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Chapter 3

Inorganic Nanoparticles: Innovative Tools for Antimicrobial Agents

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Additional information is available at the end of the chapter

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Abstract

Resistance of bacteria to antibiotics is an urgent problem of humanity, which leads to a lack of therapy for serious bacterial infections. Development of new antibiotics has almost ceased in the last decades—even when a new antibiotic is launched, very soon the resistance of bacteria appears. There is a long list of applications where antimicrobial protection is required to achieve effective treatment. However, if we use the same antibiotics for all these applications, we will remain caught in the "vicious circle" of constant discovery of new synthetic antibiotics and very fast development of their resistant species. Therefore, we need to find alternative strategies that will be routinely used for some specific conditions (wounds, implants, etc.). Thus, we will keep the activity of antibiotics and save them for acute conditions (pneumonia, meningitis, etc.). An option for designing alternative antimicrobial strategies is to go back to the antimicrobials that were used before the discovery of antibiotics, i.e., inorganic antimicrobial agents including ions (Ag^{+}, Cu^{+}/Cu^{2+}, Zn^{2+}, Ga^{3+}, etc.) or nanoparticles (Ag/AgO, $Cu/Cu_2O/CuO$, ZnO, Ga/Ga_2O_3, TiO_2, MgO, V_2O_3, etc.). Here we are going to summarize the main properties of inorganic antimicrobials as well as advantages, disadvantages and perspectives for their application.

Keywords: Ag/AgO, $Cu/Cu_2O/CuO$, ZnO, Ga/Ga_2O_3, TiO_2, MgO, V_2O_3, functionalized Au

1. Introduction

Resistance of bacteria to antibiotics is becoming an increasingly urgent problem of the humanity. The most serious threat comes from vancomycin-resistant *Enterococcus* (VRE, mainly *E. faecium*),

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methicilin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella* (especially *K. pneumoniae*), *Acinetobacter baumanii, Pseudomonas aeruginosa*, *Enterobacter* and *Escherichia coli* (the so-called "ESKAPE" pathogens,), Gram-positive *Mycobacterium tuberculosis* and some other Gram-negative bacteria [1]. Soon there will be no available antibiotics to treat infections with these pathogens. The problem first appeared in hospitals and grew promptly as a consequence of uncontrolled application of antibiotics not only in the healthcare but also in agriculture, stock breeding, poultry breeding, etc. However, overuse and misuse are not the only factors that speed up the spread of resistance. Some mechanisms of resistance do not destroy the antibiotic and leave it active in the environment. Thus, bacteria themselves help maintain the antibiotic environment; furthermore, the drug can be released into other environments and alter them. Many precautions against drug misuse and overuse led to the reduction of antibiotic application in the last decade. Consequently, the spreading of resistance slowed down, but it did not decrease. We could get rid of the resistant strains with new antibiotics. Unfortunately, development of new antibiotics has almost ceased in the last decades. Investments in research and development of new kinds of antibiotics were minimized due to their unprofitability. And even when a new antibiotic is launched, very soon the resistance of bacteria to the new antibiotic appears.

What can we deduce from all these facts? Instead of focusing only on development of new antibiotics, which will sooner or later create resistance, we should focus on preventing the resistance itself. There is a long list of applications where antimicrobial protection is required in order to achieve effective treatment. However, if we use the same antibiotics for all these applications, we will remain caught in the "vicious circle" of constant discovery of new synthetic antibiotics and very fast development of their resistant species. Therefore, we need to find alternative strategies that will be routinely used for some specific conditions (such as insufficient and slow wound healing, rejection of medical implants during their incorporation into the body due to the presence of bacteria on the surface of the implant, unsuccessful use of autologous, allogeneic or xenografts in tissue engineering because of the development of infection, etc.). Thus, we will keep the activity of the antibiotics and save them for urgent, acute conditions (like pneumonia, meningitis, peritonitis, etc.). One option for designing these alternative antimicrobial strategies is to go back to the antimicrobials that were used before the discovery of antibiotics, i.e., inorganic antimicrobial agents. There are a lot of inorganic substances with the capacity to kill bacteria or to inhibit bacterial growth. They are applicable in the form of antibacterial ions (i.e. Ag^{+}, Cu^{+}/Cu^{2+}, Zn^{2+}, Ga^{3+}, etc.) or antibacterial nanoparticles (i.e. Ag/AgO, Cu/Cu_2O/CuO, ZnO, Ga/Ga_2O_3, TiO_2, MgO, V_2O_3, functionalized Au, etc.). The new knowledge brought especially by the emergence and progress of biomaterials science and nanotechnology might enable: (i) local, targeted action without side effects in the organism, (ii) improved transport towards and eased penetration into the pathogenic species, leading to higher efficiency, (iii) unique opportunity for development of effective medicines.

This chapter provides detailed overview of various inorganic antimicrobial agents, their physicochemical properties and various mechanisms of action on bacterial/mammalian cells.

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2. Antibacterial ions

2.1. Silver (I) (Ag^+)

Silver nitrate ($AgNO_3$) was widely used for treatment of ulcers, burn wounds and different infections until the discovery of penicillin and sulpha drugs completely drove it out from the market [2]. In 1965, the favourability of $AgNO_3$ over antibiotics was shown and burn treatment procedure that involved frequent wetting of a cotton gauze dressing with 0.5 wt.% $AgNO_3$ solution was established [2, 3]. In spite of reduced mortality from severe burns and strong action against *Staphylococcus aureus*, haemolytic streptococci, *Pseudomonas aeruginosa* and *Escherichia coli*, the 0.5% $AgNO_3$ method had several disadvantages. It required very clean wounds, so deep and large burns were prone to invasive infection (usually by *P. aeruginosa*) leading to sepsis and death. 0.5% $AgNO_3$ was hypotonic, sensitive to light, inactive against *Aerobacter*, *Paracolon*, *Klebsiella* and a number of cutaneous saprophytes and could cause methaemoglobinaemia. Although the precipitation of $AgNO_3$ with different anions resulted in low absorption of silver into the body through the wounds, it consequently caused Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- depletion in serum [2–4]. Hence, an improvement was tried by combining $AgNO_3$ with a sulpha drug to obtain silver sulphadiazine [2]. Many other ionic Ag drugs emerged, but Ag-sulfadiazine remained the most widely used, although it delays the wound healing process [2, 5]. Further development went to systems for controlled delivery of Ag^+ ions, Ag-containing wound dressings, catheters and antibacterial coatings [6, 7] which flooded the market recently [8].

The antibacterial action of Ag^+ ions is currently explained by three mechanisms:

1. Ag^+ ions react with thiol groups of the respiratory and transport proteins in the cell membrane [6, 9] so that cellular respiration and electron transfer are blocked [6, 9], membrane potential and permeability are disrupted, leading to cell death [10].
2. Ag^+ ions enter the bacterial cells either through ion channels or due to the detachment of the cytoplasm membrane [9, 11]. Once inside, they complex with nucleobases of DNA and RNA leading to DNA condensation and loss of replication ability [6, 9, 12].
3. Increased production of reactive oxygen species (ROS). Disruption of cellular respiration and inactivation of intracellular thiol-based antioxidants increases the oxidative stress caused by reactive radicals that are generated by the Fenton reactions [9, 13].

There are two forms of resistance: complexation of Ag^+ inside cells or reduced permeability to Ag^+ combined with an upgraded active efflux mechanism to pump Ag out of the cell [5]. Several Gram-negative and positive bacteria have been reported to be Ag-resistant, including *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, members of the *Enterobacteriaceae* and *Citrobacter spp*. [6]. It has been recently shown that the toxic values of $AgNO_3$ and commercial Ag-containing wound dressings for *P. aeruginosa, E. coli*, *S. aureus* and human fibroblasts are very similar and that thiol-containing molecules reduce their toxicity towards both prokaryotes and eukaryotes [14].

2.2. Copper (I, II) (Cu^{+}, Cu^{2+})

$Cu^{+/2+}$ has also been known as a sterilizing, antiseptic and antimicrobial agent [15] used to treat a variety of skin diseases, syphilis, tuberculosis and anaemia, and to fight mildew [10, 16, 17]. In modern healthcare, the antimicrobial effect of Cu is very effectively used in hospital water distribution systems [16, 17]. Recent research has focused on "contact killing" mechanism [17]. In 2008, the US Environmental Protection Agency (EPA) proclaimed Cu-surfaces as efficient antimicrobials [17]. Cu is the first metal to be awarded such a status [17]. Cu is an essential micronutrient and more than 30 types of proteins that contain Cu ions are known today [18]. In these enzymes, Cu serves as an electron donor/acceptor by alternating between the redox states Cu(I) and Cu(II) [19]. Dietary intakes of 0.9–1.4 mg of Cu for an adult (a 70-kg person) and 50 μg per kg body weight per day in infants are recommended by the WHO [16, 20]. Cu ions are also toxic to prokaryotes and eukaryotes at higher cellular concentrations, and the involvement of Cu (and Zn) in phagosomal killing of bacteria engulfed by macrophages is an important defence mechanism [10, 21].

The antibacterial/toxicity action of Cu(I, II) is currently explained by the following mechanisms:

1. Direct generation of ROS through Fenton-type reactions [19, 22]. Radicals can cause oxidative damage to proteins, nucleic acids and lipids, which lead to cell death [23].
2. Indirect generation of reactive oxygen species by inactivation of antioxidants and thiol depletion [19, 23]. Such reactions of Cu can lead to the inhibition of respiratory enzyme function and disruption of respiration leads to ROS as explained for Ag^{+} [6].
3. Competition with other metal ions for important binding sites on proteins [6, 17, 19]. Site-specific inactivation by Cu ions can also occur in Fe-S dehydratases, the cytoplasmic enzymes needed to make branched-chain amino acids [17, 23].
4. By cross-linking within and between strands of DNA, Cu may cause helical structure disorders and DNA denaturation [16, 24]. Some studies have shown that H_2O_2 was required for the DNA breakage, which questions the relevance of this mechanism [16].

Bacteria have evolved a range of mechanisms to protect themselves from the toxic effects of excess Cu ions: exclusion by a permeability barrier; intra- and extracellular sequestration of Cu ions by cell envelopes and metallothionein-like Cu-scavenging proteins in the cytoplasm and periplasm; active transport membrane efflux pumps; reduction in the sensitivity of cellular targets to Cu ions; extracellular chelation or precipitation by secreted metabolites including Cu; and adaptation and tolerance via up-regulation of necessary genes in the presence of Cu [16, 19, 25]. Active extrusion of Cu from the cell appears to be the chief mechanism of Cu tolerance in bacteria and has been extensively studied in Gram-positive and Gram-negative bacteria. However, due to the multiple targets and mostly non-specific mechanisms of damage exerted by Cu, this bacterial tolerance is relatively low, as compared to the resistance to antibiotics (i.e., 10-fold lower sensitivity to Cu as opposed to 1000-fold less sensitivity to methicillin, for example, by methicillin-resistant *S. aureus*).

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2.3. Zinc(II) (Zn^{2+})

Zn^{2+} is also an essential micronutrient for the development, growth and differentiation of all living systems, including bacteria, and exhibits antibacterial action only at higher concentrations when its homeostasis is overcome. The adult human body contains approximately 1.5–2.5 g of Zn^{2+} [22, 26–28] with essential role in cell membrane integrity, development and maintenance of the body's immune system, managing insulin action and blood glucose concentration, bone and teeth mineralization, normal taste and wound healing [22]. Zn is a constituent of more than 300 enzymes that have a central role in reconstruction of the wound matrix [26, 29]. Zn in castor oil has a special place in the treatment of nappy (diaper) rash [26]. A vast range of zincated bandages, dressings, emollients, shampoos and creams are available commercially. In normal wound healing, body creates a higher amount of Zn^{2+} in the wound margin at a certain stage—during the formation of granulation tissue, scar tissue and re-epithelialization. It is believed that the addition of Zn at this stage might accelerate wound healing. Experimental studies have shown that topical ZnO reduced the initial haemorrhagic phase and promoted the regrowth of damaged skin and hair [26]. The antibacterial properties of Zn^{2+} ions are exploited especially in oral healthcare for prevention of caries, gingivitis and periodontitis. Zn− salts are used in mouthwashes and toothpastes [30]. The effect of Zn^{2+} ions is most probably only bacteriostatic, so oral-care products are designed for frequent use, while bactericidal action can be obtained in combinations with fluoride or Triclosan [30–33].

The antibacterial action of Zn− ions is a consequence of the following mechanisms [6, 30–32]:

1. Inhibition of enzymes that contain sulfhydryl groups and require Mg^{2+} ions; competitive inhibition and reaction of Zn(II) with sulfhydryl groups [30–32].
2. Zn^{2+} ions inhibit the utilization of the bacterial carbon source. They can disrupt the metabolism of sugars as well as the amino acid metabolism.
3. Zn^{2+} reduces the acid tolerance of *S. mutans* by inhibiting the transmembrane proton-translocating F-ATPase, which is the main engine for acid tolerance [30].
4. Zn(II) binds to the membranes and slows down the growth of organisms [6], inhibits protease-induced adhesion [34] and reduces the net negative charge on the cell surface and, hence, increases co-aggregation [34].

Resistance of bacteria to toxic levels of Zn^{2+} can be due to extracellular accumulation, sequestration by metallothioneins, intracellular physical sequestration, and/or can be efflux based [35]. A recent study compared the Cu and Zn resistance of MRSA and methicillin-susceptible *S. aureus* in a global collection of species [36]. While there was no difference in their Cu− susceptibility, there were significantly more Zn-resistant MRSA strains, which also had an encoded Zn resistance [36]. Similarly to Ag, recent progress of Zn− antimicrobials has gone in the direction of ZnO nanoparticles and incorporation of ionic Zn into zeolites, polymers, bioactive ceramics and glasses to achieve better efficiency and local action [6].

2.4. Gallium(III) (Ga^{3+})

Antibacterial properties of Ga^{3+} were first mentioned in 1931 [37]. Initially, it was mainly investigated for cancer diagnosis and treatment [38, 39]. Intensive research of Ga(III) as an antibacterial agent in the 2000s revealed great efficacy against *M. tuberculosis* [40] and *P. aeruginosa* [41, 42]. A recent study has shown that $Ga(NO_3)_3$ at safe therapeutic dosage (10 mg/kg) protects mice from *M. tuberculosis* infection [43]. A Phase-1 clinical study is being conducted since 2010, which tests Ganite in human patients suffering from cystic fibrosis, and chronically infected by *P. aeruginosa* [37, 38, 44]. Current results show that intravenous Ganite infusion for 5 days decreases the amount of *P. aeruginosa* in the lung without any serious adverse effect [38, 44]. Subcutaneous application of Ga-maltolate was effective in reducing *S. aureus*, *A. baumannii* and *P. aeruginosa* colonization in burn wounds of thermally injured mouse model [42]. These data support a potential use of Ga-maltolate *in vivo*, especially in topical administration for the prevention and treatment of wound infections. Besides $Ga(NO_3)_3$ and Ga-maltolate, some other forms of Ga(III) have also been used, i.e., chloride [45], citrate [46], desferriox-amine B and other complexes [46–48].

The following is currently known about the mechanism of antibacterial action of Ga^{3+} ions:

1. Ga^{3+} follows uptake and transport pathways for Fe^{3+}; unlike Fe(III), it cannot be reduced to the oxidation state (+2); small amounts of non-bound Ga can exist in solution at physiological conditions, versus insignificant amounts of non-bound Fe^{3+}, permitting biological interactions for Ga^{3+} that would not be possible for Fe^{3+} [49, 50].
2. Most bacteria require Fe for growth [37]. If bacteria use Ga instead of Fe, it will prevent their multiplication, which is crucial for harming the organism (as observed in bacterial ferric-binding protein and non-ribosomal peptide microbial siderophores [49]).
3. Ga(III) can affect the synthesis of siderophores by regulation of gene expression [68] leading to shortage of Fe inside cell and inhibition of many Fe-requiring enzymes.
4. Increased production of H_2O_2 was noticed due to Ga^{3+} antibacterial action [51]. However, Ga^{3+} quenches the superoxide ion signal [51], and it is not yet clear whether the ROSs are the main reason or only a consequence of the Ga^{3+} antibacterial action.

Considerable progress has been recently made in the development of Ga delivery systems using phosphate-based glasses [52–54], cellulose [55], scaffolds [56], phosphosilicates [57, 58] and titanium implants [59]. Because bacteria cannot discriminate between Fe(III) and Ga(III), they will not sense an increase of Ga^{3+} concentration and a decrease of Fe^{3+}. However, since Ga(III) enters microbial cells by exploiting specific Fe(III)-uptake mechanisms, mutations in these pathways could block Ga from reaching its cellular targets, ultimately making bacteria less susceptible to Ga's inhibitory activity, as it has been observed in laboratory studies of Ga(III) antibacterial mechanism, in which resistant strains were created by genetic modification of *P. aeruginosa* [60, 61]. Nevertheless, such mutations could never completely prevent Ga^{3+} entrance into bacterial cells and only 2–4 times higher Ga(III) concentrations were already effective against the resistant strains.

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3. Antibacterial nanoparticles

3.1. Ag nanoparticles

Ag nanoparticles show bactericidal action in both Gram-positive and Gram-negative bacteria, with higher efficiency induced by smaller particles [12, 32, 62] and quite intriguing dependence of the efficiency on the shape, falling in the order: triangular nanoplates, nanospheres, nanowires [63]. In Ag nanoparticles, there are three sources of bactericidal activity: Ag, Ag ions and nanosize. As the three sources are interlacing, it is difficult to determine what effect comes from each of them. Ag nanoparticles come into contact with bacterial cells. Positively charged Ag nanoparticles attach to bacterial membrane by electrostatic interactions, while negatively charged ones attach due to high affinity of Ag (soft acid) for P- and S-containing molecules (soft bases) [63–65]. Then, Ag ions are released into the cell and inhibit respiratory enzymes, which facilitates the generation of ROS and consequently damages the cell membrane [66]. The uptake of Ag can be recognized by irregular pits. They can be dissolved by oxygen or H_2O_2. A Fenton-like reaction was suggested to account for the observed generation of OH• radicals at pH below 7.4 [67]. Then, S-containing proteins in the membrane or inside the cells and P-containing elements like DNA are likely to be the preferential sites for Ag nanoparticle binding. Disruption of membrane morphology may cause a significant increase in permeability, leading to leaking of the internal components resulting in cell death [63].

Exposure of murine macrophages to Ag NPs showed mitochondrial damage, apoptosis and cell death abrogated in the presence of Ag ion-reactive, thiol-containing compounds suggesting the central role of Ag ions in Ag NP toxicity [68]. Further research showed that Ag^+ ions were the only active part of the Ag NPs [69]. Testing under anaerobic conditions (ion release was negligible) showed that Ag NPs were ineffective against *E. coli* K12. If the Ag NPs were exposed to air prior to the antibacterial test under anaerobic conditions, their antibacterial properties were enhanced and bacterial survivability depended on released ions.

However, the amount of released ions from the Ag NPs at their MIC was always lower than the MIC of Ag^+ ions [70–72]. Recent research [73, 74] showed much higher intracellular dissolution of Ag NPs compared to extracellular ones. The ion release from the Ag NPs is size-specific and surface-dependent. The toxicity of 20–80-nm Ag NPs follows this size dependence and is mainly assigned to the released ions. However, the 10-nm Ag NPs are much more toxic. Importantly, immobilized Ag NPs were more efficient than Ag^+ ion-releasing substrates, even though they released much lower amount of ions and the immobilized Ag NPs were not internalized [72, 75]. Ag NPs can change the lipid composition of the membrane, anchor and incorporate into the outer membrane, and it is currently believed that the outer membrane damage is mainly "nano-specific" [72]. Ag NPs enhance the transport of Ag^+ ions into the cell and could avoid bacterial resistance that involves efflux systems. However, *E. coli* easily develop resistance to Ag NPs as well as Ag^+ after 100–200 generations of exposure to Ag NPs [76] and several studies have shown low efficiency of Ag NPs against Ag-resistant bacteria [71, 72].

3.2. Cu/CuO nanoparticles

In Cu nanoparticles, there is a coincidence of antibacterial effect of ions and nano-sized particles. The efficiency of Cu was improved by decreasing the dimensions, but it was higher for Gram-positive bacteria [32]. Cu nanoparticles have great affinity for amines and carboxyl groups, so they bind to the ones on the surface of bacteria and release the ions inside. These ions can then interact with DNA molecules and intercalate with nucleic acid strands [77]. It is believed that here, the role of ROS is much larger than in Ag nanoparticles, since they can be generated by CuO as well as the released Cu^{+}/Cu^{2+} ions by their dissolution [78]. Some scientists, on the other hand, emphasize the role of the released ions more [32]. Both Cu and CuO antibacterial nanoparticles cause lipid peroxidation, cell wall and membrane damage and oxidative damage to DNA. They generate ROS in the absence of any cells, in extracellular as well as intracellular environment. CuO nanoparticles are much more toxic to mammalian cells than Cu^{2+} ions and also much more cytotoxic than ZnO and TiO_2 NPs [79]. In general, it has been shown that trends in bactericidal activity were similar to trends in cytotoxicity, i.e. more powerful bactericidal agents [80] were more toxic towards human cells [81].

3.3. ZnO nanoparticles

ZnO has so far been found to be the most effective metal oxide antimicrobial, with efficiency comparable to Ag [32]. If ZnO nanoparticles are shined with UV light, their antibacterial effect can become strongly bactericidal as a consequence of photocatalysis. ZnO is a semiconductor with a direct 3.3-eV band gap [82]. Absorption of light with energy greater than 3.3 eV induces the electron transfer from the valence to the conduction band and separation of charge, generating a hole (h^{+}) in the valence band and an electron (e^{-}) in the conduction band [82, 83]. At the surface of the excited ZnO particle, the valence band holes abstract electrons from water and/or hydroxide ions, generating hydroxyl radicals (OH•). Electrons can reduce O_2 to produce the superoxide anion O−•. The obtained OH• and O−• can induce lipid peroxidation in membranes, DNA damage due to strand breakage or oxidized nucleotides and oxidation of amino acids and protein catalytic centres [83]. Negative charge of OH• and O−• prevents these species from passing through the membrane into the cell, so they can exert only outside damage. They can also combine with H^{+} to create H_2O_2, which can pass into the cell and create internal damage leading to cell death [82]. ZnO nanoparticles show bactericidal properties as well as ROS generation also in complete absence of light. This effect has been tried to be explained by surface defects and the oxidative role of oxygen or halogens adsorbed on their surfaces [31, 82]. Such a mechanism would be enhanced in an aerobic environment and it was observed that oxygen annealing and formation of nanoholes on the surface, which both stimulated a high amount of adsorbed oxygen atoms on the ZnO surface, increased the ROS production and enhanced the antibacterial properties [31, 82]. ZnO nanorods are stronger antimicrobials than nanospheres, and flower-shaped nanoparticles with exposed polar are even stronger [82].

3.4. TiO_2 nanoparticles

TiO_2 nanoparticles can account for their antibacterial effect as a consequence of production of OH• radicals. They do not possess any antibacterial properties in the absence of UV light due

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to weak interaction with bacterial surface because of negative charge. In contrast, recent studies have shown that TiO_2 particles exhibited high tendency to bond to the *E. coli* membrane via Van der Walls and receptor-ligand interactions. The extent of these interactions was more pronounced than in the case of ZnO nanoparticles. As a result, when illuminated with UV light, TiO_2 was more powerful than ZnO. As opposed to TiO_2, it did not show up-regulation of ROS-related proteins but rather caused membrane damage via direct transfer of ROS molecules from particle surface towards the bacterial membrane [84]. Of interest is metal doping (e.g. with Ag) of TiO_2, which can improve its antibacterial properties significantly and enable visible-light-induced photocatalytic activity [85, 86].

3.5. Functionalized Au nanoparticles

Au nanoparticles alone are considered biocompatible and bioinert [87–89]. Only Au NPs with size below 3 nm are cytotoxic due to their irreversible binding to key biopolymers [90]. Internalization of Au NPs into a cell is size-, shape- and charge-dependant. The fastest uptake was observed for 40–50 nm size; it was higher for nanospheres vs nanorods and positively charged NPs penetrate more easily [91–93]. Au NPs can be used as antibacterial agents only if they are irradiated with NIR light (photothermal treatment) or if some antibacterial component is added to them [77, 85, 89]. Interestingly, some studies have also shown antibacterial activity of Au nanoparticles with non-antibacterial components added to them, like C/Au core shell [94] or functionalized Au nanoparticles [95–97]. Au nanoparticles as carriers enable entry of the added molecules into bacterial cells, where they can directly affect some important molecules, otherwise protected by the cell wall and membrane. Concentration of otherwise inactive molecules on the surface of Au nanoparticle enables (or increases) some interactions that lead to bacterial death [95]. In this way, 4,6-diamino-2-pyrimidinethiol was able to chelate Mg^{2+} ions when attached to the Au nanoparticle [95] and induced damage of the outer membrane, leading to increased permeability of the cellular membrane. Nanoparticles entered the cell, where chelation of Mg^{2+} and interaction of the particles with DNA resulted in inhibition of protein synthesis. Cell death followed as a consequence of leakage of intracellular contents [95]. The antibacterial action of Au/4,6-Diamino-2-pyrimidinethiol NPs involves changing the membrane potential and inhibition of ATP synthase activities to decrease the ATP level and inhibition of the ribosome subunit for tRNA binding, indicating a collapse of biological process [98]. Alternatively, in amino acid-functionalized Au NPs, a structure similar to antimicrobial peptides was created and enabled strong electrostatic interactions between cationic functionalization at Au NPs and bacterial membrane resulting in damage of the membrane compactness and structure which provided antibacterial action in *E. coli* and *S. aureus* [97].

3.6. Gallium-containing nanoparticles

Investigation of Ga-based antibacterial nanoparticles has begun only very recently and it started with Ga_2O_3 nanoparticles (100 nm) showing their anti-biofouling properties against *E. coli* and *S. aureus* [99]. However, concentrations up to 25 mg/L (133 μM) exhibited only very weak (towards *S. aureus*) or no inhibition (towards *E. coli*) of planktonic growth. Further investigation showed antibacterial action of Ga_2O_3 nanorods (50×200 nm) which created an inhibition zone in *E. coli* already at 25 mg/L concentration, whereas at least 50 mg/L

concentration was needed for an inhibition zone in *S. aureus* [100]. By contrast, bulk Ga_2O_3 did not create any inhibition zone. They also presented good photocatalytic properties of this semiconductor with a band gap of 4.9 eV, but photocatalysis was not responsible for the observed antibacterial action, since the test was performed in dark and Ga_2O_3 nanoparticles can create reactive oxygen species (only OH• radicals) only under direct visible light illumination [101]. Antibacterial activity was shown also for GaN nanoparticles (50 nm) [102] and anti-biofouling activity was observed towards *S. aureus*, *E. coli*, *P. aeruginosa* and *Pseudomonas putida*. Another study about a new Ga(III) delivery system in the form of $KGa[Fe(CN)_6]$/PVP NPs (average size of 15 nm) was published, which demonstrated their very good biocompatibility with HeLa cells until at least 1.1 mM concentration, their most probable endocytotic penetration into the cells and untargeted distribution in the cytoplasm, and *in vitro* exchange of Ga(III) by Fe(III) from Fe(II) suggesting their ability to sequester Fe(II) and consequently release Ga(III) [103]. A very recent study on eutectic GaIn alloy nanoparticles (average size around 100 nm) has also shown their low *in vitro* cytotoxicity against HeLa cells for at least 21 mg/L (0.2 mM Ga) concentration and endocytosis, fusion and degradation of the eutectic GaIn nanoparticles with release of Ga^{3+} ions inside HeLa cells [104]. The *in vivo* injection of these nanoparticles into mice caused no tissue damage, no allergic reaction, exhibited very low acute toxicity (maximum tolerated dose of 700 mg/kg), while Ga and In were excreted with both faeces and urine [104]. However, the antibacterial properties of KGa[Fe(CN)6]/PVP and eutectic GaIn alloy nanoparticles have not been evaluated. On the other hand, Narayanasamy et al. incorporated Ga(III)-tetraphenyl porphyrin into polymer nanoparticles (average size of 300 nm) and demonstrated their efficiency against *Mycobacterium smegmatis* as well as against HIV in macrophages, and did not show any sign of cytotoxicity for macrophages even at 2 mM concentrations despite internalization of the nanoparticles into all compartments of the cells [105]. Another way for the local delivery of Ga(III)-tetraphenyl porphyrin was by its conjugation to Pt nanoparticles (average size around 30 nm) [106]. Bactericidal properties against *S. aureus* were demonstrated under visual light illumination. However, the non-conjugated Pt nanoparticles were not tested, so it is not clear how large their contribution was and to what extent they were only deliverers of Ga(III)-tetraphenyl porphyrin. First investigation on antibacterial performances of elemental Ga nanoparticles [107] confirmed activity against *P. aeruginosa* with MIC at 0.1 mg/ml, low toxicity at this concentration and wide therapeutic window, which gives a good promise to this material for further investigations and design for biomedical applications.

3.7. Nanostructured MgO

MgO exhibits a broad range of antimicrobial activities against both Gram-positive and Gram-negative bacteria comparable to ZnO [107]. The molecular mechanism of MgO's antibacterial activity is still unclear. Some reports show dominant role of ROS which cause lipid peroxidation and diffuse inside the cell and cause cell death [108–110]. Other reports show non-ROS mechanism and suggest polar interaction with components of the cell wall (e.g. lipopolysaccharides), which cause membrane disintegration and bacterial death [111] similar to antimicrobial peptides [112]. In both mechanisms' descriptions, the surface defect

sites were related to the production of ROS. In the latter case, generation of ROS species was attributed to defects in general [111]. In the former case, oxygen could be reduced at the surface oxygen vacancy [109]. This is consistent with the mechanism of ROS species generation at the surface of MgO. However, for this to happen, energy is required to support electron transfer from vacancy towards the molecular oxygen [113]. Other mechanism of ROS generation was completely ignored in explanation of MgO antibacterial activity [113]. Till date, the following properties of MgO are known: (i) MgO exhibits contact-based antibacterial action [108]; (ii) increasing the pH in bacterial suspension due to MgO hydration did not contribute to its antibacterial activity [108]; (iii) dissolved Mg^{2+} were not causing harm to bacteria [108]; (iv) AFM and SEM morphology studies confirmed deterioration of bacterial membrane, which indicated membrane leakage [111, 114]; (v) TEM study showed lack of MgO particle internalization in bacteria, which indicated that MgO particles are "doing the damage" outside of the bacteria [111]. It has been shown that the bactericidal potential of MgO is proportional to its specific surface area. The MgO with the highest specific surface area (BET) exhibited the most effective antibacterial activity [115]. Improvement in the effectiveness of the bacteria/surface contact is also achieved by Li-doping which enhanced the creation of oxygen vacancies and improved antibacterial activity [116]. The strength of the nanoparticle interaction is inversely proportional to the size, i.e. smaller particles exhibited stronger agglomeration [117]. The agglomeration could strongly influence the further processing of MgO nanoparticles [118]. MgO particles containing microrods exhibited moderate antibacterial activity, while nano-textured microrods showed strongly improved antibacterial activity. As-prepared particles exhibited reduced agglomeration, lower specific surface area and improved bactericidal potential when compared to the commercial MgO nanoparticles. We attributed the difference in antibacterial activity to a reduced concentration of non-emissive defects at the surface of nano-textured MgO microrods [118]. Magnesium is the second most abundant intracellular cation in the human body [119] essential in many physiological processes like enzyme activity, membrane processes, functioning of muscle and neural tissue, and so on. [119]. The clinical study showed the ability of MgO to reduce hypertension (1g for 21 days) [120]. Although *in vitro* studies pointed out toxic effect of MgO on human cells [121], at a concentration of 0.2 mg/ml in suspension MgO particles were able to eliminate bacteria while at the same time showed potential to exhibit bioactive properties on the cells. In this context, there is a possibility to exploit multifunctional properties of MgO to design medicine-relevant devices, which exhibit both bioactive and antimicrobial properties.

3.8. Nanostructured V_2O_5

Recent studies have highlighted the ability of nanostructured V_2O_5 to mimic the myeloperoxidase activity [122, 123]. The activity is a characteristic of enzyme in human neutrophils, which eliminate bacteria via the catalysis of the hydrogen-peroxide-to-hypochlorite transformation in the presence of chloride ions [124]. This biomimetic property of V_2O_5 was effectively utilized for the processing of an anti-biofouling ship-hull coating using sea water as a source of hydrogen peroxide (100 nM) [123]. However, it has been shown that V_2O_5 generates ROS on its own [125], which indicated the possibility to perform a unique mode

of antibacterial activity with a two-step mechanism: (i) generation of ROS and (ii) transformation of the generated ROS to antibacterially more potent hypochlorite ions. The use of V_2O_5 in medicine is limited by its relatively high solubility in aqueous media (>1 g/L). So-formed, high concentrations of vanadate ions are toxic to human cells [126, 127]. *In vitro* studies also showed their bi-phasic nature, as these ions stimulate proliferation of various types of mammalian cells at low concentrations (up to 10 μM) [128, 129]. They exhibit an insulin-mimicking action via the inhibition of tyrosine phosphatase [130]. Orally administered vanadates in rat models stimulated the orientation of the fibroblasts in parallel arrays early in the tissue-repair process, i.e., vanadate ions can accelerate tissue repair [131–133]. Vanadates improved the bone-formation rate, mechanical strength and mineralization [134], while the pro-oxidant potential of vanadates was not revealed in erythrocytes [135]. These studies confirmed the bioactive potential of vanadate ions when they are properly delivered, which might be effectively applied when designing the antibacterial drug-delivery system to enable controlled delivery of vanadate ions.

4. Concluding remarks

Antibacterial ions are prone to similar problems as antibiotics, i.e., biodistribution and bacterial resistance. Nevertheless, they offer new options, especially for local delivery, and the antibiotic resistant bacteria are not always resistant also to antibacterial ions, even though Cu- and Ag-resistance genes have been found associated with antibiotic resistance genes in a few cases. On the other hand, the major problem of nanoparticles is their non-selectivity and consequent toxicity for eukaryotic cells. For this reason, current findings are still far from a good substitution of antibiotics. It is very good that nanomaterials have many targets as opposed to antibiotics. This implies that they could be the solution for antibiotic resistance. But, the problem is that many of the targets are not specific for bacteria, in contrast to antibiotics. Particularly, the production of free radicals and reactive oxygen species in the absence of any cells needs to be avoided. Designing a wide therapeutic window (antibacterial activity at low concentrations and cytotoxicity at high concentrations of inorganic agent) is one of the greatest challenges for the application of inorganic antimicrobial agents. The possibility to modulate therapeutic window has the decision-making role in the perspective of inorganic antimicrobial agents as an alternative antimicrobial strategy.

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