections, coupled with their potential for bacterial theranostics, is also highlighted (Scheme 1).

## ANTIBACTERIAL MECHANISMS OF METAL OXIDE NANOPARTICLES

Antibiotics are the current standard to combat bacterial infections by altering prokaryotic cell components that are not present in eukaryotic cells. Their antibacterial activity is susceptible to bacteria-associated resistance that mainly results from chromosomal mutation. At present, how to solve the microbes resistance is a vital problem. As a substitute for antibiotics, metal oxide
The metal oxide nanoparticles have inherent capacity in antimicrobial stewardship. (A) A variety of strategies have been developed to reduce the biotoxicity and immunogenicity of metal oxide nanoparticles via surface coating and hybridization for improved bacterial eradicating and tracing. (B) The metal oxide nanoparticles can not only target the cell wall, membrane, and cytoplasmic contents to disrupt cellular homeostasis, but can also generate reactive oxygen species (ROS) highly cytotoxic for virtually all microorganisms without resistance concern. (C) By taking advantage of inherent imaging characteristics and facile surface functionalization with specific imaging moieties, metal oxide nanoparticles show great promise in the bacteria tracing.

nanoparticles typically show less potential to induce resistance due to nonspecific antibacterial mechanisms (Figure 1). Here, in this section, we systemically introduce the antibacterial mechanisms of metal oxide nanoparticles.

### 2.1 Disrupting bacterial cell wall

As the most distinctive feature compared with the mammalian cells, bacterial cell wall is a thick, tough, and slightly elastic structure present outside of the cell membrane. It has a great capacity to determine the antigenicity of bacteria, to maintain the morphology of bacteria, to protect bacteria from the low-permeability environment, and as a potential defense against hazardous substances for bacteria. The main component of bacterial cell wall is peptidoglycan layer, also known as mucopeptide that decides the mechanical strength of cell wall. Depending on the different structure of cell wall, bacteria can be divided into gram-positive bacteria (G⁺ bacteria) and gram-negative bacteria (G⁻ bacteria). Generally, the cell wall of G⁺ bacteria is relatively thick (20-80 nm), and their chemical compositions are simple. By contrast, the cell wall of G⁻ bacteria is thin (10-15 nm), and has a multi-layer structure (peptidoglycan and lipopolysaccharide layers, etc). Both of the bacteria exhibit an overall negative charge out of the cell surface, mainly attributed to the lipopolysaccharide in G⁻ bacteria and the teichoic acids in G⁺ bacteria. Such anionic segment provides great potential to connect with positively charged metal oxide nanoparticles. As a typical example, the bare CuO nanoparticles with positive charge at neutral pH can effectively adhere to the
negatively charged bacterial cell wall via electrostatic interactions, and then revealing excellent activity inhibition on various bacteria, including *Escherichia coli*, *Streptococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*.\(^{33}\) Apart from the CuO, other metal oxide nanoparticles such as Ag\(_2\)O, Al\(_2\)O\(_3\), ZnO, MgO, and TiO\(_2\) also have inherent ability for cell wall anchoring and further release cationic ions in solution.\(^{34}\) With the concentration increasing, such cationic ions perform high affinity to the functional groups in bacterial cell wall, thereby interfere its biological functions and cause bacterial death.

### 2.2 Influencing bacterial cell membrane

Biological membranes are composed of a phospholipid double layer and particular proteins embedded, not only acting as a semipermeable barrier between the cytoplasm and outside medium, but also providing potential communication channels with surrounding environment.\(^{35}\) Specifically, the membrane proteins are often involved in basic cell behaviors, such as solute and ion transport, sensory stimuli transduction, and information processing.\(^{8}\) Hence, the membrane integrity is critical for cell functions, and the specific proteins expressed on the membrane surface are important drug targets for therapeutics and diagnosis.

It has been proved that metal oxide nanoparticles can influence the cell membrane potential and integrity of bacteria through electrostatically binding to the bacterial cell wall and/or releasing metallic ions. These interactions disrupt the bacterial membrane and produce increased oxidative stress to damage bacterial proteins. Due to the breakage of cell membrane, abundant amount of water is released from bacterial cytosol.\(^{14}\) To compensate this water loss, bacteria effectively activate proton efflux pumps and electron transport. However, the chain reactions cause cascaded severe damage to these transmembrane systems and finally break the intracellular homeostasis. Overall,
The production of ROS by binding with specific cytoplasmic targets

Bacterial cell cytoplasm is a primary location for bacterial metabolism, mainly including nucleic acid, protein, lipid, water, inorganic salt, and so on. The antibiotics such as macrolides, aminoglycosides, and chloramphenicol, can interact with nucleic acid to hinder its regular physiological function. However, rapid and continuing evolution facilitates bacteria to generate enzyme for antibiotics inactivation or induce these antibiotics efflux from cell. To overcome such resistance mechanism, metal oxide nanoparticles with specific characteristics have been developed. Such nanoparticles with size below 30 nm can easily penetrate into cellular environment of bacteria, facilitate toxic metal ions ionizing, and target various cytoplasmic sites, especially intracellular proteins, enzymes, and DNA. Along with the binding interaction between metal ions and cytoplasmic contents, bacteria gradually lose their cell integrity and normal metabolic activity, ultimately resulting in death. For example, the Ag$^+$ was reported to have the ability of entering bacterial cell. It can destroy the hydrogen bonding through inserting itself between pyrimidine and purine base pairs, thereby damaging the DNA molecule. Likewise, CuO nanoparticles after passing through the bacterial cell membrane can inhibit cytoplasmic enzyme activity and bind to bacterial DNA chain, followed by biochemical disorder and bacterial inactivation. Benefiting from such cytoplasm-mediated action mechanism, CuO nanoparticles showed superior antimicrobial ability against multiple microbes including E. coli, P. aeruginosa, Klebsiella pneumonia, Enterococcus faecalis, Shigella flexneri, Salmonella typhimurium, Proteus vulgaris, and S. aureus.

2.4 Generating reactive oxygen species

Although antibacterial mechanism of metal oxide nanoparticles is still under controversial, the formation of reactive oxygen species (ROS) is thought to be the main mechanism of their antibacterial activity. Generally, ROS include hydrogen peroxide (H$_2$O$_2$), free radicals such as hydroxyl radical (•OH), singlet oxygen (1O$_2$), and superoxide ions (O$_2$•$^-$), which exhibit different levels of antimicrobial activity. Compared with H$_2$O$_2$ and O$_2$•$^-$, the •OH and 1O$_2$ are more poisonous, and are hardly inactivated by various enzymes in biological environment. At present, many studies have illustrated the high lethal activity of ROS toward bacteria via impacting the bacterial cell membrane, protein, DNA, and electron transport chain. Owing to nonspecific action mechanism and low mutagenic potential, ROS have been shown to be highly cytotoxic for virtually all microorganisms with no resistance concern. It is noted that the production of ROS by metal oxide nanoparticles mainly relies on endogenous reactions and exogenous physical excitation (Figure 2), which is originally considered as the basic classification profile in the following discussion.

2.4.1 The production of ROS by endogenous catalysis

Similar to the ROS-generating capability of hydrogen peroxidase, the released metal ions from metal oxide nanoparticles also possess the ability to catalyze hydrogen peroxide into destructive hydroxyl radical, which is defined as Fenton reaction and Fenton-like reaction. Such ROS production process is widely observed on Fe$_2$O$_3$ nanoparticles because they readily provide reductive Fe$^{2+}$ ion to trigger Fenton reaction in the low acidic microenvironment of bacterial infection lesions (Figure 3). In addition to Fe$^{2+}$ ion, some redox-active transition metals including Cu, Cr, Co, V, and Ni can catalyze Fenton chemistry to produce ROS in vitro. Recently, MgO nanoparticles were verified to continuously produce H$_2$O$_2$.
FIGURE 2  Schematic illustration of the generation of reactive oxygen species (ROS) on metal oxide nanoparticles antibacterial surface and sterilization mechanisms. (A) Mechanism of ROS generation on the surface of titanium or zinc oxides. (B) ROS have the ability to penetrate the bacteria cell envelope and cell wall; plasma membrane as well as nucleoid will have great influence by ROS-induced damage. (Reproduced with the permission. 55 Open access.)

FIGURE 3  Scheme of •OH free radical generation by iron oxide nanoparticles in biomicroenvironment. (A) The detailed process of •OH generation on the interface of Fe2O3 nanoparticles. (B) Detailed chemical process diagram of •OH generation by Fe2O3 nanoparticles with the existence of main ingredients (H2O2 and H+). (C) Diagram of intracellular action and process of iron oxide nanoparticles. After the magnetic iron oxide nanoparticles entered into the cells via endocytosis, the dissolution or reductive dissolution will occur in the acid lysosomal microenvironment. Subsequently, these decomposed free Fe ions or nanoparticles release into the cytoplasm and specifically react with hydrogen peroxide or superoxide in mitochondrion to produce reactive •OH via Fe(II)/Fe(III) catalytic Fenton/Haber–Weiss type reaction. (Reproduced with the permission. 56 Copyright 2013 American Chemical Society.)
via inherent catalytic activity on glucose oxidase. Benefiting from the strong ROS-generating activity, the minimal inhibitory concentrations of MgO nanoparticles to $10^4$ colony-forming unit/mL (CFU/mL) of Campylobacter jejuni, E. coli O157:H7, and Salmonella Enteritidis were determined to be 0.5, 1, and 1 mg/mL, respectively.\textsuperscript{59}

2.4.2 | The production of ROS by exogenous physical excitation

Exogenous physical excitation is the other important strategy to generate ROS by metal oxide nanoparticles. This approach mainly depends on photodynamic therapy that utilizes specific wavelength of light to activate photosensitive metal oxide nanoparticles for ROS production.\textsuperscript{60} It is worth noting that the metal oxide nanoparticles themselves have no inhibitive activity and are very low in toxicity. They are bioactive only when exposed to light stimulation. Moreover, the applied light irradiation is nonionizing and can be accurately confined to the lesion site, without any damage to adjacent normal tissues. Thus, the potential systemic toxicity that frequently troubles antibiotic treatment is promisingly avoided.\textsuperscript{61} Titanium dioxide (TiO$_2$) nanoparticles as a typical photocatalyst have been widely investigated in antibacterial territory. It has been proved that TiO$_2$ nanoparticles can effectively absorb UV spectrum for high quantum yield of ROS due to the recombination of electron-hole pairs.\textsuperscript{62–64} When replacing light with low-frequency ultrasound excitation, a potent antibacterial sonodynamic therapy was performed by TiO$_2$ nanoparticles.\textsuperscript{65,66} Compared with photo-induced antibacterial therapies that are limited to skin lesion, such sonodynamic therapy takes advantage of superior tissue penetrability of ultrasonic wave, showing great potential in deeply seated diseases.\textsuperscript{67,13}

3 | PHYSICOCHEMICAL CHARACTERISTICS OF METAL OXIDE NANOPARTICLES FOR ANTIBACTERIAL THERAPY

It is known that the antibacterial efficiency of metal oxide nanoparticles is closely related to various physicochemical factors, primarily including size, morphology, surface charge, solubility, agglomeration, dispersity, and crystal structure.\textsuperscript{18} Up to now, a diversity of metal oxide nanoparticles have been proved to perform the bactericidal effect depending on different physicochemical characteristics. For example, the modulation of size, morphology, and surface of ZnO nanoparticles was demonstrated to effectively control the sterilizing effect, although their antibacterial mechanisms remain controversial.\textsuperscript{68} In this section, we mainly focus on how physicochemical characteristic influence the functionality and application of the commonly used metal oxide nanoparticles.

3.1 | Size, morphology, and surface charge

Compared with small biological molecules, nanoparticles tend to play a more effective role on antibacterial agents. It has been extensively recognized that the smaller size of metal oxide nanoparticles possesses the larger surface area to volume ratio, which can promote nanoparticles permeation into bacterial cell more easily, resulting in better antibacterial performance.\textsuperscript{14} However, the defects of over-small nanoparticles should also be taken into account, because the decreased size leads to aggregation that is detrimental to antibacterial effect of nanoparticles.\textsuperscript{69} Apart from therapeutic activity, the size of nanoparticles is also closely related to their toxicity. Appropriate size may allow for effective toxicity control. If the size of nanoparticles is too small, their toxicity expectantly increases.\textsuperscript{70} Other than size, the morphology of nanoparticles also has been widely investigated and different shapes such as spheres, triangles, sheets, plates, tubes, cubes, and rods directly affect antibacterial efficiency.\textsuperscript{15} In general, the spherical nanoparticles with size about 5 nm were reported to be most effective in antibacterial treatment due to the intimate interaction between nanoparticles and bacterial surface at such morphology parameters.\textsuperscript{14,71} Nevertheless, the phenomenon that spherical nanoparticles represent superior effect is not absolute; different morphologies of nanoparticles have their own unique properties. In one research, CuO nanoparticles with plate-shape seem to be more active compared with other morphologies, because the plate-like CuO nanoparticles promote the release of Cu$^{2+}$, leading to greater cytotoxicity on bacterial cells.\textsuperscript{72} As for photodynamic therapy, the phototoxicity of metal oxide nanoparticles is deeply affected by morphology as well. It has been showed that TiO$_2$ nanoparticles with rod or sphere shape exhibit more phototoxicity than tube and sheet shapes, resulting in higher photocatalytic activity and more ROS production.\textsuperscript{73} This is mainly because that nanoparticles morphology has a significant impact on the interaction between nanoparticles and bacterial cell outer shell. Other than the size and morphology, the surface charge of metal oxide nanoparticles is another key factor deciding antibacterial performance.\textsuperscript{74} It is known that the bacterial cell membrane and cell wall with negative charge can closely interact with positively charged nanoparticles via electrostatic binding.\textsuperscript{75} When the nanoparticles bind with cell envelope via electrostatic attraction, the function
of bacterial wall will be deeply influenced and cause an efficient permission of metal oxide nanoparticles into the bacterial cells to induce serious damage. In addition, the positively charged metallic ions released by metal oxide nanoparticles are able to effectively penetrate into bacterial cell membrane, and then trigger morphological change and membrane leakage of bacteria, ultimately impairing bacterial growth. Therefore, changing and controlling the size, morphology and surface charge are promising to fabricate desirable metal oxide nanoparticles that can not only perform highly efficient antibacterial therapy but also show minimal or even no side effects.

3.2 Solubility, agglomeration, and dispersity

Although the nanosized metal oxide nanoparticles are available to significantly combat bacteria, the inherent characteristics of low solubility cause a great loss of antibacterial ability and induce an increase in toxicity. Solubility deeply determines many important properties of nanoparticles, especially their surface area, which can control the interaction between nanoparticles and microorganism. Until now, many researches have testified that employing the surfactants to modify nanoparticles can remarkably increase their solubility and improve the bacterial inhibition and killing capacity. In addition, the nanoparticles tend to aggregate when the solubility is below a specific level and the agglomeration dictates the behavior and biotoxicity of nanomaterials within a system. The explanation is that agglomeration increased the size and decreased the surface area to volume ratio, both of them enormously limit the connection between nanoparticles and bacteria. Moreover, high agglomeration further prevents nanoparticles from getting access to bacterial cell wall and cell membrane. On the other hand, many metal oxide nanoparticles have the ability to generate a large amount of ROS, but the agglomeration will bring a sharp decrease of ROS production. Therefore, tactics for keeping metal oxide nanoparticles away from aggregation and endowing them with great dispersity in living system are expected to make nanoparticles more effective and achieve excellent quantum yield of ROS for complete sterilization.

3.3 Others

Other than abovementioned characteristics, the influence factors of antibacterial activity also include crystal structure and the concentration of metal oxide nanoparticles. It has been reported that crystal structure of titanium nanoparticles significantly governs their antibacterial activities. For instance, TiO₂ nanoparticles have two most common crystal structures, rutile and anatase form. Compared with rutile crystal, anatase TiO₂ form shows more potent antibacterial ability due to its higher photocatalytic property to absorb UV light for hydroxyl radical generation. Besides, the concentration of nanoparticles plays an important role in antibacterial effect. With the concentration rising, there is increased chance for nanoparticles to adhere and interact with bacterial cell wall. As a typical example, MgO nanoparticles exhibit their bacterial activity in a concentration-dependent manner. In the case of high drug concentration, the long-time adhesion between nanoparticles and bacteria induces cell membrane damage and cellular content leakage. Furthermore, the productivity of ROS is also relevant to nanoparticles concentration. Unequivocally, MgO nanoparticles used in high concentration produce more superoxide, which effectively suppress E. coli and S. aureus growth. Nevertheless, the side effects for healthy tissues and organs raise with the higher concentration. In conclusion, antibacterial application of metal oxide nanoparticles is affected by various aspects, which may provide us an innovative method to optimize nanoparticles for more effective microbe inactivation.

4 DECORATION OF METAL OXIDE NANO Particles FOR ANTIBACTERIAL THERAPY

Nanomaterials have numerous applications in areas ranging from catalysis, photonics, energy storage, and molecular imaging to disease treatment. Compared to their microsized counterparts, nanoscaled materials exhibit larger surface-to-volume ratio, which provides unsaturated and thus more reactive surface atoms. Considering nanoparticles for biological applications, such as drug delivery, bacterial imaging, and therapy, several key requirements have to be fulfilled. The first is to elaborate the engineered nanoparticles with well-characterized composition, size, crystallinity, and morphology. The second involves the manipulation of stabilized, nonagglomerated nanoparticles for facile dosage control. Finally, the most crucial prerequisite is their biocompatibility. Despite the fast development of bionanotechnology in the last 30 years, many challenges are still plaguing these three requirements.

As one kind of potential next-generation antibacterial agents, metal oxide nanoparticles are promising to resolve the obstacle of bacterial resistance. In practice, pristine metal oxide nanoparticles without any decoration constitute several problems, such as agglomeration, potential cytotoxicity, and protein crown formation, resulting in
bacterial cell out of nanoparticle recognition. Moreover, metal oxide nanoparticles are synthesized through multistep chemical methods, which may import harmful substances to damage cell health. To achieve more effective theranostic activity, the exterior coating, functional groups, or compounds for bioconjugation are widely introduced as the excellent methods for surface modification of nanoparticles. Here, in this section, some excellent decoration tactics for reducing the biotoxicity and enhancing their antibacterial performance will be highlighted.

4.1 | Surface coating for reducing biotoxicity

As mentioned above, the inherent biotoxicity is the most vital conundrum limiting the application of metal oxide nanoparticles. Collaborative using non-biotoxic or less toxic substance for surface modification will be one of the most effective strategies to improve these situations. For example, Bharath et al applied rutin-modified chitosan to coat iron oxide nanocomposite. In this new strategy, the surface coating effectively improved the therapeutic index of metal oxide nanoparticles due to the potent antimicrobial properties, low toxicity, great biodegradability, and biocompatibility of chitosan. Rigorous experimental results demonstrated that both $G^+$ bacteria and $G^−$ bacteria could be effectively eradicated by such chitosan-modified nanoparticles. Apart from the chitosan for surface decorating, other materials such as hydroxyapatite (HA), polymethyltrimethoxysilane, polydopamine, and carbon nano tubes have been widely used for metal oxide nanoparticles surface coating.

4.2 | Hybrid structures for enhancing antibacterial performance

Apart from reducing their biotoxicity and immunogenicity via surface coating method, another common decoration method is to synthesize hybrid nanomaterials, such as multi-metal oxide nanoparticles and metal oxide nanoparticles-doped hybrids. In the case of multi-metal oxide nanoparticles, they could integrate multiple beneficial metal components, thereby stimulating diverse mechanisms against bacteria without resistance generation. A representative paradigm is Fe/Cu oxide nanoparticles. Depending on the favorable antibacterial property of Fe and Cu components, this nanoplateform exhibited stronger inhibition zones for *Bacillus subtilis*, *S. aureus*, *S. typhimurium*, and *E. coli* than commercial antibiotic gentamycin. Similar improvement on antibacterial effects has also been proved in graphene oxide/Ag nanoparticles and AgCl/ZnO or Mg/Ag alloy. As for metal oxide nanoparticles-doped hybrids, they are efficient platforms to combine multiple therapeutic modalities with synergetic effects, and therefore are promising for enhancing antibacterial efficiency and possibly reducing the needed concentration of bactericidal reagents. For example, ZnO-doped carbon on graphene (TRB-ZnO@G) nanosheets with phase transformable thermal-responsive brushes (TRB) polymer layers was developed recently (Figure 4A). With the aid of multiple function materials, such hybrid provided many unique characteristics such as the localized massive Zn$^{2+}$ ions penetration for bacterial damage, physical cutting, and NIR-triggered photothermal effect (Figure 4B), which result in synergistically enhanced disruption of bacterial membranes and intracellular substances, thus achieving broad-spectrum and robust antibacterial activities.

5 | BACTERIAL DIAGNOSIS OF METAL OXIDE NANOPARTICLES

In recent years, nanomaterials are not only developed as therapeutic drugs for antibacterial management, but also examined in the context of pathogen labeling and tracking. It is well known that nanoparticles tend to locate in tumor or inflammation site due to the enhanced permeability and retention effect. Such disease foci-targeted high accumulation, coupled with inherent imaging characteristics, facilitates metal oxide nanoparticles as promising diagnosis agents to detect bacterial infections. From this perspective, magnetic nanoparticles such as Fe$_3$O$_4$ nanoparticles represent a great paradigm. In clinic, MRI is a commonly used imaging method that can aid disease diagnosis and is well established for in vivo application, providing outstanding spatial resolution and significantly avoiding radiation-associated risk in other current clinical approaches. Depending on the inherent magnetic and electronic characteristic, Fe$_3$O$_4$ nanoparticles have been extensively explored in detecting a range of inflammatory pathologies. For example, Baraki and colleagues using FeraTrack superparamagnetic iron oxide nanoparticles successfully labeled neutrophil granulocytes through the passive uptake of granulocytes-mediated phagocytic phenotype. After injecting such magnetic nanoparticles-loaded cells into *S. aureus*-infected rats, the granulocytes effectively migrated to infected tissues and were clearly observed by MRI technique. This excellent approach effectively facilitates diagnosis of bacterial infections, while providing a biological insight into the process of infection. A similar strategy was adopted by Hoerr et al, who labeled *S. aureus* with iron oxide nanoparticles before in vivo inoculation, thereby allowing
real-time visualization of the infected organs and the resulting host inflammatory response.\textsuperscript{101} In addition to MRI function, Fe\textsubscript{3}O\textsubscript{4} nanoparticles after gold coating can serve as a sensitive surface-enhanced Raman scattering (SERS) platform for bacterial detection.\textsuperscript{102} As shown in Figure 5, these Fe\textsubscript{3}O\textsubscript{4}@Au magnetic nanoparticles, with the aid of dual recognition by vancomycin and aptamers, showed rapid capture of bacteria from complex solutions with an efficiency as high as 88.89\% for \textit{S. aureus} and 74.96\% for \textit{E. coli} within 15 min. Notably, the limits of detection were as low as 20 cells/mL for \textit{S. aureus} and 50 cells/mL for \textit{E. coli}, which indicates great potential applications of nanoparticles for accurate diagnosis of pathogens, especially in clinical samples.

Although metal oxide nanoparticles have proved promising in diagnosing bacterial infection, this approach is still at the preliminary stage and its related researches are mostly limited to magnetic Fe\textsubscript{3}O\textsubscript{4} nanoparticles.
As discussed before, metal oxide nanoparticles possess extremely high flexibility, and a large variety of materials and preparation technologies are available. The chemical composition and architecture of nanoparticle matrices can be easily designed to accommodate application requirements. More importantly, their large surface area provides great opportunity for functionalization. In the modification process using imaging probes such as indocyanine green, the metal oxide nanoparticles are expected to perform fluorescence and photoacoustic imaging modalities that are more attractive than clinically used MRI and nuclear imaging in the price, safety, and operational simplicity. Further elaborately fine-tuning, metal oxide nanoparticles can be readily tailored with bacteria-targeted ligands, implementing a precise signal readout for bacteria detection and imaging.

6 | BACTERIAL THERANOSTICS OF METAL OXIDE NANOPARTICLES

The alarming evolution and spread of MDR bacteria make antimicrobial stewardship more challenging. Clinicians are gradually going beyond the traditional “all-in-one” strategy of medicine toward a new era of personalized approach to antibacterial treatment. By integrating therapeutic and diagnostic capability in a single system, theranostics is expected to contribute significantly to the ever-growing field of personalized medicine, because medical treatments in such approach can be tailored to the specific characteristics of individual patients. Currently, metal oxide nanoparticles as theranostic platforms have been developed widely in oncology. Within the infectious diseases domain, bacterial theranostics that combines both therapeutic and diagnostic potential against bacteria, although relatively new, is rapidly developing. Such emerging antibacterial management serves as a valuable tool in the selection of a safe and effective dose, real-time monitoring of the therapeutic progress, and preserving our ability to control and optimize current antimicrobial stewardship. Further considering the interindividual variability in therapeutic response, this patient-specific treatment may offer better therapeutic responses, while minimizing patient discomfort and undesirable side effects.

As a typical paradigm, bull serum albumin-stabilized ZnO nanoparticles (ZnO@BSA-PEP-MPA) were functionalized with bacteria-targeted peptide fragment, and a near infrared dye for bacteria-selective optical imaging (Figure 6). Based on this theranostic platform, vancomycin was subsequently immobilized on its surface for more efficient antibacterial activity and lower biotoxicity relative to free vancomycin. Importantly, this ZnO-based nanoplatform was also proved to be potent optical imaging agent that can distinguish bacterial infection not only from oligochitosan- or lipopolysaccharide-induced sterilized inflammation but also from malignant tumor with high selectivity. Further decoration with antibiotic methicillin, such as theranostic strategy, revealed significant capability to combat the MDR bacteria. A similar approach was adopted by Ag/ZnO/reduced graphene oxide (Ag/ZnO/rGO) nanocomposites, which successfully integrate with photocatalytic activity of ZnO nanoparticles, high specific surface area, and near infrared photothermal conversion property of reduced graphene oxide, as well as the bacteria-killing capability of Ag nanoparticles. By taking advantage of the surface-enhanced Raman scattering property of Ag nanoparticles, this theranostic composite could also detect E. coli effectively.

7 | CONCLUSIONS AND FUTURE PERSPECTIVES

The ever-increasing microbial resistance to common disinfectants and antibiotics facilitates an urgent need for new multi-mechanism drugs against MDR bacteria. As one kind of desired alternative antibacterial agents, metal oxide nanoparticles provide a novel-acting treatment tactics outside of the traditional antibiotic pipeline. Compared with the prior antibiotics, employing metal oxide nanoparticles for bacteria tracing and eradicating shows many advantages. First, metal oxide nanoparticles have innate ability for discriminating between prokaryotic and eukaryotic cells, which is conducive to avert biotoxicity. Second, as a functional material of nanometer grade, metal oxide nanoparticles possess a built-in capacity for bacteria-targeted treatment through facilely regulating their structural and physical properties such as shape, size, and zeta potential. Third, metal oxide nanoparticles involve a variety of natural antibacterial mechanisms, including disrupting the rigid of cell wall and membrane to affect homeostasis, releasing toxic metal ions to target intracellular proteins, enzymes, and DNA for inducing abnormal physiological metabolisms, and producing destructive ROS under endogenous reactions and exogenous physical excitation. Intriguingly, the generated ROS are highly cytotoxic for virtually all microorganisms with no resistance concern due to nonspecific action mechanism. Finally, in addition to inherent imaging characteristics such as MRI, metal oxide nanoparticles can also serve as efficient carrier systems for specific imaging moieties loading. Further coupled with highly effective antimicrobial activity, it will be an efficient portfolio for metal oxide nanoparticles to accomplish bacterial theranostics.
Despite the multitudinous advantages mentioned above, some intrinsic and potential risks are still unsolved for metal oxide nanoparticles, significantly limiting their further applications. For example, the metal poisonousness on human cells still exists, although metal oxide nanoparticles have the capability in discriminating prokaryotic and eukaryotic cell types. New innovative modification tactics such as surface engineering to prevent and minimize their toxic effects are highly demanded in the clinical setting. Moreover, how to avert accumulation toxicity remains to be discovered. With further in-depth studies, it is expected to see the successful clinical translation of metal oxide nanoparticles for better safety and effectiveness.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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