INTERNATIONAL JOURNAL OF HEALTH & MEDICAL RESEARCH

ISSN(print): 2833-213X, ISSN(online): 2833-2148

Volume 05 Issue 07 July 2026

DOI : 10.58806/ijhmr.2026.v5i7n17

Page No. 699-711

Comparative Antibacterial Mechanisms of Metal Oxide Nanoparticles (Zno, Nio, Ceo_2, And Fe_2O_3): A Critical Review of Structure-Activity Relationships and Emerging Applications

Ghanem Abdulnabi Kazem*

Ministry of Education / Karbala Education Directorate

ABSTRACT

The global problem of the increase of antibiotic resistance has motivated several studies on alternative bactericidal agents. The promising materials include metal oxide nanoparticles (MONPs) due to their multi-mechanistic antibacterial action, chemical stability, and customizable physicochemical features. In this review, we critically and comparatively examined the antibacterial mechanisms of four extensively studied MONPs, namely zinc oxide (ZnO), nickel oxide (NiO), cerium oxide (**CeO_2**), and iron oxide (Fe_2O_3), emphasizing the impact of particle size, morphology, surface area, crystal phase, and synthesis route on their biological activity.The four bactericidal processes addressed here, ROS generation , membrane rupture , metal ion release , and intracellular DNA damage , are comparatively analyzed across the oxide systems with emphasis on mechanistic contrasts and overlaps. The action against Gram-positive and Gram-negative bacteria is established in parallel to the demonstrated efficacy against resistant clinical infections, especially MRSA. MIC statistics reported are extensively reviewed and cytotoxicity profiles essential for biological translation are assessed for each substance.

This review provides an integrated view of mechanistic, structural and biological data to identify major knowledge gaps and to propose directions for further research, in particular on standardization of antibacterial testing protocols and the importance of strong in vivo efficacy studies.

KEYWORDS: Metal oxide nanoparticles, Antibacterial mechanisms, ZnO, NiO, **CeO_2**, Fe_2O_3, Reactive oxygen species, Antimicrobial resistance, Structure activity connection

1.INTRODUCTION

Antimicrobial resistance (AMR) is one of the most serious public health threats of the twenty-first century. AMR has been declared as a worldwide priority problem by the World Health Organization (WHO). In 2019, drug-resistant bacteria were directly responsible for an estimated 1.27 million fatalities.[1] Under a business-as-usual scenario, this burden might rise to almost 10 million deaths per year by 2050, surpassing the deaths related to cancer.

The rapid development of resistance has not been matched by the discovery of new antibiotics, resulting in an ever widening therapeutic gap and a need for radically new ways to deal with infections. Metal oxide nanoparticles (MONPs) have become an interesting scientific alternative to conventional antibiotics. Unlike single-target pharmacological agents, MONPs function through multifarious bactericidal mechanisms, including production of oxidative stress through reactive oxygen species (ROS), physical disruption of cell membranes, release of toxic metal ions and interference with DNA replication and enzymatic activity.

Resistance to MONPs is fundamentally difficult to acquire since bacteria would have to acquire simultaneous mutations on many separate targets, a highly unlikely event.[6] In addition to their lethal effects, MONPs are thermally stable, active against a broad spectrum of pathogens and can be readily surface modified. These properties make them attractive candidates for wound care products, antimicrobial packaging, water purification units and coatings for implantable medical devices [7,8].

The most studied MONPs among them are zinc oxide (ZnO), nickel oxide (NiO), cerium oxide (**CeO_2**) and iron oxide (Fe_2O_3). Each of these oxides has a particular electrical structure, band gap and surface chemistry that combined dictate their antibacterial nature. Among the most investigated are ZnO nanoparticles, showing a significant activity under both UV and visible light irradiation by means of photocatalytic ROS production.NiO nanoparticles are far less studied yet exhibit significant activity by releasing Ni2+ ions and generating superoxide radicals [10].

The unique redox-switchable Ce3+/Ce4+ pair of **CeO_2**nanoparticles can act as antioxidants or pro-oxidants depending on the circumstances.[11] Fe 2 O 3 nanoparticles has magnetic properties and Fenton type catalytic activity, which produces hydroxyl radicals that are fatal to bacterial cells [12].

Notwithstanding this large quantity of data, the literature on MONPs antibacterial action is fragmented. Most existing evaluations treat various oxide systems separately and relatively few have tried to compare mechanisms systematically across different oxides [13,14].

The large range in MIC values reported for the same material (sometimes differing by two to three orders of magnitude) is a major problem and is a result of the differences in nanoparticle characterisation, bacterial strain selection, growth medium composition and test methodology.Fifteen The lack of standardization severely affects cross-study interpretation and hampers logical material design.The present review fills these gaps by a structured, critical evaluation of ZnO, NiO, **CeO_2**and Fe_2O_3nanoparticles in a uniform mechanistic and structure-activity context. This study aims: (i) to delineate the main antibacterial mechanisms for each oxide; (ii) to investigate the effect of physicochemical parameters on antibacterial efficacy; (iii) to determine the differential activity against Gram-positive and Gram-negative organisms; (iv) to assess the activity against resistant clinical strains; and (v) to critically assess cytotoxicity data relevant to biomedical translation. This review is meant to be a comprehensive reference for the researchers interested in understanding the molecular basis of MONP antibacterial activity and designing the future generation of the antimicrobial nanomaterials.

2. A Review on the Synthesis of Metal Oxide Nanoparticles and Its Effect on Antibacterial Properties

The synthesis procedure used for the production of MONPs is directly and significantly affecting the particle size, morphology, surface chemistry, crystal phase purity and defect density which are all the key parameters in determining the antibacterial activity . The knowledge of this relationship is vital in order to evaluate the heterogeneity found in the published antibacterial data and to design materials with improved efficacy [16].

2. CHEMICAL SYNTHESIS METHODS

The main chemical processes used for the production of MONPs are co-precipitation, sol-gel processing and hydrothermal synthesis. Co-precipitation method includes simultaneous precipitation of metal hydroxides from aqueous salt solutions with a base and thermal degradation to the oxide phase. Thus, nanoparticles are formed with relatively narrow size distributions, but with limited control over the morphology.17 The sol-gel synthesis consists of hydrolysis and condensation of metal alkoxide or inorganic salt precursors in order to generate amorphous or semi-crystalline intermediates, which are then converted into crystalline oxides through calcination.

The sol-gel method allows great control of composition and synthesis at relatively low temperatures.[18] Hydrothermal synthesis is a method that employs sealed autoclaves under high temperature and pressure conditions to generate highly crystalline products with well-defined morphologies, including nanorods, nanosheets, nanoflowers and hierarchical structures. The morphological diversity that can be obtained under hydrothermal conditions is of particular interest for antibacterial applications, since the particle shape has a profound effect on the surface area and the interaction dynamics with bacteria [19].

3. GREEN SYNTHESIS APPROACHES

Green synthesis using plant extracts, fungal filtrates or algal biomass has received considerable importance as an eco-sustainable alternative to the conventional procedures. Polyphenols, flavonoids, terpenoids and alkaloids are among the phytochemicals found in plant extracts, which work as reducing and capping agents in this process. The phytochemicals are responsible for lowering the metal ions and stabilizing the formed nanoparticles to avoid agglomeration.[20,21] The nanoparticles formed typically contain bioactive surface ligands that, in addition to the intrinsic properties of the oxide core, can operate independently to improve antibacterial activity. It has been shown in many studies that green-synthesized ZnO and **CeO_2**nanoparticles have a higher antibacterial effect than chemically synthesized ones of the same size. This is due to synergistic effects between the oxide surface and the remaining phytochemical capping agents [22,23].

The reproducibility of green synthesis processes remains a serious concern. The composition of plant extracts varies with the season and geography leading to differences in the features of nanoparticles prepared from different batches that impedes systematic research and scaling.A full physicochemical characterisation of each batch is necessary for a complete comparison of antibacterial efficacy between green- and chemically generated MONPs.

4. EFFECT OF SYNTHESIS PARAMETERS ON ANTIBACTERIAL EFFICACY

Irrespective of the synthesis route, several parameters consistently modulate the antibacterial properties of the resulting MONPs. Calcination temperature influences crystal phase, grain size, and oxygen vacancy concentration; higher temperatures generally promote crystal growth and reduce surface area, with concomitant effects on ROS generation capacity.[25] Precursor concentration

and pH during synthesis govern particle size and morphology. Post-synthetic doping with transition metals (e.g., Ag, Cu, Co) or non-metals (e.g., N, F) has been shown to narrow the band gap, extend light absorption into the visible range, and enhance photocatalytic antibacterial activity.[26] Table 1 summarizes the key synthesis-property-activity relationships for the four oxide systems reviewed herein.

Table 1. Summary of synthesis methods and their influence on antibacterial properties of selected MONPs.

Oxide	Common Routes	Typical Size (nm)	Morphologies Reported	Key Synthesis–Activity Relationship
ZnO	Green, hydrothermal, co-precipitation	10-100	Spheres, rods, flowers	Smaller particles → higher ROS; flower morphology ↑ surface area → ↑ activity
NiO	Sol-gel, green, co-precipitation	15-80	Spheres, sheets	Lower calcination → more surface defects → ↑ Ni^{2+} release
CeO_2	Hydrothermal, green, sol-gel	5–50	Spheres, cubes, rods	Higher Ce^{3+}/Ce^{4+} ratio → ↑ ROS under acidic conditions
Fe_2O_3	Co-precipitation, hydrothermal	20-100	Spheres, rods, spindles	Fenton activity ↑ with smaller size; H_2O_2 concentration critical

5. ANTIBACTERIAL MECHANISMS OF METAL OXIDE NANOPARTICLES

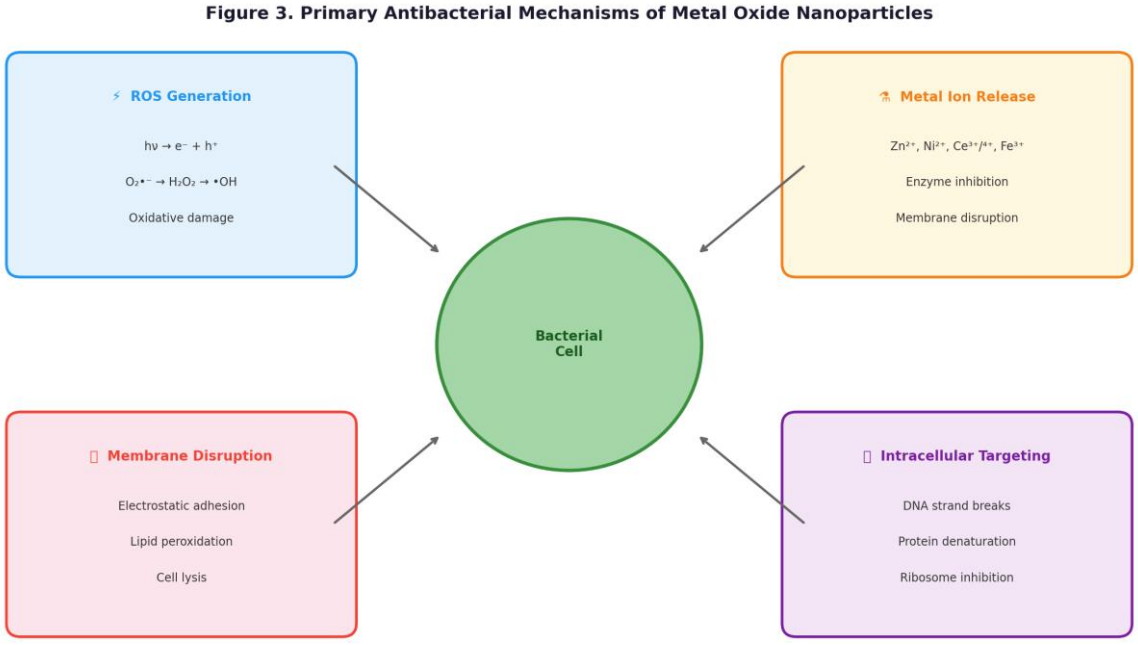

Figure 1. Schematic representation of the four main antibacterial processes of metal oxide nanoparticles: (1) ROS generation, (2) metal ion release, (3) membrane rupture and (4) intracellular targeting.

MONPs do not kill bacteria by a single mechanism. Rather, they work in concert through a variety of interconnected mechanisms, the relative importance of which depends on oxide type, particle size, shape, ambient circumstances and bacterial species. We describe the four major pathways as ROS-mediated oxidative stress, membrane rupture, metal ion release and intracellular targeting followed by a comparative mechanistic study below [27,28].

5.1 Reactivation of Reactive Oxygen Species (ROS) The main antibacterial mechanism of most Metal Oxide Nanoparticles (MONPs) is considered to be the production of ROS, especially under light. Photoexcitation with energy equal to or larger than the band gap promotes electrons from the valence band to the conduction band and so forms electron-hole pairs. These charge carriers

react with adsorbed water and molecular oxygen on the surface to provide superoxide radicals (O2•−), hydrogen peroxide (H2O2), and hydroxyl radicals (•OH).

These chemicals are highly reactive and non-selectively oxidize lipids, proteins and nucleic acids, leading to permanent damage to cells.ZnO has a band gap of about 3.37 eV allowing good photoactivation under UV light and also doped under visible light. The larger band gap of NiO (~3.6-4.0 eV) results in the formation of reactive oxygen species mainly by surface-mediated reactions rather than bulk photocatalysis, with superoxide radicals being the main species [31]. **CeO_2**works in a unique manner through the redox pair Ce3+/Ce4+: it works as an antioxidant under oxidizing conditions (alkaline pH) and, under the acidic pH typical of bacterial microenvironments, the Ce3+ state is dominant and allows for the Fenton-like formation of •OH. Fe_2O_3generates The Fenton reaction (Fe2+ + H2O2 → Fe3+ + •OH + OH−) produces •OH, which is particularly effective in environments with high concentrations of H2O2 [33].

5.2 Membrane Disruption

Another important bactericidal mechanism is the physical contact of MONPs with the bacterial cell membrane. Electrostatic attraction between the positively charged nanoparticle surfaces and the negatively charged bacterial cell wall (mediated by teichoic acids in Gram-positive species or lipopolysaccharides in Gram-negative species) enhances nanoparticle adherence and membrane penetration.[34] Nanoparticles can affect the fluidity of the lipid bilayer, cause lipid peroxidation by localized ROS formation, and physically disrupt the membrane by mechanical stress upon interaction with the membrane. This leads to loss of membrane integrity, outflow of cytoplasmic contents, dissipation of the proton motive force and finally cell death [35,36] .

The membrane rupture efficiency is strongly size and shape dependent. The peptidoglycan layer of Gram-positive bacteria and the outer membrane of Gram-negative bacteria are more easily penetrated by nanoparticles smaller than 20 nm than by larger particles [37]. High curvature surface characteristics of anisotropic shapes such as nanorods and nanoflowers concentrate mechanical stress at the points of membrane contact, which can enhance disruption compared to smooth spherical particles [38].

5.3 Release of Metal Ions

The release of metal cations from dissolution of MONPs in aqueous biological environments has separate bactericidal effects. For ZnO, Zn2+ ions at sub-toxic concentrations impede the activity of important enzymes in bacteria such as alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase and ATP synthase thereby affecting energy metabolism and glycolysis.[39] Ni2+ ions produced from NiO substitute for critical divalent cations (Mg2+, Ca2+) at membrane transport proteins and catalytic sites, therefore impairing membrane integrity and enzymatic performance [40]. The release of Ce3+/Ce4+ ions from **CeO_2**plays a comparatively little effect in the bactericidal activity compared to the formation of ROS at the surface but can contribute to the overall antibacterial activity at greater concentrations [41]. Fe3+ released from Fe_2O_3can bind bacterial siderophores, hence reducing the availability of iron for bacterial development [42].

5.4 Intracellular Targeting

MONPs that manage to get into the bacterial cell, provide extra death effects through intracellular pathways. Once in the cytoplasm, nanoparticles or dissolved metal ions can directly interact with DNA, leading to strand breakage, base alterations, and cross-linking that inhibit replication and transcription.[43] Binding to ribosomal subunits inhibits protein synthesis and contact with thiol-containing enzymes leads to conformational alterations that eliminate catalytic activity. The areas of ROS production inside the cell are similarly vulnerable to ROS produced in structures equivalent to mitochondria in bacteria (i.e., the respiratory chain embedded in the inner membrane), further increasing the metabolic disturbance [44].

6. MECHANISTIC COMPARISON STUDY: ZNO, NIO, CEO2 AND FE_2O_3

To appreciate their respective strengths, limits and application applicability, a direct mechanistic comparison of the four oxide systems is necessary. Table 2 gives a systematic mechanistic comparison, and the following subsections examine important distinctions and points of convergence.

Table 2. Comparative mechanistic profiles of ZnO, NiO, **CeO_2**and Fe_2O_3NPs against bacteria.

Mechanism	ZnO	NiO	CeO_2	Fe_2O_3
Membrane Disruption	+++	++	++	+
Metal Ion Release	+++ (Zn^{2+})	++ (Ni^{2+})	+ ($Ce^{3+/4+}$)	+ (Fe^{3+})

ROS Generation	+++ (UV/Vis)	+ (surface $O_2^{\bullet-}$)	++ (pH-dependent •OH)	+++ (Fenton •OH)
DNA Damage	++	+	+	+++
Metal Ion Release	+++ (Zn^{2+})	++ (Ni^{2+})	+ ($Ce^{3+/4+}$)	+ (Fe^{3+})
Light Dependency	Yes (UV)	Partial	Yes (pH)	Partial (H_2O_2)
Gram(+) Efficacy	+++	++	++	++
Gram(−) Efficacy	++	++	++	++
MRSA Activity	++ (reported)	+ (limited)	+ (limited)	++ (reported)
Cytotoxicity Concern	Moderate	Moderate-High	Low-Moderate	Low

+++ Strong; ++ Moderate; + Weak/Limited. Ratings based on aggregate literature assessment.

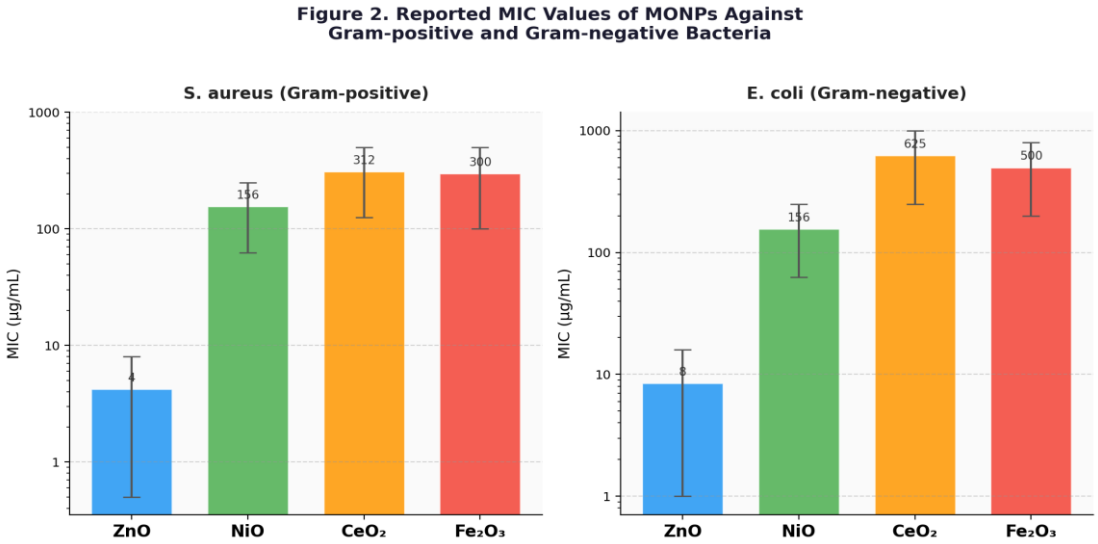

Figure 2. Reported minimum inhibitory concentration (MIC) values (µg/mL) of the four metal oxide nanoparticles against S. aureus (Gram-positive) and E. coli (Gram-negative). Error bars indicate the range of reported values

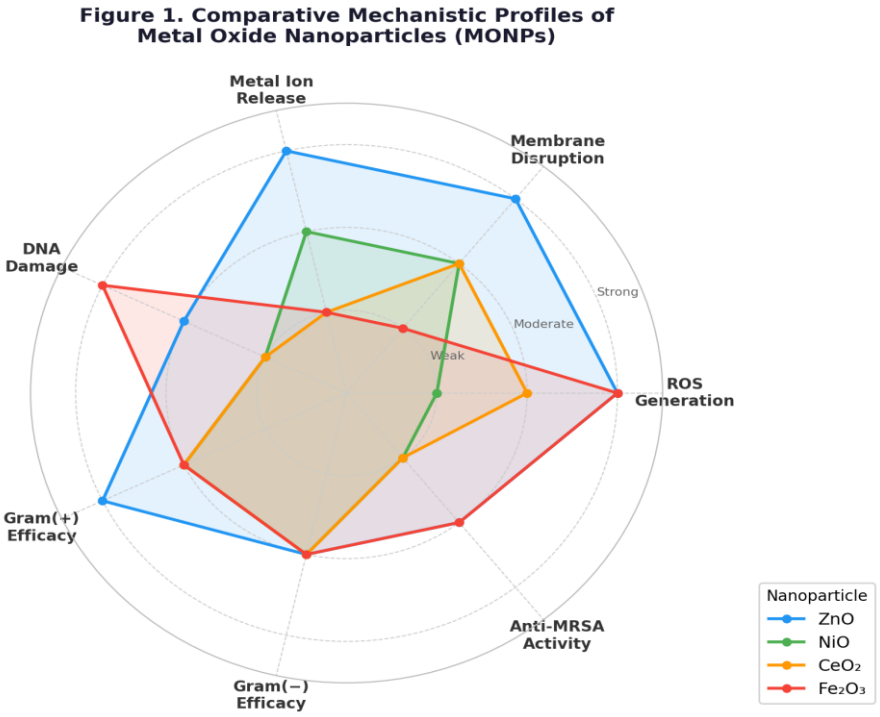

Figure 3. Radar graphic comparing the mechanistic profiles of ZnO, NiO, $CeO_2$and Fe2O3 nanoparticles with respect to seven antibacterial characteristics.

6.1 ZnO Nanoparticles

The best researched antibacterial metal oxide is ZnO nanoparticles . The great effectiveness of ZnO nanoparticles testifies to a near-optimal confluence of all four bactericidal processes . Under dark conditions, the Zn2+ ion release alone has been shown to account for 20-45% of the observed antibacterial activity, while under light the photocatalytic ROS generation dominates [45] .The major advantage of ZnO is its action against both Gram-positive and Gram-negative organisms with Gram-positive organisms such as S. aureus generally more vulnerable because to the lack of the outer membrane barrier seen in Gram-negative species.[46] The reported MIC values for ZnO are between 0.5 and 8 mg/mL for S. aureus and 1 and 16 mg/mL for E. coli, however there is a large degree of inter-study variability, which can be attributed to changes in particle size, crystallinity and test conditions [47].

6.2 Nanoparticles of NiO

NiO nanoparticles are considerably less studied in the antibacterial literature and their molecular foundation is less well described. The evidence suggests that the primary mechanism of action of NiO is due to the bactericidal effect of released Ni2+ ions and surface-catalyzed superoxide production, with membrane rupture being a secondary mechanism [48]. NiO has a modest cytotoxic profile compared to ZnO, as a result of the longer dissolution kinetics and lower peak metal ion concentrations in biological mediums.[49] The MIC values of the green produced NiO nanoparticles against S. aureus and E. coli have been observed in the range of 62.5-250 µg/mL, but the standardized comparison data is still lacking.[50] One of the strategies offered to improve the ROS formation and expand the antibacterial activity into the region of visible light is the doping of NiO with copper or cobalt [51].

6.3 CeO_2Nanoparticles

One of distinct antibacterial metal oxides is CeO2 nanoparticles, which possess intrinsic redox duality. The Ce3+ and Ce4+ oxidation states co-exist on the nanoparticle surface and the relative ratio between the two states is determined by the oxygen vacancy concentration. The oxygen vacancy concentration determines the ability of CeO2 to scavenge or create ROS depending on the pH of the surrounding environment and the availability of oxidants.[52] Acidic microenvironments produced by bacterial metabolism promote the formation of hydroxyl radical via Fenton-like chemistry driven by Ce3+ centres. [52] Conversely, under neutral or alkaline circumstances, CeO2 can paradoxically protect host cells from oxidative stress.[53] This pH-dependent behaviour has important implications for selectivity in mixed host-pathogen situations, and is a potentially exploitable characteristic for tailored antibacterial therapy. Generally, the antibacterial activity of CeO2 is weaker than ZnO or Fe2O3, however surface doping with silver or copper significantly improves its potency [54].

6.4 Nanoparticles of Fe2O3

Fe2O3 (hematite) nanoparticle combines the bactericidal characteristics of iron oxide with the added functionality of magnetic responsiveness for targeted delivery and recovery from complicated media.[55] The main antibacterial action is based on a Fenton-type chemistry where Fe2+ (produced by surface reduction of Fe3+) combines with endogenous or external H2O2 to generate highly reactive •OH radicals.[56] DNA strand breakage was shown to be the main route of bacterial killing, which distinguishes $Fe_2O_3$from ZnO and NiO, where membrane-associated processes are predominant.[57] A common constraint is the need for a sufficient H2O2 concentration to sustain the Fenton activity that may not always be present in all biological settings.[58] In order to overcome this constraint, coating $Fe_2O_3$with ZnO or embedding them in composite structures with photocatalytic oxides have been investigated [59].

7. STRUCTURE ACTIVITY RELATIONSHIP

7.1 Size of particles

The particle size factor is the most dependably recorded element affecting the antibacterial activity of MONPs. The well-known inverse size-antibacterial effect is due to a number of mechanisms: smaller particles have a larger surface area per unit mass, which increases ion dissolution and the production of reactive oxygen species; they can more easily penetrate the bacterial membranes; they are more likely to be internalized and reach intracellular targets.Raghupathi et al. showed that ZnO nanoparticles less than 12 nm demonstrated antibacterial activity at far lower concentrations than 45 nm or 302 nm particles, and the relationship closely followed an inverse power law [62]. Similar size dependent trends exist for NiO and Fe_2O_3, but their quantitative correlations are not as well characterized [63].

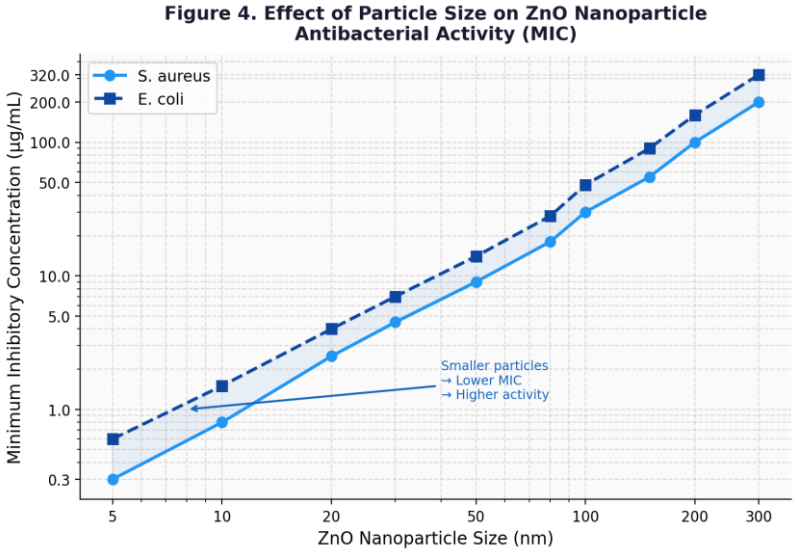

Figure 4. Inverse connection between the size (nm) of ZnO nanoparticles and the minimum inhibitory concentration (MIC, µg/mL) against S. aureus and E. coli.

7.2 Morphology

The effect of particle morphology on antibacterial activity is mainly attributed to the effect of particle morphology on surface area, surface energy and geometry of contact between nanoparticles and bacterial membrane. Rod-shaped and plate-like nanoparticles have been found to be more potent than spheres of same volume for antibacterial activity due to higher aspect ratio interaction with bacterial surface and the existence of high energy facets with enhanced catalytic activity [64] .In several investigations, hydrothermally synthesized ZnO and **CeO_2**nanoflower morphologies have very high surface areas and have showed remarkable antibacterial efficiency [65].

7.3 Morphology

The effect of particle morphology on antibacterial activity is mainly attributed to the effect of particle morphology on surface area, surface energy and geometry of contact between nanoparticles and bacterial membrane. Rod-shaped and plate-like nanoparticles have been found to be more potent than spheres of same volume for antibacterial activity due to higher aspect ratio interaction with bacterial surface and the existence of high energy facets with enhanced catalytic activity [64]. ZnO and **CeO_2**can also be synthesized by hydrothermal synthesis to yield nanoflower morphologies with extraordinarily high surface areas which have shown improved antibacterial activity in several investigations [65]. However, the independent contribution of morphology to antibacterial activity, dissociated from size effects, remains difficult to isolate in experimental systems, and represents an area in need of more controlled exploration [66].

7.4 Surface doping and functionalization

Surface functionalization and elemental doping are two important ways to improve intrinsic antibacterial capabilities. Silver doping of ZnO, NiO and **CeO_2**nanoparticles boosts the antibacterial activity consistently due to the increased Ag+ ion release, narrowing of the band gap and improved charge carrier separation [67] .Copper doping in a similar fashion boosts the visible-light photocatalytic activity via mid-gap state generation.[68] Surface coating with natural polymers like as chitosan or polyethylene glycol (PEG) enhances colloidal stability in physiological media and can lower non-specific cytotoxicity while preserving antibacterial activity.[69] The construction of composite nanostructures e.g. ZnO/ Fe_2O_3or CeO_2 /Ag heterostructures, allows for the synergistic combination of the distinct oxide processes, typically resulting in higher than additive antibacterial effects [70].

Differential Susceptibility Gram Positive vs Gram Negative Bacteria

The structural differences between Gram-positive and Gram-negative bacteria cell envelopes significantly alter their susceptibility to MONPs. Gram-positive bacteria have a large peptidoglycan layer (20-80 nm) outside the cytoplasmic membrane but no outer membrane of lipopolysaccharide. In contrast, gram-negative bacteria have a thin peptidoglycan layer that lies between an inner cytoplasmic membrane and an outside membrane with lipopolysaccharide (LPS) [71].

The outer membrane of Gram-negative bacteria presents a major permeability barrier, hindering the entry of nanoparticles and reducing the efficacy of ion release as a bactericidal mechanism. Thus many MONPs, especially ZnO, are slightly less active (higher MIC) against Gram-negative microbes as opposed to Gram-positive species under similar circumstances [72]. But this is not a general difference: Fe_2O_3nanoparticles exhibit generally similar action toward both bacterial groups, potentially because to DNA

damage through diffusible •OH radicals do not need to penetrate the membrane.[73] CeO_2 nanoparticles also demonstrate reduced Gram-type selectivity, consistent with a process driven by extracellular ROS production instead of membrane contact [74].

It should also be noted that a huge number of published antibacterial studies use only one or two model organisms (most commonly S. aureus and E. coli) and results from these strains are routinely generalized across bacterial groups without proper rationale. A major void exists in the literature about systematic research utilizing a broader panel of clinically relevant Gram-positive and Gram-negative bacteria such as Streptococcus pneumoniae, Klebsiella pneumoniae and Pseudomonas aeruginosa [75].

8. FIGHTING ANTIBIOTIC-RESISTANT BACTERIA

Since the main therapeutic motive for the development of MONP-based antibacterials is the treatment of drug resistance infections, the absence of research explicitly testing MONPs against resistant clinical isolates constitutes a significant lacuna in the literature. Most of the published work uses normal laboratory strains maintained under optimal culture conditions that may not adequately reflect the behaviour of resistant clinical isolates with altered membrane composition, efflux pump expression or biofilm-forming potential [76].

MRSA has been the most studied resistant microbe in MONP antibacterial investigations. Several studies have shown that ZnO nanoparticles had action against MRSA at doses of 500-1000 µg/mL with smaller particles (<20 nm) displaying activity at lower concentrations.[77] ZnO or Ag composites of Fe_2O_3 have also demonstrated promising anti-MRSA efficacy, which was linked to synergistic Fenton and ion-release processes [78]. Data on the activity of NiO and CeO_2 against MRSA are limited, and there are no adequately powered trials investigating the activity of these oxides against extended-spectrum beta-lactamase (ESBL)-producing organisms or carbapenem-resistant Enterobacteriaceae, which are among the most clinically urgent resistance threats [79].

Another barrier of resistance is biofilm development, particularly relevant to medical device associated illnesses. Bacterial biofilms are surrounded by a polysaccharide matrix that severely restricts the entry of antibiotics and creates microenvironments with low pH and low oxygen tension that may also alter MONP function. ZnO and Fe_2O_3nanoparticles showed moderate anti-biofilm activity in vitro although the concentrations of nanomaterials required to kill established biofilms are typically 4-16 times higher than the minimum inhibitory dosages of planktonic cells [80]. This points to a major translational hurdle not sufficiently discussed in the literature.

9. CONSIDERATIONS OF CYTOTOXICITY AND BIOCOMPATIBILITY

The therapeutic potential of MONPs depends on selectivity, that is, bactericidal efficacy at concentrations lower than those that cause significant damage to the host mammalian cells. The cytotoxicity data for the four oxides discussed here give a complicated and sometimes conflicting picture that reflects the dependency of the toxicological outcome on particle size, surface chemistry, cell type, exposure time and assay method.In general, ZnO nanoparticles are more cytotoxic to mammalian cells than Fe_2O_3or CeO_2, which is mainly attributed to the production of Zn^{2+} ions at amounts hazardous for eukaryotic cells . IC50 values of ZnO to human cell lines are typically in the range of 10-100 µg/mL, while antibacterial MIC values are usually between 500 and 2000 µg/mL, indicating a poor therapeutic index for systemic administration [82]. The coating of ZnO with SiO_2 or PEG significantly decreases the cytotoxicity but still retains some antibacterial activity [83].

On the other hand, NiO nanoparticles present some hazards of cytotoxicity related to the established carcinogenicity of nickel compounds in occupational exposure situations. The in vitro cytotoxicity of NiO nanoparticles is minimal at the levels important for antibacterial applications, but, the genotoxicity data indicates DNA strand breakage at concentrations as low as 25 µg/mL in mammalian cells, therefore requiring careful consideration [84].

Among the 4 oxides, CeO_2 nanoparticles had the most favourable cytotoxicity profile with demonstrated antioxidant action toward mammalian cells under non-infectious conditions [53]. This characteristic may provide host-protective advantage in inflammatory infection scenarios [85]. Fe_2O_3nanoparticles are generally well tolerated in vitro and in vivo at relevant concentrations, and their current use in MRI contrast agents and iron supplementation formulations provides a positive regulatory precedent [86].

10. NEW APPLICATIONS

10.1 Applications in Biomedicine

Antibacterial MONPs have potential biomedical uses such as wound treatment, orthopedic implant coating, catheter surface modification and topical antimicrobial formulation. ZnO-containing wound dressings have been shown in animal models to expedite healing and decrease bacterial colonization . This is because of the combined antibacterial and pro-angiogenic properties of Zn^{2+} ions.[87] Fe_2O_3nanoparticles have been examined as magnetically steerable antibacterial treatments for deep tissue infections, however the requirement of external magnetic field application limits their practical utilization. CeO_2 nanoparticles have been of interest for managing chronic wounds with high oxidative stress, where their combined antioxidant-antibacterial nature may be of particular utility [88,89].

10.2 Packaging and Food Safety

Antimicrobial food packaging materials have been intensively studied with MONPs as active components. Studies have shown that ZnO nanoparticles in polymer films (chitosan, polyethylene, starch) have reduced microbiological deterioration of meat, fish, and produce, extending shelf life by 2-5 days at refrigeration temperatures.[90] The Fe_2O_3nanoparticles are used in packaging to take use of their oxygen scavenging and antibacterial characteristics at the same time . Regulatory acceptability of food contact products containing MONP is still being evaluated by the European Food Safety Authority (EFSA) and the U.S. Food and Drug Administration (FDA) [91].

10.3. Water treatment

Photocatalytic MONPs are also very suitable for water disinfection applications, where the formation of ROS under visible light can inactivate waterborne pathogens without the halogenated by-products associated with conventional chlorination. Laboratory scale reactors under solar irradiation have shown log10 reductions of 4-6 of E. coli and Enterococcus faecalis by ZnO and Fe_2O_3.[92] CeO_2 -based photocatalysts have been reported to be active against antibiotic-resistant bacteria in hospital wastewater treatment.Scale-up, recovery of nanoparticles and their long-term environmental fate are still difficulties to be overcome for practical deployment [93,94].

11. CRITICAL GAPS AND FUTURE RESEARCH DIRECTIONS

Several key gaps in the current research on MONP antibacterial activity have been highlighted in this study that need to be filled in order to move the field forward into clinical and commercial translation.

First, the protocols of antibacterial testing need to be standardized promptly. The existing diversity in MIC reporting, where the same nanoparticle versus the same organism might span orders of magnitude, makes comparison between studies mostly worthless. Adoption of defined procedures similar to CLSI or EUCAST protocols for conventional antibiotics, modified to nanomaterial specific aspects (agglomeration state, particle characterisation, medium composition) will greatly increase data comparability [95].

Second, there is an essential absence of systematic in vivo evaluation of MONPs in relevant infection models. Most of the published data are in vitro. The translation in vivo of in vitro results is often poor for nanomaterials due to protein corona formation, agglomeration in physiological fluids and interactions with the immune system that change the behaviour of the nanoparticles [96] . Before any MONP-based antibacterial may be considered as a significant therapeutic candidate, in vivo research in relevant clinical infection models (wound infection, catheter-associated infection, pneumonia) are needed.

Thirdly, mechanistic research on NiO nanoparticles is much less developed than ZnO and Fe2O3. The unusual electrical properties and the genotoxic potential of NiO make a dedicated effort to understand its antibacterial mechanism, and to determine the concentration threshold between antibacterial and cytotoxic effects scientifically justified and clinically relevant [97] .

Fourth, studies on antibiotic-resistant clinical isolates, notably ESBL-producing Gram-negative bacteria and carbapenem-resistant species, are severely underrepresented. The validation of the therapeutic importance of MONP-based treatments requires proving the efficacy against these essential resistance threats [98].

Finally, the fate of MONPs exposed into aquatic and terrestrial ecosystems for longer periods deserves more attention. Such problems require a full lifecycle assessment, not just single toxicity endpoints. Prior to any large-scale deployment, researchers must systematically evaluate the effects of these materials on non-target animals and if linked heavy metals accumulate progressively via food webs [99].

12. FINAL NOTES

A detailed and critical comparative assessment on the antibacterial mechanisms, structure-activity connections and applications of the ZnO, NiO, CeO_2 and Fe_2O_3nanoparticles is presented. Each oxide exhibits a unique mechanistic signature, including ZnO exhibiting strong photocatalytic ROS generation and Zn2+ ion-mediated toxicity, NiO acting mainly via Ni2+ release and surface superoxide formation, CeO_2 utilizing an unusual pH-responsive Ce3+/Ce4+ redox switch, and Fe_2O_3producing potent DNA damage via Fenton-type hydroxyl radical generation. Particle size, morphology, surface chemistry, and synthesis process together govern the antibacterial potency of each system within and between oxide types.

We have observed several key limitations including the absence of standardized testing methodologies, paucity of in vivo efficacy data, restricted research in clinically relevant resistance isolates and lack of mechanistic characterization of NiO . The joint use of standardized methodologies, rigorous in vivo research, and focused mechanistic studies to fill these gaps is necessary to translate the significant in vitro promise of MONPs into clinically effective antimicrobial medicines. This translation has never been more necessary, with the growing worldwide burden of antibiotic resistance.

12. Conclusion

Metal oxide nanoparticles (MONPs), particularly ZnO, NiO, CeO_2, and Fe_2O_3, have emerged as promising alternatives to conventional antibiotics because of their broad-spectrum antibacterial activity and multi-target mechanisms of action. Unlike traditional antimicrobial agents, these nanoparticles simultaneously induce reactive oxygen species (ROS) generation, membrane disruption, metal ion release, and intracellular damage, making the development of bacterial resistance considerably more difficult.This review demonstrates that the antibacterial performance of MONPs is strongly governed by their physicochemical characteristics, including particle size, morphology, crystal structure, surface chemistry, and synthesis method. Among the reviewed materials, ZnO nanoparticles exhibit the most comprehensive antibacterial profile owing to their efficient ROS generation and Zn^{2+} ion release, whereas Fe_2O_3 nanoparticles provide unique advantages through Fenton-mediated oxidative damage. CeO_2 nanoparticles display pH-dependent redox behavior with favorable biocompatibility, while NiO nanoparticles remain relatively underexplored despite their promising antibacterial potential. Despite the encouraging in vitro findings, several critical challenges continue to limit the clinical translation of MONPs. These include the lack of standardized antibacterial testing protocols, insufficient in vivo validation, limited studies involving multidrug-resistant clinical isolates, incomplete understanding of long-term toxicity, and concerns regarding environmental safety. Addressing these issues through standardized experimental methodologies and comprehensive biological evaluation will be essential for successful biomedical applications. Overall, this review provides an integrated comparison of the structure–activity relationships, antibacterial mechanisms, and biomedical prospects of ZnO, NiO, CeO_2, and Fe_2O_3 nanoparticles. It also identifies current research gaps and highlights future directions that may facilitate the rational design of safer, more effective, and clinically translatable nanoparticle-based antimicrobial therapies to combat the growing global challenge of antimicrobial resistance.

REFERENCES

1) Murray CJ, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629–655.
2) O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. Review on Antimicrobial Resistance. London: HM Government; 2016.
3) Dadgostar P. Antimicrobial resistance: implications and costs. Infect Drug Resist. 2019;12:3903–3910.
4) Slavin YN, et al. Metal nanoparticles: understanding the mechanisms behind antibacterial activity. J Nanobiotechnology. 2017;15(1):65.
5) Wang L, et al. Nanoparticles displaying microbial antigens. Nanomedicine. 2017;12(4):415–429.
6) Hemeg HA. Nanomaterials for alternative antibacterial therapy. Int J Nanomedicine. 2017;12:8211–8225.
7) Beyth N, et al. Alternative antimicrobial approach: nano-antimicrobial materials. Evid Based Complement Alternat Med. 2015;2015:246012.
8) Galdiero S, et al. Silver nanoparticles as potential antiviral agents. Molecules. 2011;16(10):8894–8918.
9) Sirelkhatim A, et al. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-Micro Lett. 2015;7(3):219–242.
10) Elumalai K, Velmurugan S. Green synthesis, characterization and antimicrobial activities of zinc oxide nanoparticles. Appl Surf Sci. 2015;345:329–336.
11) Alpaslan E, et al. pH-Controlled cerium oxide nanoparticle inhibition of gram-positive and gram-negative bacteria growth. ACS Biomater Sci Eng. 2015;1(11):1096–1103.
12) Arakha M, et al. Antimicrobial activity of iron oxide nanoparticle upon modulation of nanoparticle-bacteria interface. Sci Rep. 2015;5:14813.
13) Dizaj SM, et al. Antimicrobial activity of the metals and metal oxide nanoparticles. Mater Sci Eng C. 2014;44:278–284.
14) Pelgrift RY, Friedman AJ. Nanotechnology as a therapeutic tool to combat microbial resistance. Adv Drug Deliv Rev. 2013;65(13-14):1803–1815.
15) Azam A, et al. Antimicrobial activity of metal oxide nanoparticles against gram-positive and gram-negative bacteria. J Antibiot. 2012;65(9):465–469.
16) Jeevanandam J, et al. Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein J Nanotechnol. 2018;9:1050–1074.
17) Ealias AM, Saravanakumar MP. A review on the classification, characterisation, synthesis of nanoparticles and their application. IOP Conf Ser. 2017;263:032019.
18) Niederberger M. Nonaqueous sol-gel routes to metal oxide nanoparticles. Acc Chem Res. 2007;40(9):793–800.
19) Wang X, et al. Hydrothermal synthesis of metal oxide nanomaterials for photovoltaic applications. Chem Soc Rev. 2012;41(22):7512–7535.

20) Kharissova OV, et al. The greener synthesis of nanoparticles. Trends Biotechnol. 2013;31(4):240–248.
21) Singh P, et al. Biological synthesis of nanoparticles from plants and microorganisms. Trends Biotechnol. 2016;34(7):588–599.
22) Vijayakumar S, Vaseeharan B. Antibiofilm and antivirulence properties of ZnO nanoparticles. Microb Pathog. 2018;127:1–8.
23) Thill A, et al. Cytotoxicity of CeO2 nanoparticles for Escherichia coli. Environ Sci Technol. 2006;40(19):6151–6156.
24) Iravani S. Green synthesis of metal nanoparticles using plants. Green Chem. 2011;13(10):2638–2650.
25) Zhang L, et al. Synthesis and antibacterial properties of ZnO nanoparticles. MedChemComm. 2014;5(8):1047–1053.
26) Lam SM, et al. Visible light responsive Ag/ZnO nanoparticles against bacteria. Desalin Water Treat. 2012;41(1-3):214–220.
27) Rai M, et al. Metal nanoparticles: the protective nanoshield against virus infection. Crit Rev Microbiol. 2016;42(1):46–56.
28) Chatterjee AK, et al. A broader spectrum of antibacterial activity of amorphous ZnO. Nanotechnology. 2015;26(8):085101.
29) Fujishima A, Zhang X, Tryk DA. TiO2 photocatalysis and related surface phenomena. Surf Sci Rep. 2008;63(12):515–582.
30) Dwivedi S, et al. Reactive oxygen species mediated bacterial biofilm inhibition via zinc oxide nanoparticles. RSC Adv. 2014;4(99):55985–55993.
31) Akhlaghi N, Najafpour-Darzi G. A comprehensive review on biological synthesis of nickel oxide nanoparticles. J Ind Eng Chem. 2021;103:292–305.
32) Heckert EG, et al. The role of cerium redox state in the SOD mimetic activity of nanoceria. Biomaterials. 2008;29(18):2705–2709.
33) Fenton HJH. Oxidation of tartaric acid in presence of iron. J Chem Soc Trans. 1894;65:899–910.
34) Goodman CM, et al. Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. Bioconjug Chem. 2004;15(4):897–900.
35) Seil JT, Webster TJ. Antimicrobial applications of nanotechnology: methods and literature. Int J Nanomedicine. 2012;7:2767–2781.
36) Morones JR, et al. The bactericidal effect of silver nanoparticles. Nanotechnology. 2005;16(10):2346–2353.
37) Raghupathi KR, Koodali RT, Manna AC. Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. Langmuir. 2011;27(7):4020–4028.
38) Cha SH, et al. Shape-dependent biomimetic inhibition of enzyme by nanoparticles and their antibacterial activity. ACS Nano. 2015;9(9):9097–9105.
39) Xie Y, et al. Antibacterial activity and mechanism of action of zinc oxide nanoparticles against Campylobacter jejuni. Appl Environ Microbiol. 2011;77(7):2325–2331.
40) Dey A. Jewellery and nanotechnology: nickel sensitization. Asian Pac J Trop Biomed. 2012;2(4):315–318.
41) Jiang W, et al. Lanthanide nanoparticle-induced cellular toxicity. J Mater Chem. 2009;19(40):7525–7532.
42) Neilands JB. Siderophores: structure and function of microbial iron transport compounds. J Biol Chem. 1995;270(45):26723–26726.
43) Applerot G, et al. Understanding the antibacterial mechanism of CuO nanoparticles: revealing the route of induced oxidative stress. Small. 2012;8(21):3326–3337.
44) Huh AJ, Kwon YJ. 'Nanoantibiotics': a new paradigm for treating infectious diseases using nanomaterials. J Control Release. 2011;156(2):128–145.
45) Li M, et al. Investigating the antimicrobial activity of ZnO nanoparticles against an avirulent strain of E. coli. J Biomed Mater Res B. 2012;100(8):2097–2106.
46) Premanathan M, et al. Selective toxicity of ZnO nanoparticles toward gram-positive bacteria and cancer cells. Nanomedicine. 2011;7(2):184–192.
47) Sirelkhatim A, et al. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-Micro Lett. 2015;7(3):219–242.
48) Fakhri A, et al. Synthesis and characterization of NiO nanoparticles by wet chemical method and evaluation of the antibacterial activity. J Appl Chem Res. 2015;9(2):22–28.
49) Ditto AJ, et al. The pharmacology of locally delivered nickel nanoparticles. J Nanopart Res. 2011;13(11):6157–6163.
50) Anantharaman A, Ling SK. Synthesis of NiO nanoparticles with enhanced antibacterial activity. Mater Today Proc. 2021;43:1662–1666.
51) Nouri A, et al. Visible light photocatalytic activity of Cu-doped NiO nanoparticles. Ceram Int. 2016;42(5):5813–5821.
52) Celardo I, et al. Pharmacological potential of cerium oxide nanoparticles. Nanoscale. 2011;3(4):1411–1420.

53) Karakoti AS, et al. Nanoceria as antioxidant: synthesis and biomedical applications. JOM. 2008;60(3):33–37.
54) Sreejivungsa K, et al. Light-controlled release of antibacterial agents from photoresponsive CeO2/Ag composites. J Photochem Photobiol A. 2016;324:10–16.
55) Mahmoudi M, et al. Superparamagnetic iron oxide nanoparticles (SPIONs): development, surface modification and applications in chemotherapy. Adv Drug Deliv Rev. 2011;63(1-2):24–46.
56) Rufus A, et al. Synthesis of biogenic hematite (α-Fe2O3) nanoparticles for antibacterial and nontoxic applications. RSC Adv. 2016;6(96):94206–94217.
57) Arakha M, et al. Interfacial activity of the iron oxide nanoparticle impacts in vitro antibacterial activity. Sci Rep. 2016;6:29,628.
58) Pereira C, et al. Superparamagnetic MFe2O4 (M = Fe, Co, Mn) nanoparticles: tuning the particle size and magnetic properties. J Phys Chem C. 2012;116(30):15806–15819.
59) Barreto RA, et al. ZnO/Fe2O3 composites: preparation and evaluation of photocatalytic and antibacterial activities. Mater Lett. 2020;270:127705.
60) Nel AE, et al. Understanding biophysicochemical interactions at the nano-bio interface. Nat Mater. 2009;8(7):543–557.
61) Jiang J, et al. Does nanoparticle activity depend upon size and crystal phase? Nanotoxicology. 2008;2(1):33–42.
62) Raghupathi KR, Koodali RT, Manna AC. Size-dependent bacterial growth inhibition. Langmuir. 2011;27(7):4020–4028.
63) Sundrarajan M, et al. Plant extract mediated biogenic synthesis of NiO nanoparticles by agathosma betulina. J Ind Eng Chem. 2017;53:224–230.
64) Stankic S, et al. Pure and multi metal oxide nanoparticles: synthesis, antibacterial and cytotoxic properties. J Nanobiotechnology. 2016;14(1):73.
65) Ong CB, Ng LY, Mohammad AW. A review of ZnO nanoparticles as solar photocatalysts: synthesis, mechanisms and applications. Renew Sustain Energy Rev. 2018;81:536–551.
66) Padmavathy N, Vijayaraghavan R. Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study. Sci Technol Adv Mater. 2008;9(3):035004.
67) Lam SM, et al. Visible light responsive Ag/ZnO nanoparticles. Desalin Water Treat. 2012;41(1-3):214–220.
68) Saber O, et al. Preparation and characterization of Cu-doped ZnO nanoparticles. J Alloys Compd. 2014;615:826–832.
69) Roque-Ruiz JH, et al. Preparation and characterization of PEG-coated magnetic iron oxide nanoparticles. Mater Chem Phys. 2020;239:121998.
70) Barreto RA, et al. ZnO/Fe2O3 composites for photocatalytic and antibacterial applications. Mater Lett. 2020;270:127705.
71) Silhavy TJ, Kahne D, Walker S. The bacterial cell envelope. Cold Spring Harb Perspect Biol. 2010;2(5):a000414.
72) Tayel AA, et al. Antibacterial action of zinc oxide nanoparticles against foodborne pathogens. J Food Saf. 2011;31(2):211–218.
73) Arakha M, et al. Antimicrobial activity of iron oxide nanoparticle upon modulation of nanoparticle-bacteria interface. Sci Rep. 2015;5:14813.
74) Thill A, et al. Cytotoxicity of CeO2 nanoparticles for Escherichia coli. Environ Sci Technol. 2006;40(19):6151–6156.
75) Franci G, et al. Silver nanoparticles as potential antibacterial agents. Molecules. 2015;20(5):8856–8874.
76) Boucher HW, et al. Bad bugs, no drugs: no ESKAPE! An update from the IDSA. Clin Infect Dis. 2009;48(1):1–12.
77) Ansari MA, et al. Synthesis and characterization of ZnO nanoparticles and their antimicrobial property against methicillin-resistant Staphylococcus aureus. Appl Microbiol Biotechnol. 2012;96(4):1065–1073.
78) Barreto RA, et al. ZnO/Fe2O3 nanocomposites: antibacterial activity against MRSA. Mater Lett. 2020;270:127705.
79) Pendleton JN, Gorman SP, Gilmore BF. Clinical relevance of the ESKAPE pathogens. Expert Rev Anti Infect Ther. 2013;11(3):297–308.
80) Applerot G, et al. ZnO nanoparticle-coated surfaces inhibit biofilm formation of drug-resistant bacteria. ACS Appl Mater Interfaces. 2012;4(11):6468–6473.
81) Nel A, et al. Toxic potential of materials at the nanolevel. Science. 2006;311(5761):622–627.
82) Brunner TJ, et al. In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. Environ Sci Technol. 2006;40(14):4374–4381.
83) Nair S, et al. ZnO nanoparticle-coated surfaces reduce bacterial biofilm formation. ACS Nano. 2009;3(7):1815–1823.
84) Dunnick JK, et al. Characterization of biological responses of rats and mice to inhaled nickel oxide nanomaterials. Nanotoxicology. 2015;9(1):9–21.
85) Celardo I, et al. Pharmacological potential of cerium oxide nanoparticles. Nanoscale. 2011;3(4):1411–1420.
86) Laurent S, et al. Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. Chem Rev. 2008;108(6):2064–2110.

87) Hamdan S, et al. Nanotechnology-driven therapeutic interventions in wound healing. ACS Nano. 2017;11(11):10424–10432.
88) Mahmoudi M, Laurent S. Iron oxide nanoparticles for biomedical applications. Amsterdam: Elsevier; 2012.
89) Caputo F, et al. Measuring oxidoreductase-like activity of nanoceria. Methods Enzymol. 2017;590:1–28.
90) He X, Deng H, Hwang HM. The current application of nanotechnology in food and agriculture. J Food Drug Anal. 2019;27(1):1–21.
91) Bumbudsanpharoke N, Ko S. Nano-food packaging: an overview of market, migration research, and safety regulations. J Food Sci. 2015;80(5):R910–R923.
92) Gupta G, et al. Solar photocatalytic disinfection of water. Adv Mater Lett. 2014;5(9):501–508.
93) Bousiakou LG, et al. CeO2 nanoparticles for the removal of antibiotics from hospital wastewater. Sci Total Environ. 2019;657:1144–1153.
94) Keller AA, et al. Global life cycle releases of engineered nanomaterials. J Nanopart Res. 2013;15(6):1692.
95) CLSI. M07-A10: Methods for dilution antimicrobial susceptibility tests for bacteria. Wayne, PA: CLSI; 2015.
96) Nel AE, et al. Understanding biophysicochemical interactions at the nano-bio interface. Nat Mater. 2009;8(7):543–557.
97) Ahamed M, et al. Synthesis and bioactivity of nickel oxide nanoparticles against human lung cancer cells. J Hazard Mater. 2013;261:188–195.
98) WHO. Global priority list of antibiotic-resistant bacteria to guide research. Geneva: WHO; 2017.
99) Nowack B, Bucheli TD. Occurrence, behavior and effects of nanoparticles in the environment. Environ Pollut. 2007;150(1):5–22.