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Antibacterial properties of nanoparticles

Mohammad J. Hajipour[1], Katharina M. Fromm[2], Ali Akbar Ashkarran[3], Dorleta Jimenez de Aberasturi[4,5], Idoia Ruiz de Larramendi[5], Teofilo Rojo[5], Vahid Serpooshan[6], Wolfgang J. Parak[4] and Morteza Mahmoudi[1,7]

[1] Laboratory of NanoBio Interactions, Department of Nanotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[2] University of Fribourg, Department of Chemistry, Chemin du Musée 9, CH-1700 Fribourg, Switzerland
[3] Department of Physics, Faculty of Basic Sciences, University of Mazandaran, Babolsar, Iran
[4] Fachbereich Physik and WZMW, Philipps Universität Marburg, Marburg, Germany
[5] Department of Inorganic Chemistry, UPV/EHU, Bilbao, Spain
[6] Division of Pediatric Cardiology, Department of Pediatrics, Stanford University School of Medicine, Stanford, CA 94305-5101, USA
[7] Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Antibacterial agents are very important in the textile industry, water disinfection, medicine, and food packaging. Organic compounds used for disinfection have some disadvantages, including toxicity to the human body, therefore, the interest in inorganic disinfectants such as metal oxide nanoparticles (NPs) is increasing. This review focuses on the properties and applications of inorganic nanostructured materials and their surface modifications, with good antimicrobial activity. Such improved antibacterial agents locally destroy bacteria, without being toxic to the surrounding tissue. We also provide an overview of opportunities and risks of using NPs as antibacterial agents. In particular, we discuss the role of different NP materials.

Antimicrobial NPs

Antibacterial activity is related to compounds that locally kill bacteria or slow down their growth, without being in general toxic to surrounding tissue. Most current antibacterial agents are chemically modified natural compounds [1], for instance, β-lactams (like penicillins), cephalosporins or carbapenems. Also, pure natural products, such as aminoglycosides, as well as purely synthetic antibiotics, for example, sulfonamides, are often used. In general, the agents can be classified as either bactericidal, which kill bacteria, or bacteriostatic, slowing down bacterial growth. Antibacterial agents are paramount to fight infectious diseases. However, with their broad use and abuse, the emergence of bacterial resistance to antibacterial drugs has become a common phenomenon, which is a major problem. Resistance is most often based on evolutionary processes taking place during, for example, antibiotic therapy, and leads to inheritable resistance. In addition, horizontal gene transfer by conjugation, transduction or transformation can be a possible way for resistance to build up [2]. Such antibacterial-resistant strains and species are informally referred to as superbugs and contribute to the emergence of diseases that were under good control for many years. One prominent example is bacterial strains causing tuberculosis (TB) that are resistant to previously effective antibacterial treatment. Indeed, it is estimated that nearly half a million new cases of multidrug-resistant tuberculosis (MDR-TB) occur worldwide every year [3]; along these lines, the newly identified enzyme, new Delhimetallo-β-lactamase-1 (NDM-1), is responsible for bacterial resistance to a broad range of β-lactam antibacterials, and it seems that most isolates with NDM-1 enzyme are resistant to all standard intravenous antibiotics for treatment of severe infections [4]. Thus, due to the fact that bacteria developed resistance against many common antibacterial agents, infectious diseases continue to be one of the greatest health challenges worldwide. In addition, drawbacks for conventional antimicrobial agents are not only the development of multiple drug resistance, but also adverse side effects. Drug resistance enforces high-dose administration of antibiotics, often generating intolerable toxicity. This has prompted the development of alternative strategies to treat bacterial diseases [5]. Among them, nanoscale materials have emerged as novel antimicrobial agents. Especially, several classes of antimicrobial NPs and nanosized carriers for antibiotics delivery have proven their effectiveness for treating infectious diseases, including antibiotic-resistant ones, *in vitro* as well as in animal models [6]. Why can NPs offer improved properties to classical organic antibacterial agents? One reason lies in their high surface area to volume ratio, resulting in appearance of new mechanical, chemical, electrical, optical, magnetic, electro-optical, and magneto-optical properties of the NPs that are different from their bulk properties [7]. In this case, NPs have been demonstrated to be interesting in the context of combating bacteria [8]. We first discuss particular properties of bacteria and important differences between different strains. The way to destroy bacteria is highly specific to the respective bacterial strains. We then describe the toxicity mechanisms of NPs against bacteria, and drug-resistant bacteria and their defense mechanisms. Finally we

Corresponding author: Mahmoudi, M. (Mahmoudi@biospion.com), (Mahmoudi-M@TUMS.ac.ir), (Mahmoudi@Illinois.edu).
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provide an outlook on NPs in the environment and ecosystems.

Properties of bacteria, and thus the way to destroy them, are highly specific to the respective bacterial strains

Role of the cell wall

The bacterial cell wall is designed to provide strength, rigidity, and shape, and to protect the cell from osmotic rupture and mechanical damage [9]. According to their structure, components, and functions, the bacteria cell wall can be divided into the two main categories: Gram positive (+) and Gram negative (–). The wall of Gram-positive cells contains a thick layer (i.e., 20–50 nm) of peptidoglycan (PG), which is attached to teichoic acids that are unique to the Gram-positive cell wall (Figure 1a) [10]. By contrast, Gram-negative cell walls are more complex, both structurally and chemically. More specifically, in Gram-negative bacteria, the cell wall comprises a thin PG layer and contains an outer membrane, which covers the surface membrane. The outer membrane of Gram-negative bacteria often confers resistance to hydrophobic compounds including detergents and contains as a unique component, lipopolysaccharides, which increase the negative charge of cell membranes and are essential for structural integrity and viability of the bacteria (Figure 1b) [11].

The structure of the cell wall plays an important role in tolerance or susceptibility of bacteria in the presence of NPs. For instance, vancomycin (van)-functionalized Ag@TiO_2 NPs have the capacity to target van-sensitive bacteria [12]. In the van-sensitive bacterium, *Desulfotomaculum*, the D-Ala-D-Ala structure on the surface of the cell wall can be recognized by vancomycin. By contrast, it is impossible for vancomycin to penetrate into van-resistant bacteria and access the D-Ala-D-Ala structure moiety. This is due to the fact that van-resistant bacteria have an additional outer membrane, which covers the cell surface. Bacterial cell wall properties can play a crucial role in diffusion of NPs inside biofilm matrixes [13]. The expression of the major cell-wall-anchored proteinase PrtP is responsible for altering the surface of *Lactococcus lactis* from a hydrophilic to an extremely hydrophobic one. In fact, the expression of PrtP in *L. lactis* 2 changes the physicochemical properties without architectural modifications during biofilm formation.

Role of the NP type and surface

Species sensitivity is not only related to the structure of the cell wall in Gram-positive and Gram-negative bacteria

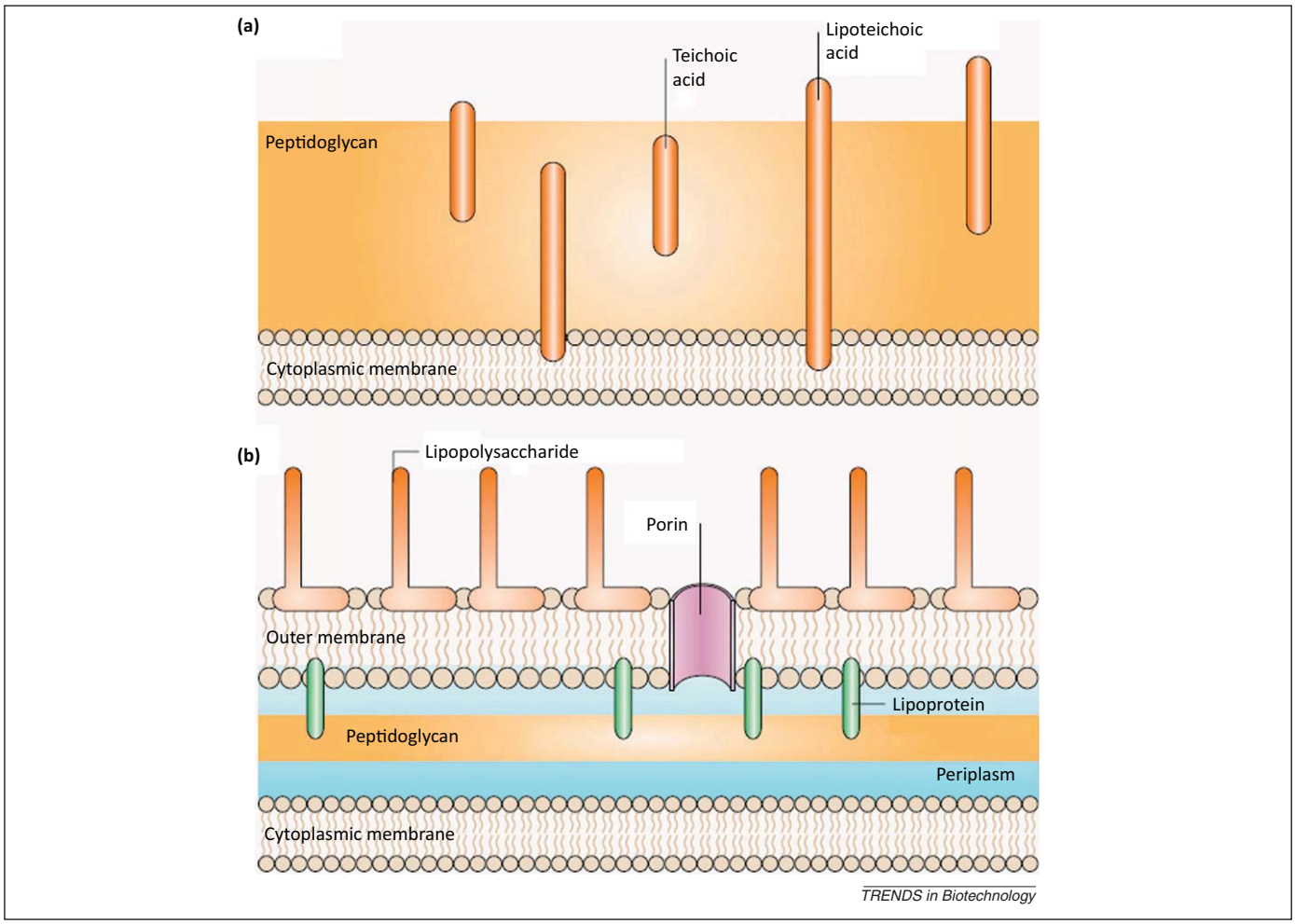

Figure 1. Bacterial cell structure. **(a)** A Gram-positive bacterial cell wall is composed of a thick and multilayered peptidoglycan (PG) sheath outside of the cytoplasmic membrane. The teichoic acids, as seen, are connected to and embedded in the PG, and lipoteichoic acids extend into the cytoplasmic membrane. **(b)** A Gram-negative bacterial cell wall is composed of an outer membrane linked by lipoproteins to thin and single-layered PG. The PG is placed within the periplasmic space that is formed between the outer and inner membranes. The outer membrane includes porins and lipopolysaccharide molecules [64].

[12]. Several additional factors can influence the susceptibility or tolerance of bacteria to NPs. For example, *Escherichia coli* (–) is highly susceptible, whereas *Staphylococcus aureus* (+) and *Bacillus subtilis* (+) are less susceptible to CuO NPs [13]. The antibacterial effect of Ag NPs is higher than Cu NPs against *E. coli* (–) and *S. aureus* (+) bacteria [14]. *S. aureus* (+) and *B. subtilis* (+) are more susceptible than *E. coli* (–) to NiO and ZnO NPs [13].

Role of growth rate

Another factor that can influence the tolerance of bacteria against NPs is the rate of bacterial growth. Fast-growing bacteria are more susceptible than slow-growing bacteria to antibiotics and NPs [15,16]. It is possible that the tolerance property of slow-growing bacteria is related to the expression of stress-response genes [14,17]. Consequently, antibacterial effects highly depend on the particular strain.

Role of biofilm formation

One of the major shortcomings of antibacterial drugs and NPs, is their failure to fight with bacteria [e.g., *S. aureus* (+)] that have the capability to produce biofilms [18,19]. Biofilms are a complex microbial community that form by adhesion to a solid surface and by secretion of a matrix (proteins, DNA, and extra-polysaccharide), which cover the bacterial cell community. Biofilms are known as a significant problem because biofilm formation protects pathogenic bacteria against antibiotics and is one of the main causes of development of chronic infections (Figure 2) [20]. The electrostatic properties of both NPs and biofilms influence how they interact. The majority of bacteria have negatively charged biofilm matrixes but *Staphylococcus epidermidis* (+) has a polycationic biofilm [21]. The uptake and bioaccumulation of Ag NPs to biofilms is increased in the presence of Suwannee River fulvic acid (SRFA) [22]. However, surprisingly, Ag NPs are able to impact biofilms only in the absence of SRFA. In all cases, the viability of bacteria is unchanged. SRFA may protect bacteria against NPs by covering the NPs and/or by intrinsic antioxidant activity, which protects the bacterial membrane from significant damage [23]. The Ag NP uptake by marine biofilms and reduction of marine biofilms are dependent on the concentration of Ag NPs [24]. Exposure to Ag NPs may prevent colonization of new bacteria onto the biofilm and decrease the development and succession of the biofilm. MgF_2 NPs have antimicrobial activity and are able to prevent the biofilm formation of common pathogens such as *E. coli* and *S. aureus* [25]. Furthermore, MgF_2 NP-modified catheters are able to restrict the biofilm formation of these bacteria significantly [26]. Moreover, they have demonstrated that glass surfaces coated with ZnO NPs are able to produce reactive oxygen species (ROS) that interfere with *E. coli* and *S. aureus* biofilm formation [27]. Among various types of NPs, superparamagnetic iron oxide NPs (SPIONs) with different surface coatings (e.g., gold and silver) show highest antibacterial activity against biofilms [18,19] (Figure 3). It is notable that magnetic NPs have considerable capability to penetrate into biofilms, using external magnetic fields [18,19].

The toxicity mechanisms of NPs against bacteria

The exact mechanisms of NP toxicity against various bacteria are not understood completely. NPs are able to attach to the membrane of bacteria by electrostatic interaction and

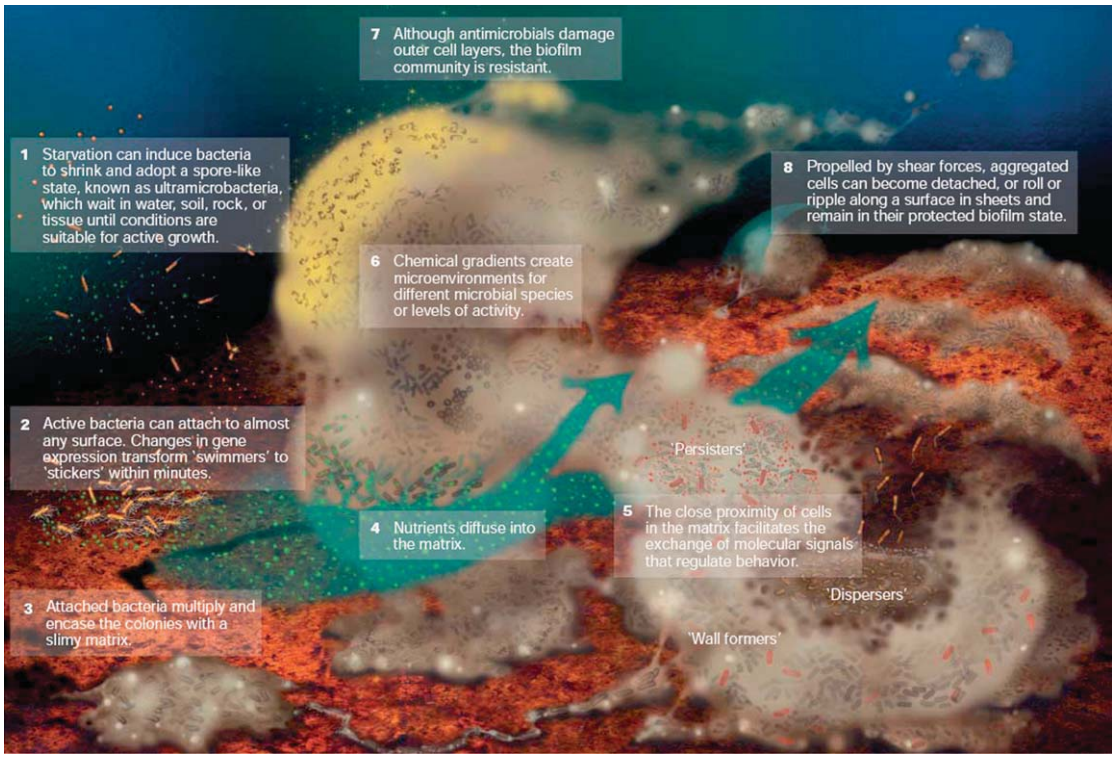

Figure 2. The stages of biofilm development [65]; (for additional information on dynamic processes of biofilm formation, see the following link: https://www.biofilm.montana.edu/biofilm-basics-section-1.html.)

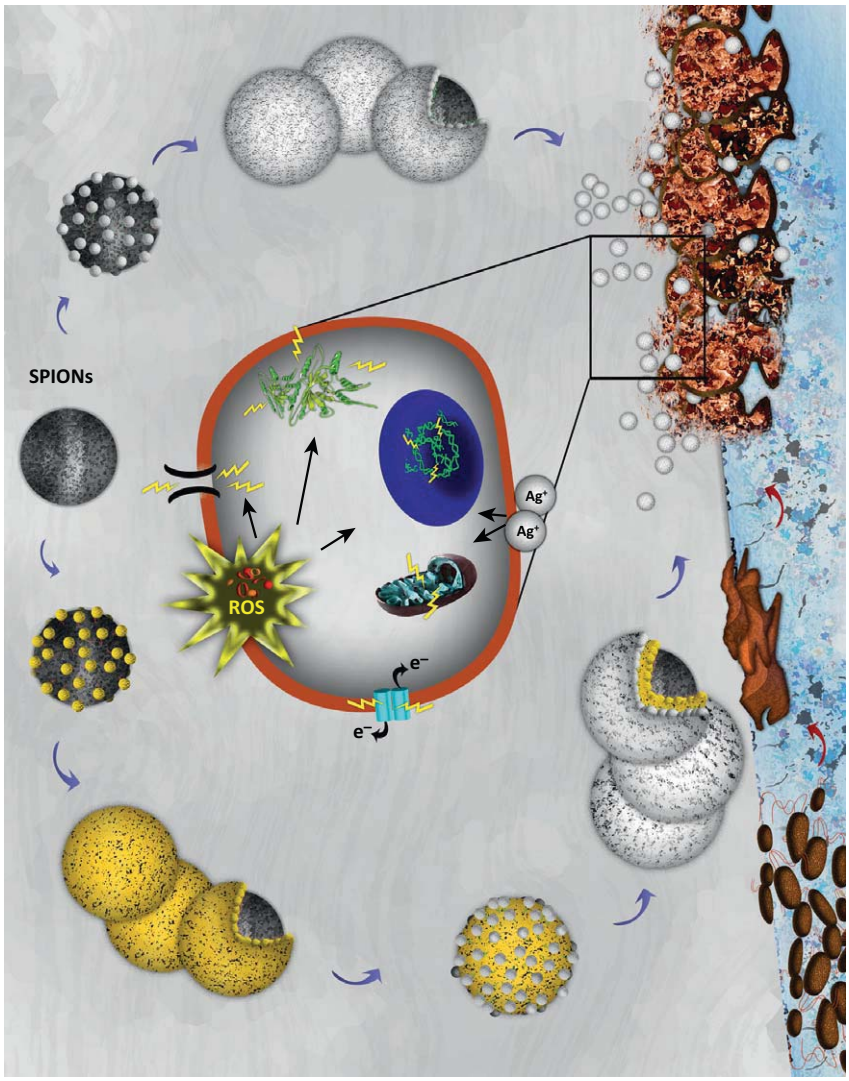

Figure 3. Schematic representation of toxicology effect of multifunctional nanoparticles (NPs) in bacterial biofilms. Monodisperse superparamagnetic iron oxide NPs (SPIONs; black spheres) are coated with silver (gray shell), gold (yellow shell), and silver ring-coated, gold-coated SPIONs; silver ring-coated SPIONs and silver ring-coated, gold-coated SPIONs have strong toxic effects on bacterial biofilms, by penetration into the biofilms. Both SPIONs cores and the intermediate gold shell have the capability to induce heat by applying alternative magnetic and laser fields, respectively; the produced heat can be used as additional means to escalate bacterial death using these NPs. The magnified section in the center illustrates the irreversible effects of NPs and their ions on the various parts of the bacteria (e.g., cell wall, DNA, and mitochondria).

disrupt the integrity of the bacterial membrane [28]. Nanotoxicity is generally triggered by the induction of oxidative stress by free radical formation, that is, the ROS, following the administration of NPs (Figure 4) [29,30]. Tables 1 and 2 summarize recently published work on antibacterial properties of nanostructured materials ranging from metallic and metal oxide NPs to semiconductors, polymers, and carbon-based materials against Gram-positive and Gram-negative bacteria.

The mechanisms of NP toxicity depend on composition, surface modification, intrinsic properties, and the bacterial species. There are many reports about the antibacterial effects of various NPs, but some reports contradict each other (for more information compare the summaries of previous reports in recent reviews [22,24,25,31,32]). These reports indicate that the mechanisms of NP toxicity are very complicated and depend on several factors (e.g., physicochemical properties of NPs). Therefore, we are not able to classify the NPs as beneficial NPs and/or adverse NPs for killing bacteria.

In the following, we describe some mechanisms of toxicity effects of NPs against bacteria. TiO_2 and ZnO NPs have weak mutagenic potential that induces frameshift mutation in *Salmonella typhimurium* (–) (TA98 and TA1537) [33]. The ability of ZnO NPs to induce frameshift mutation is dependent on the presence of S9 fraction. It is possible that the S9 fraction increases the internalization of NPs and then increases the generation of ROS that induce frameshift mutation in the bacteria. However, TiO_2 NPs induce frameshift mutation in *Sal. typhimurium* (TA98 and TA1537) independent of S9 fraction. TiO_2 NPs are toxic to *Pseudomonas aeruginosa* (–), *Enterococcus hire* (+), *E. coli* (–), *S. aureus* (+), and *Bacteroides fragilis* (–), only under UV illumination and killed approximately all bacteria in 60 min. These NPs have no toxicity in the dark [34]. TiO_2 NPs photocatalysis can increase peroxidation of

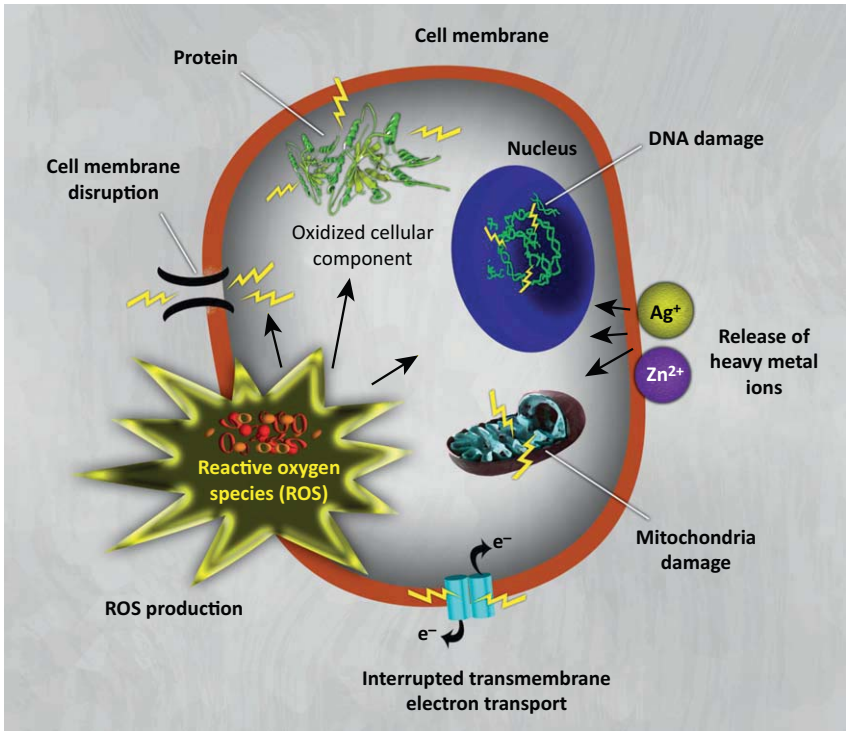

Figure 4. Mechanisms of toxicity of nanoparticles (NPs) against bacteria. NPs and their ions (e.g., silver and zinc) can produce free radicals, resulting in induction of oxidative stress (i.e., reactive oxygen species; ROS). The produced ROS can irreversibly damage bacteria (e.g., their membrane, DNA, and mitochondria), resulting in bacterial death.

the polyunsaturated phospholipid component of the lipid membrane and promote the disruption of cell respiration [35].

The toxicity of copper NPs depends on the combination of several factors such as temperature, aeration, pH, concentration of NPs, and concentration of bacteria (*E. coli*). The high temperature, high aeration, and low pH decrease the agglomeration and increase the toxicity. In fact, the lower agglomeration provides more available surface area for interaction with bacterial membranes and for solubilization of copper ions, which leads to more toxicity [36]. Metallic and ionic forms of copper produce hydroxyl radicals that damage essential proteins and DNA [37].

Au NPs in solution, prepared by using the citrate reduction method, are photomutagenic against *Sal. typhimurium* (–) strainTA102. The photomutagenicity of Au NPs is dependent on coexisting Au^{3+} ions and citrate and it is not related to their intrinsic properties. Oxidation of Au^{3+} and decarboxylation of citrate in the presence of light induce the generation of free radicals that damage essential proteins and DNA [38].

Among NPs such as CuO, NiO, ZnO, and Sb_2O_3 used against *E. coli*, *B. subtilis*, and *S. aureus*, CuO NPs have the highest toxicity, followed by ZnO (except for *S. aureus*), NiO and Sb_2O_3 NPs [39]. The toxicity of ions, which come as a result of NPs, is not significant and the toxicity strength of metal oxide NPs depends on the natural toxic properties of heavy metals. There appears to be a quantitative relation between colony size, colony number and the concentration of metal oxide NPs [39]. Also, the toxicity of oxide NPs (e.g., ZnO and CuO) does not always depend on the bacteria internalizing the NPs; these NPs can locally change microenvironments near the bacteria and produce ROS or increase the NPs solubility, which can induce bacterial damage [40].

Biogenic Ag NPs, which are produced by living organisms or biological processes, have synergistic effects with antibiotics such as erythromycin, chloramphenicol, ampicillin, and kanamycin against Gram-negative and Gram-positive bacteria [41]. The combination of biogenic Ag NPs with antibiotics has efficient antibacterial activity. In fact, the ampicillin damages the cell wall and mediates the internalization of Ag NPs into the bacteria. These NPs bind to DNA and inhibit DNA unwinding, which leads to cell death. Moreover, Ag NPs modified with titanium are toxic to *E. coli* and *S. aureus*. Ag NPs naturally interact with the membrane of bacteria and disrupt the membrane integrity, and silver ions bind to sulfur, oxygen, and nitrogen of essential biological molecules and inhibit bacterial growth [42]. The aforementioned studies show that suitable NPs can be selected to fight against specific bacteria.

NPs against drug-resistant bacteria

The emergence of antibiotic- and/or multidrug-resistant bacteria is recognized as a crucial challenge for public health. Killing of antibiotic-resistant bacteria requires multiple expensive drugs that may have side effects. As a result, treatments are costly and require more time. NPs can offer a new strategy to tackle multidrug-resistant bacteria [43]. Four types of silver carbon complexes (SCCs) with different formulations including micelles and NPs have efficient toxicity against medically important pathogens such as

Table 1. Different nanostructured materials and their toxic effects in Gram-positive bacteria

Bacteria	Bacterial property	NP Composition	Physicochemical Properties of NPs	Applied dosage	Mechanism of Toxicity Action	Remarks	Refs
S. aureus	Biofilm formation, normal flora of skin, production a matrix of exopolymeric substances	Carboxyl-grafted SPIONs	10–20 nm (size defined by TEM) ZP: –15.4±0.5 mV	0.35 mg/ml	An external magnetic field could target carboxyl-grafted SPIONs into a biofilm and increase antibacterial efficacy	Carboxyl-grafted SPION, APTES-grafted and bare SPION Internalized into the cell but does not affect mammalian cell adhesion and spreading	[66]
		APTES-grafted SPIONs	10–20 nm (size defined by TEM) ZP: +32.6±0.3 mV	0.35 mg/ml	ROS generation, electrostatic interaction between NPs and bacteria		
		Bare SPIONs	10–20 nm (size defined by TEM) ZP: +43.7±1.7 mV	0.35 mg/ml			
		PEGylated SPIONs	10–20 nm (size defined by TEM) ZP: –7.71±0.9 mV	0.35 mg/ml	No bacterial toxicity	PEGylated SPION does not internalize into the cell	
		Ag-coated SPIONs	15–20 nm (size defined by TEM)	80 μg/ml	Bacterial toxicity by penetration within the biofilm and increase of the bacterial toxicity in the presence of external magnetic field, ROS generation, electrostatic interaction, and physical damage of bacteria	Ag-coated SPION and Ag–Au-coated SPION are fully compatible with the cell	[67]
		Ag–Au-coated SPIONs	20–30 nm (size defined by TEM)	80 μg/ml			
		Au-coated SPIONs	25–40 nm (size defined by TEM)	80 μg/ml	Spion-Au NPs have slight antibacterial activity only in the presence of external magnetic field due to penetration within biofilm of bacteria		
S. epidermidis	Biofilm formation, normal flora of skin, production a matrix of exopolymeric substances, gentamicin-resistant	NO-releasing MAP3(N-methyl amino propyltrimethoxysilane) Si NPs	80–100 nm (size defined by AFM)	8 mg/ml	Biofilm killing due to electrostatic properties of NO-releasing NPs and increase NO delivery to biofilm-based microbes	Rapid delivery of NO may be more effective at biofilm killing than slow NO delivery	[68]
(Halophilic) *bacterium* sp. EMB4	Non-pathogen Gram-positive halophilic, has a thicker PG layer with higher percentage of neutral phosphatidylglycerol	ZnO	<100 nm (Cat. No. 544906, Sigma–Aldrich)	2 or 5 mM	Electrostatic interaction, morphological changes in the presence of bulk and nano ZnO, increase in membrane permeability and ZnO accumulation in the cytoplasm		[69]
		Ag	<100 nm (Cat. No. 576832, Sigma–Aldrich)	2 or 5 mM	Bulk Ag and nanosized Ag did not affect the growth and cell wall		
Vancomycin-resistant *Enterococcus*	Medically important pathogens, vancomycin-resistant	Ag Caron Complex-L-tyrosine polyphosphate NP(SCC23-LTP NPs)	700–800 nm (size defined by DLS)	(MBC) NA (MIC) 10 mg/l			[43]

Table 1 (*Continued*)

Bacteria	Bacterial property	NP Composition	Physicochemical Properties of NPs	Applied dosage	Mechanism of Toxicity Action	Remarks	Refs
B. subtilis	Non pathogen, protective endospore forming	ZnO	<100 nm (Cat. No. 544906, Sigma–Aldrich)	10 mM	Bulk and nanosized forms of ZnO and Ag have marginal reduction in the specific growth rate and viable count	Toxicity towards Gram-positive cells is significantly less, because of the presence of thicker PG layer	[69]
		Ag	<100 nm (Cat. No. 576832, Sigma–Aldrich)	10 mM			
		Ag	2–4 nm (size defined by TEM)	ND	Release of Ag^{1+} and Cu^{2+}, electrostatic interaction, cell wall damage, rupture of the plasma membrane, and disrupt biochemical process	There are more amines and carboxyl groups on cell surface of *B. subtilis* and therefore bind to NPs	[70]
		CuO	8–10 nm (size defined by TEM)	ND			
		Al_2O_3	40–70 nm (purchased from Zhejiang Hongsheng Material Technology Co., China) ZP: +30 mv	20 mg/l	bacterial attachment (electrostatic interaction) Damage to the bacterial cell wall and increase the permeability	Toxicity of NPs is from their higher tendency to attach to the cell walls	[71]
		TiO_2	40–60 nm (purchased from Zhejiang Hongsheng Material Technology Co) ZP: –21 mv		TiO_2 has no toxicity in dark condition		
M. smegmatis	Non pathogen	Cu-doped TiO_2 NPs	~20 nm (size defined by TEM)	20 mg/l	Release of Cu^{2+}, decreased enzymatic activity NADPH production, no cell damage, no internalization of NPs	In the presence of EDTA, the antibacterial activity of Cu-doped TiO_2 decreases significantly	[51]

Abbreviations: AFM, atomic force microscopy; APTES, 3-aminopropyltriethoxysilane; DLS, dynamic light scattering; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; NA, not available; ND, not determined; TEM, transmission electron microscopy; ZP, zeta potential.

Table 2. Different nanostructured materials and their toxic effects in Gram-negative bacteria

Bacteria	Bacterial property	NP composition	Physicochemical properties of NP	Applied dosage	Mechanism of aoxicity action	Remarks	Refs
K. pneumoniae	Medically important pathogens, nitrogen fixation, extended-spectrum β-lactamase production Encapsulated	Ag Caron complex-L-tyrosine polyphosphate NP (SCC23-LTP NPs)	~ 800 nm (size defined by DLS)	(MBC) NA (MIC) >10 mg/l			[43]
		Ag NPs	43 nm (Hydrodynamic size in XRD) Surface area: 26 m^2/g	30 mg/l	Electrostatic interaction, adsorption, and penetration of NPs and toxicity	The adsorption of Ag NPs increases at 20 °C compared to 37 °C	[72]
		NO NPs	10–15 nm (size defined by TEM)	(MIC) 10 mg/ml in 24 h	Alteration of the bacterial membrane, antimicrobial effects via nitrosation of protein thiols and the nitrosylation of metal centers	NO oxidized to RNS, which exert antimicrobial effects	[47]
P. aeruginosa	Opportunistic, normal flora of skin and intestine, biofilm formation	NO NPs	8–15 nm (size defined by TEM)	(MIC) 10 mg/ml in 16h	Alteration of the bacterial membrane, antimicrobial effects via nitrosation of protein thiols and the nitrosylation of metal centers	NO oxidizes to RNS which exert antimicrobial effects	[47]
		NO-releasing MAP3 (N-methyl amino propyltrimethoxysilane) Si NPs	80–100 nm (size defined by AFM)	8 mg/ml	Biofilm killing due to electrostatic properties of NO-releasing NPs and increased NO delivery to biofilm-based microbes	Rapid delivery of NO may be more effective at biofilm killing than slow NO delivery	[68]
		TiO_2	10–25 nm (ST-01, Ishihara Sangyo Kaisha Ltd., Osaka, Japan)	10 mg/l	Photoactivation of TiO_2 promotes bactericidal effect Peroxidation of the polyunsaturated phospholipid of membrane, loss of respiratory activity		[73]
		Ag	1–10 nm (size defined by TEM)	25–100 mg/l	Disturbs permeability, respiration, and cell division, interacts with cell membrane and sulfur- and phosphorus-containing compounds		[74]
		ZnO	10–20 nm (size defined by TEM)	1–4.25 mM in 100 ìL of LB	Bacterial attachment by Electrostatic interaction, ROS generation, membrane disruption, and disturbance of permeability		[75]

Table 2 (*Continued*)

Bacteria	Bacterial property	NP composition	Physicochemical properties of NP	Applied dosage	Mechanism of aoxicity action	Remarks	Refs
E. coli		Extracellular biogenic synthetic Ag NPs by *Trichoderma viride* fungi	5–40 nm (size defined by TEM)	(MIC) 30 μg/ml	Ag NP–ampicillin leads to cell wall lysis, penetration of Ag NPs, and prevents DNA unwinding	Biogenic Ag NPs have synergistic effects with antibiotics	[76]
		NO-releasing MAP3 (N-methyl amino propyltrimethoxysilane) Si NPs	80–100 nm (size defined by AFM)	8 mg/ml	Biofilm killing due to electrostatic properties of NO-releasing NPs and increased NO delivery to biofilm-based microbes	Rapid delivery of NO may be more effective at biofilm killing than slow NO delivery	[68]
		Al_2O_3	50–70 nm (Zhejiang Hongsheng Material Technology Co.) +30 mV	20 mg/l	Bacterial attachment (electrostatic interaction) Damage to the bacterial cell wall and increased permeability	Toxicity of NPs is from their high tendency to bind to the cell walls	[71]
		ZnO	~20 nm (Zhejiang Hongsheng Material Technology Co.) –5 mV	20 mg/l			
		TiO_2	~50 nm (Zhejiang Hongsheng Material Technology Co.) –21 mV	20 mg/l	No toxicity in dark condition		
		NiO	~20–30 nm (NanoAmor, Houston, USA)	20 mg/l	Growth inhibition (in aqueous medium). Significant damaged cellular functions, physical/ mechanical stresses on cellular structure integrity (in aerosol exposure)	Synergistic effect between the soluble ion stress and the nano-related stress (in aerosol exposure of NiO, ZnO, CuO)	[77]
		zero valent Cu NPs (ZVCN)	~25 nm (Sun Innovations, USA)		Release of Cu ions and generation of hydroxyl radical in the cytoplasm		[78]
		TiO_2	20 nm ST-01 (Ishihara Sangyo Kaisha Ltd.)	10mg/l	Photoactivation of TiO_2 promotes bactericidal effect. Peroxidation of the polyunsaturated phospholipid of membrane, loss of respiratory activity		[73]
		Ag	1–10 nm (size defined by TEM)	25–100 mg/l	Disturbed permeability, respiration, and cell division Interacts with cell membrane and sulfur- and phosphorus-containing compounds		[74]
Sal. typhimurium		ZnO	25–40 nm (core size in TEM)	8 and 80 ng/ml	ZnO: cellular uptake, ROS generation and has no significant toxicity. Frameshift mutation in the presence of metabolic activation system (S9).		[79]
		TiO_2	40–60 nm (core size in TEM)	8 and 80 ng/ml	TiO_2: cellular uptake, ROS generation and has no significant toxicity. Frameshift mutation independent of metabolic activation system(s9)		

Table 2 (*Continued*)

Bacteria	Bacterial property	NP composition	Physicochemical properties of NP	Applied dosage	Mechanism of aoxicity action	Remarks	Refs
Waste water biofilm bacteria	Biofilm formation	Ag	5–100 nm (Sky-Spring Nanomaterials, Houston, USA)	1–200 mg/l	Wastewater biofilms with original EPS are highly tolerant to the Ag-NP. After removing of EPS, the bacteria are vulnerable to Ag NPs	EPS and microbial community interactions in the biofilms play important roles in inhibition of Ag NPs toxicity	[80]
Sh. oneidensis MR-1	Reduce poisonous heavy metals, resistant to heavy metals such as iron and uranium	Cu-doped TiO_2 NPs	~20 nm (size defined by TEM)	20 mg/l	Cu-doped TiO_2 NPs do not affect *Sh. oneidensis* MR-1 growth	*Sh. oneidensis* MR-1 tolerate against NPs due to production of a large amount of EPS and reduction of ionic Cu	[51]
P. putida KT2442	Non-pathogen, biofilm formation, beneficial soil bacterium	Ag	~10 nm Sigma–Aldrich, St. Louis, MO, USA	1 mg/l	Cell membrane damage and bactericidal effect	Nano-Ag and nano-CuO NPs have different targets for killing bacteria	[58]
		CuO	25–40 nm Sigma–Aldrich	10 mg/l			
		ZnO	50–70 nm Sigma–Aldrich	10 mg/l	Bacteriostatic effect		
C. metallidurans CH34	Non-pathogen, resistant in the presence of several forms of heavy metal	TiO_2	<25 nm (size defined by TEM)		*C. metallidurans* CH34 is resistant to NPs. TiO_2 and Al_2O_3 can internalize in this bacterium but these NPs do not cause death	The resistance of *C. metallidurans* CH34 may be related to overexpression of protective components or by efflux systems	[53]
		Al_2O_3	<25 nm (size defined by TEM)				
		Multiwalled-carbon nanotubes (MWCNTs)	<25 nm (size defined by TEM)		*C. metallidurans* CH34 is resistant to NPs. The MWCNTs cannot internalize in this bacterium		

Abbreviation: LB, lysogeny broth; XRD, X-ray diffraction.

P. aeruginosa (–), *Burkholderia cepacia* (–), methicillin-resistant *S. aureus*, multidrug-resistant *Acinetobacter baumannii* (–), and *Klebsiella pneumoniae* (–) in the range of 0.5–90 mg/l [43]. The SCCs are able to inhibit the growth of bio-defense bacteria such *B. subtilis* and *Yersinia pestis* (–) [43].

Targeting bactericidal NPs to specific bacteria or specific infected tissue is an efficient prospect in treating infection because this phenomenon minimizes side effects and enhances antibacterial activity [44,45]. In this case, multifunctional NPs can be very useful; for instance, multifunctional IgG–Fe_3O_4@TiO_2 magnetic NPs are able to target several pathogenic bacteria and have efficient antibacterial activity under UV irradiation. The IgG and TiO_2 play a critical role in the targeting and killing properties of these NPs respectively. These NPs are toxic to *Streptococcus pyogenes* M9022434 and M9141204 [46].

Nitric-oxide-releasing NPs (NO NPs) are broad spectrum antibacterial agents that are able to inhibit the growth of many antibiotic-resistant and sensitive clinically isolated bacteria such as *K. pneumoniae*, *Enterococcus faecalis* (+), *Str. pyogenes*, *E. coli*, and *P. aeruginosa* (–). The toxicity of these NPs depends on the delivery of NO to the target. These NPs are able to change the structure of the bacterial membrane and produce reactive nitrogen species (RNS), which lead to modification of essential proteins of bacteria [47]. Beside NO NPs, ZnO NPs are toxic to antibiotic (methicillin)-resistant bacteria such as *Streptococcus agalactiae* (+) and *S. aureus*. These NPs are able to disorganize and damage the cell membrane and increase the permeability, which leads to cell death. The polyvinyl alcohol (PVA)-coated ZnO NPs are able to internalize the bacteria and induce oxidative stress [48]. The toxicity of ZnO NPs is concentration-dependent and these NPs are mildly toxic at low concentration [49].

NPs in water can significantly promote the horizontal conjugative transfer of multidrug-resistance genes mediated by the RP4, RK2, and pCF10 plasmids [50]. Here, nanoalumina can promote the conjugative transfer of the RP4 plasmid from *E. coli* to *Salmonella* spp. by up to 200-fold compared with untreated cells. The nanoalumina is able to induce oxidative stress, damage bacterial cell membranes, enhance the expression of mating pair formation genes and DNA transfer and replication genes, and depress the expression of global regulatory genes that regulate the conjugative transfer of RP4 [50].

Defense mechanisms of tolerant bacteria against NPs

Several naturally adapted bacteria are tolerant to specific toxins or NPs that are present in the environment. Cu-doped TiO_2 NPs are able to inhibit the growth of *Mycobacterium smegmatis* (+), but have no effect against *Shewanella oneidensis* MR-1(–) [51]. These NPs release Cu^{2+} ions, which might be the main cause of toxicity, because the antibacterial activity of Cu-doped TiO_2 NPs was decreased in the presence of chelating agents such as EDTA. *Sh. oneidensis* MR-1 has excellent resistant against several concentrations of Cu^{2+} and Cu-doped TiO_2 NPs because of the production of extracellular polymeric substances (EPSs) under NP stress. This bacterium is able to absorb NPs on the cell surface and to decrease the amount of ionic Cu in the culture medium. Therefore this bacterium can be regarded as a promising candidate for cleaning of metal oxide NPs from the environment.

B. subtilis and *Pseudomonas putida* (–) can physically adapt to nC_{60} [buckminsterfullerene (C60) introduced as colloidal aggregates in water] [52]. *P. putida* increases cyclopropan fatty acids and decreases unsaturated fatty acid levels, but *B. subtilis* increases the transition temperature and membrane fluidity in the presence of nC_{60}. These physiological adaptation responses of bacteria help to protect the bacterial membrane against oxidative stress. TiO_2 and Al_2O_3 NPs are able to be internalized by *E. coli* and *Cupriavidus metallidurans* CH34, but these NPs are toxic only against *E. coli* [53]. The resistance mechanism of *C. metallidurans* CH34 is not yet understood completely. The tolerance mechanism of this bacterium may be related to physical properties of their PG layer and/or products of genes that are located in the plasmids and are able to stabilize the plasma membrane or efflux of NPs.

Many bacteria are able to tolerate NO NPs using various mechanisms. For example *P. aeruginosa, E. coli*, and *Sal. typhimurium* induce the expression of genes that are responsible for repairing of DNA and altering the metal homeostasis in the presence of NO NPs [54–56]. In this condition, *K. pneumoniae* produces the enzyme flavohemoglobin, which neutralizes nitrosative stress [57].

NPs against environment and ecosystems

Extensive use of NPs in biological science, medical science, and commercial products leads to leakage and accumulation of NPs in the environment (e.g., soil and water). Protection of the environment and beneficial bacteria from NPs is very important because, for example, the indiscriminate use of nanosized Ag materials leads to release of Ag into the environment. The leakage of NPs into the environment is one of the most serious threats to beneficial microbes, microbial communities in ecosystems, and public health [58]. Many microbes benefit the environment and the ecosystem, because they play an important role in bioremediation, element cycling, and nitrogen fixation for plant growth [59–61]. For instance, in the nitrification process, ammonium nitrogen is converted to nitrite and then to nitrate by ammonia- and nitrite-oxidizing bacteria, respectively; the nitrifying bacteria are spread in the regions that have a high amount of ammonia; Ag NPs (<5 nm) have toxicity against nitrifying bacteria by interaction with the bacterial membrane, which contains ammonia-oxidation enzymes and by generation of ROS. The deletion of these bacteria from the environment leads to decreased nitrogen removal and interferes with plant growth [62]. As another example, the exposure of *E. coli* and MS2 phages (in a binary system) to Ag NPs and ZnO NPs leads to an increase in the transportation of MS2 phages into bacteria by 2–6 orders of magnitude. Therefore, Ag NPs and ZnO NPs facilitate the internalization of MS2 phages into bacteria. This can be a serious problem because these NPs may mediate the internalization of phages with drug-resistant genes into the bacteria and thus facilitate multidrug resistance development in the bacteria [63]. Therefore, the scientific community should pay attention to the adverse effects of the NPs on the

environment and human health, in spite of their beneficial commercial use.

Concluding remarks

Antibacterial activities of NPs depend on two main factors: (i) physicochemical properties of NPs and (ii) type of bacteria. Although there are good trends of correlation in a few aspects of antibacterial activity of NPs (e.g., for biofilms), individual studies are difficult to generalize. This is mainly due to the fact that the majority of researchers perform experiments based on available NPs and bacteria, rather than targeting specific, desired NPs or bacteria. In particular, often poorly defined and characterized NPs are used and thus correlation with basic physicochemical properties is not possible. Without agreement on standard NPs and bacteria as reference systems, which should be included in future studies, there is still a long way to go in order to unravel systematically the antibacterial properties of NPs.

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