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Abstract: This review details the antimicrobial applications of inorganic nanomaterials of mostly metallic form, and the augmentation of activity by surface conjugation of peptide ligands. The review is subdivided into three main sections; of which the first describes the antimicrobial activity of inorganic nanomaterials against gram-positive, gram-negative and multidrug-resistant bacterial strains. The second section highlights the range of antimicrobial peptides and the drug resistance strategies employed by bacterial species to counter lethality. The final part discusses the role of antimicrobial peptide-decorated inorganic nanomaterials in the fight against bacterial strains that show resistance. General strategies for the preparation of antimicrobial peptides and their conjugation to nanomaterials are discussed, emphasizing the use of elemental and metallic oxide nanomaterials. Importantly, the permeation of antimicrobial peptides through the bacterial membrane is shown to aid the delivery of nanomaterials into bacterial cells. By judicious use of targeting ligands, the nanomaterial becomes able to differentiate between bacterial and mammalian cells and thus, reduce side effects. Moreover, peptide conjugation to the surface of a nanomaterial will alter surface chemistry in ways that lead to reduction in toxicity and improvements in biocompatibility.

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In Turku, Finland, 23.12.2019

Dear Editor and Referees of *International Journal of Pharmaceutics*,

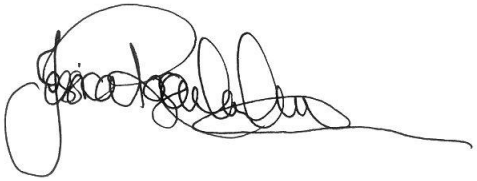
We are hereby submitting our review manuscript titled "*Anti-bacterial effects of inorganic nanomaterials and their antimicrobial peptide conjugates against resistant and non-resistant pathogens*" by Dinesh M. Pardhi, Didem Şen Karaman, Juri Timonen, Wei Wu, Qi Zhang, Saurabh Satija, Meenu Mehta, Nitin Charbe, Paul McCarron, Hamid A. Bakshi, Murtaza Tambuwala, Jarkko Rautio and Jessica M. Rosenholm, to be considered for publication in the Special Issue "**Pharmaceutical Technology in Europe**" in *International Journal of Pharmaceutics*.

Our report represents a comprehensive review on the antibacterial activity of inorganic nanomaterials and antimicrobial peptides, and how concomitant use of the two can effectively tackle a range of bacterial infections which is a rapidly escalating issues in public health care worldwide. We believe this is of particular current interest with regard to "antimicrobial resistance" being declared one of the top-10 global health threats in 2019 by the WHO.

In this group of authors, we have teamed up within the NordForsk-funded university hub Nordic POP (Patient Oriented Products), which we wish to showcase with this contribution. We are currenlty carrying out joint research supported by this network within the topic of the review, so we view this a valuable contribution also within the dissemination of Nordic POP activities.

We appreciate your consideration and look forward to hearing from you.

Sincerely,



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***Response to Reviewers**

Ms. Ref. No.: IJP-D-19-02907R1
Title: Anti-bacterial effects of inorganic nanomaterials and their antimicrobial peptide conjugates against resistant and non-resistant pathogens
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Reviewers' comments:

Reviewer #2:

The paper must be very carefully checked for various inconsistencies, wordings, inconsistent numbering and so on.

It has not been prepared with sufficient care.

Comments from the editor:

As reviewer #2 points out, you must very carefully double check the entire paper.

I had a quick look and found the following examples:

a) The title now reads: "Anti-bacterial activity of inorganic nanoparticles/nanoparticles and their antimicrobial peptide conjugates against resistant and non-resistant pathogens".

b) Abstract: "The review is subdivided into four sections, the first of which describes antimicrobial activity of inorganic nanoparticles against gram-positive, gram-negative and multidrug-resistant bacterial strains. The second section highlights the range of antimicrobial peptides and the drug resistance strategies employed by bacterial species to counter lethality. The final part discusses the role of antimicrobial peptides in the fight against bacterial strains that show resistance."
FOUR sections: First section, second section, final part. Where is section 3?

c) Section numbering:
"3. Antimicrobial peptides (AMP)
3.1 Antimicrobial action of AMP
Membrane disruption
Intracellular targets
3.1.3 Modulation of immune responses"

"3.1.1" and "3.1.2" are missing.

d) Wordings like "The European Commission's definition of a nanomaterial is definable as materials with,…".

I expect that there are many more issues like these.

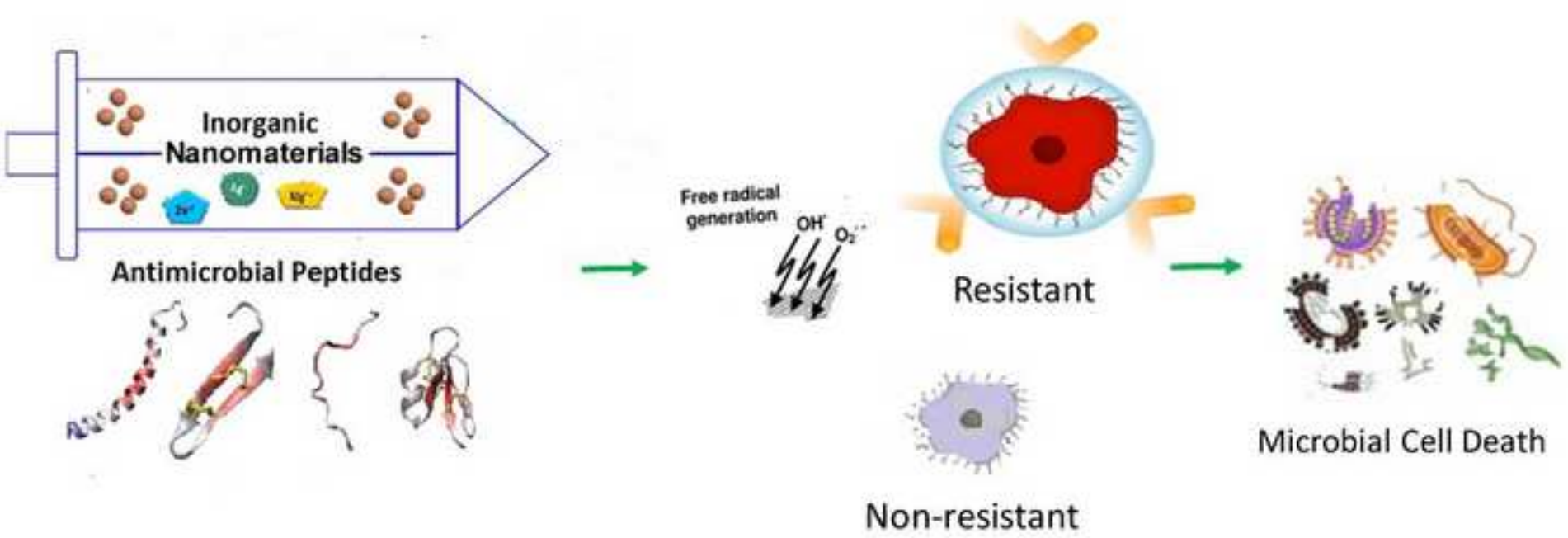
Please note that I cannot accept your manuscript, if it has not very carefully been prepared.

Please highlight all changes you make in yellow.

Response to the Reviewer’s and Editor’s comments:

We thank the Reviewer and Editor for constructive comments and profusely apologize for these omissions in our original revision. We have re-read the manuscript and re-checked the numbering to correct any such mistakes in the new revision. We deeply hope we managed to catch them all this time around.

*Graphical Abstract (for review)



Anti-bacterial activity of inorganic nanomaterials and their antimicrobial peptide conjugates against resistant and non-resistant pathogens

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Abstract

This review details the antimicrobial applications of inorganic nanomaterials of mostly metallic form, and the augmentation of activity by surface conjugation of peptide ligands. The review is subdivided into three main sections; of which the first describes the antimicrobial activity of inorganic nanomaterials against gram-positive, gram-negative and multidrug-resistant bacterial strains. The second section highlights the range of antimicrobial peptides and the drug resistance strategies employed by bacterial species to counter lethality. The final part discusses the role of antimicrobial peptide-decorated inorganic nanomaterials in the fight against bacterial strains that show resistance. General strategies for the preparation of antimicrobial peptides and their conjugation to nanomaterials are discussed, emphasizing the use of elemental and metallic oxide nanomaterials. Importantly, the permeation of antimicrobial peptides through the bacterial membrane is shown to aid the delivery of nanomaterials into bacterial cells. By judicious use of targeting ligands, the nanomaterial becomes able to differentiate between bacterial and mammalian cells and thus, reduce side effects. Moreover, peptide conjugation to the surface of a nanomaterial will alter surface chemistry in ways that lead to reduction in toxicity and improvements in biocompatibility.

Keywords: Nanomaterial, antimicrobial, conjugates, antimicrobial peptides, antibiotics

Contents

1. Introduction

Unnecessary and frequent use of antibiotics has caused a worrying and wide-ranging rise in bacterial resistance, which has led to serious and life-threatening restrictions in their clinical use (Shimanovich and Gedanken, 2016)(Rizzo *et al.*, 2013). Microbes are adept at developing antibiotic resistance, and they do this by employing one or more evasive mechanisms. These are diverse and include (i) drug target alteration, (ii) enzymatic degradation of antibiotic compounds, (iii) efflux-pump of antibiotic molecules from the cell and (iv) biofilm formation (Ahmed, Raman and Veerappan, 2016)(Alekshun and Levy, 2007)(Salouti and Ahangari, 2014)(Huang *et al.*, 2015; Fidler and Fidler, 2016). Resistance has become serious and is now a global concern. It will most likely be responsible for at least 10 million deaths by 2050 (O'Neill, 2014). According to the WHO, methicillin-resistant *Staphylococcus Aureus* (MRSA) infection is fatal and multidrug-resistant. Therefore, the development of innovative strategies for combating bacterial infection is of pressing need (Wang *et al.*, 2010). The options under investigation for addressing this issue are numerous. Among other strategies, antimicrobial peptides (AMP) have attracted much interest due to favorable biocompatibility and a low probability of inducing bacterial resistance (Baltzer and Brown, 2011)(L. Peng *et al.*, 2016).

AMP works in efficient ways that are not overly specific. For example, the formation of pores may be a general outcome following use with no specificity to bacterial type. They exhibit a range of toxicities to bacteria, fungi, parasites, and viruses. They are capable of bypassing and disintegrating into bacterial cell surfaces of multidrug-resistant bacteria (Wang *et al.*, 2016)(Yount *et al.*, 2006). Some AMP, such as Bactericin and Cap-18, are stable in the presence of proteases, elevated temperatures, and pH; properties found to be responsible for a long-term

bacterial resistance (Ebbensgaard *et al.*, 2015) (Hassan *et al.*, 2014). Nevertheless, several pathogens have developed resistance against antibiotics by modifying the cellular surface, expression of efflux pumps, and proteolytic degradation by microbial enzymes (Joo Fu and Otto, 2016)(Andersson, Hughes and Kubicek-Sutherland, 2016). Therefore, AMP are used as commercially available antimicrobials with potential as alternatives to traditional cell wall inhibitors, nucleic acid inhibitors, plasma membrane inhibitors, and protein synthesis inhibitors (Peters, Shirtliff and Jabra-Rizk, 2010; Tillotson and Theriault, 2013).

Different kinds of inorganic, mostly metal, nanomaterials (NM) have to date been used as antimicrobial agents. A significant benefit of metal and metal oxide NM is their various modes of action and lack of traditional therapeutic targets, which is why it is challenging for microbes to develop resistance against them (Karaman *et al.*, 2017). According to The European Commission, NM are materials with at least one external dimension in the size range 1-100 nm. In this review, we are going to discuss inorganic nanomaterials with focus on metals and metal oxides, and their inherent antimicrobial activity. For instance, to date many different shapes of Au and TiO_2 NM have been studied as antimicrobial agents (Bhattacharya and Mukherjee, 2008)(Khan *et al.*, 2011). Furthermore, other metal oxides, such as Copper (II) oxide (CuO), Magnesium oxide (MgO), and Zinc oxide (ZnO), exhibit affinity towards the bacterial surface and interfere with bacterial integrity. (Bhattacharya and Mukherjee, 2008)(Richards *et al.*, 2000). The selective nature of NM in bacterial cells over mammalian cells is due to their differentiated perception for these two cells, e.g., from cell wall composition, ribosomes, and Ergosterol composition (Lemire, Harrison and Turner, 2013).

Although NM show promise for treatments against microbial infections, several essential requirements must be met before they can be used for clinical therapies (Casals *et al.*, 2019). The first is to address the specific physicochemical properties of NM, such as composition, size, crystallinity, and morphology (Kumar *et al.*, 2012) since they are strongly related to the activity of NM. Secondly, stable and non-agglomerating NM is engineered to monitor the toxicity. NM has a known physical and chemical impact on their toxicity, which can also be severely altered depending on their surroundings, for instance, because NM in biological fluids tend to agglomerate (Hajipour *et al.*, 2012) (Sutariya *et al.*, 2014). The last one is the biocompatibility of NM (Yen, Hsu and Tsai, 2009). The combination of these strategies with AMP allows the creation of unique designs that unleashes the promising potential to use the AMP's natural functionalities for microbial infections with increased effecacy.

Namely, AMP-conjugated NM can address the disadvantages of free AMP, such as proteolytic degradation and low permeability across biological barriers (Rajchakit and Sarojini, 2017b). Synergistic behavior can be rendered by amplifying the AMP's anti-microbial strength with that of the NM carrier, not only through the conjugation with NM but also, the therapeutic efficacy of AMP can be increased. For instance, since AMP can further show selectivity for species, researchers have started using NM combined with AMP to carefully release NM from the body into the coat of arms where pathogens can be hyperthermally killed (Zharov *et al.*, 2006).

This review illustrates the chemistry, biology, interfacial science, and utilization of AMP-conjugated NM, not only to hinder the growth but also to kill the bacteria based on their inherent action. We will provide the reader with an overview of the antimicrobial mechanisms of action

of inorganic NM, AMP, and their conjugates for antibacterial treatments. This review aims to summarise the latest promising findings and propose future approaches for building peptide conjugated NM for bacterial infection therapy.

2. NM as antimicrobial agents

*2.1. **Mechanisms of action of NM***

NM can exert a beneficial antimicrobial effect due to sub-micrometer scales of size and high surface-to-volume ratios. These properties enhance the contact area able to interact with pathogens. For this reason, NM exhibit increased biological and chemical activity and can be used to target different bacterial structures (Holban and Andronescu, 2016). Figure 1 illustrates the general mechanisms of antimicrobial activity exerted by different NM.

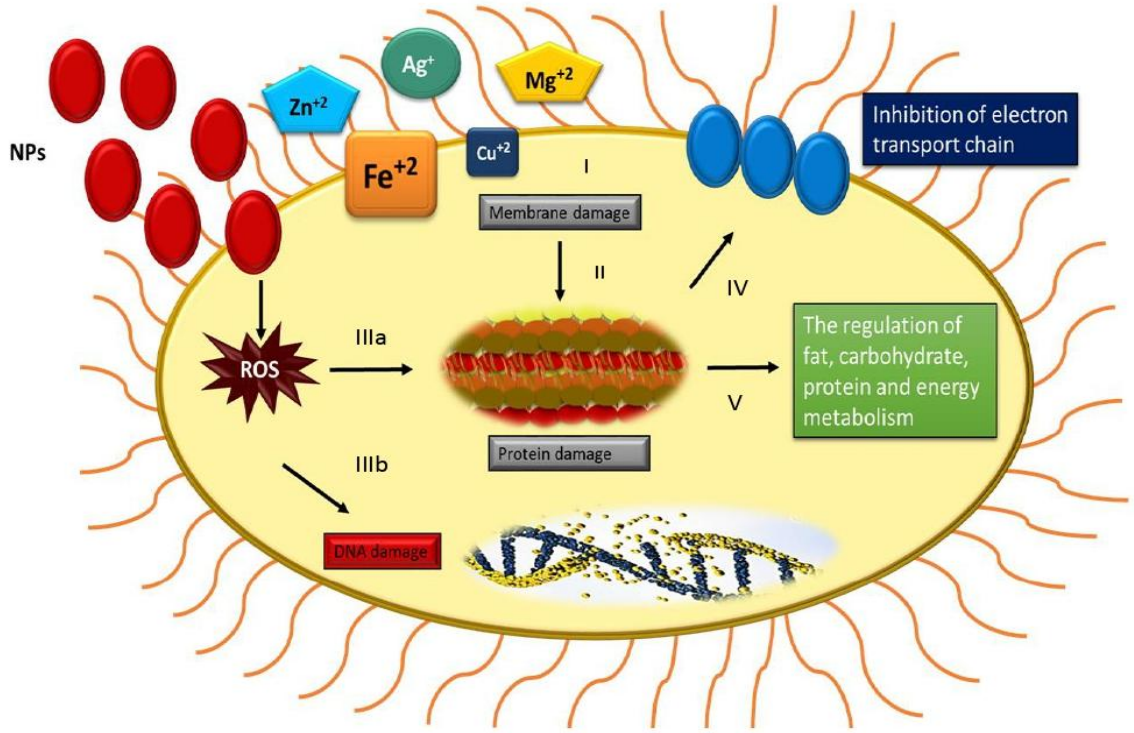

Fig.1 Different mechanisms of antibacterial action by NM. I) Metal ions released from their respective NM electrostatically bind and disturb the phospholipid bilayer of the bacterial membrane, causing membrane damage. II) Oxidative stress generated by the membrane disruption responsible for the bacteria protein damage. III) ROS is generated by NM, which is accountable for the IIIa) damage of the cell's protein, and IIIb) DNA damage. Protein damage leads to loss of metabolic activity through IV) the disruption of the transmembrane electron transport chain. V) Protein damage interferes with fat, carbohydrate, protein, and energy metabolism. Adapted with the permission from (Wang, Hu and Shao, 2017) Copyright © 2017 Dovepress.

Impaired cell membrane function

Bacterial membranes are negatively charged with a high binding affinity for positively charged metal ions (Lemire, Harrison and Turner, 2013)(Palza, 2015). Several researchers have investigated the bacterial toxicity of Ag NM (Yamanaka, Hara and Kudo, 2005)(Sondi and Salopek-Sondi, 2004) and Au NM (Yaganza *et al.*, 2004) against *E. coli* and *S. aureus*, and found that both induce damage to the plasma membrane. Another research by Marius et al. (Marius *et al.*, 2011) revealed that Ag NM deposited on the bacterial cell wall surface form clusters, leading to bacterial death through cell lysis. Furthermore, Sondi and Salopek-Sondi (Sondi and Salopek-Sondi, 2004), as well as Prabhu and Poulose (Prabhu and Poulose, 2012) explained that the formation of pores in bacterial cell membranes was due to the NM deposition on the bacterial cell surface. Other evidence suggests that the antimicrobial activity of ion release from NM surfaces is connected with interruption of the electron transport chain of the membrane (Rainnie and Bragg, 1974)(Gordon *et al.*, 2010). For example, micromolar concentrations of Ag^{+} interact with NADH: ubiquinone oxidoreductase (NQR) enzyme, a component of the respiratory chain of bacteria, and inhibit energy-dependent Na^{+} transport resulting in energy depletion and

pathogen death (Travan *et al.*, 2007). Lipid peroxidation is another mechanism of Cu^{2+} and Cd^{2+} toxicity in bacteria (Hong *et al.*, 2012)

Reactive oxygen species (ROS) production

NM induce reactive oxygen species (ROS) directly when they interact with aerobically grown bacteria, which ultimately leads to necrotic and apoptotic bacterial death (Acker and Coenye, 2016)(Held and Instruments, 2015). The redox transition of the ROS is carried out using reaction mechanisms of Fenton in biologically based systems, including Si, Fe, Cu, Cr, V and Ni. (Huang, Wu and Aronstam, 2010)(Kirisits, 2015)(Ubini, 2003)(Tee *et al.*, 2016). Hydrogen peroxide (H_2O_2), which is toxic to biological molecules during the Fenton reactions, oxidizes transition metal ions such as Fe^{2+} to produce (HO^-) and highly relational hydroxyl radicles (OH). (Thannickal and Fanburg, 2000).

When exposed to the acidic environment in lysosomes, metal NM produce ions (Ag^+, Cd^{2+}, $Fe^{2+/3+,}$ $Au^{1+/3+),}$ that can induce different chemical reactions from ROS species (Li *et al.*, 2010)(Pokhrel *et al.*, 2009). Furthermore, NM can communicate directly with redox active proteins such as NADPH oxidase, and stimulate large scale production of ROS in immune cells, including macrophages and neutrophils (Manke, Wang and Rojanasakul, 2013). Many recent studies clarify the antimicrobial activity of metal NM through the production of ROS. Ag NM are well known for their ROS production through surface oxidation or release of Ag^+ in biological medium (Ivask *et al.*, 2010). Moreover, chitosan-coated iron oxide (Fe_2O_3) NM (IONM) also induced significant production of ROS and thus, exhibited bactericidal activity against *E. coli* and *B. subtilis* (Arakha *et al.*, 2015). Significant intracellular ROS production by CuO NM in *E. coli* was attributed to the release of Cu cations (Ivask *et al.*, 2010)(Meghana *et*

al., 2015). Vijayaraghavan et al. (Padmavathy and Vijayaraghavan, 2008) studied the formation of ROS in terms of superoxides ($^{\cdot}O^{-}_{2}$), OH^{-} and H_2O_2, when ZnO NM was in contact with microbial cells. Because of their negative charge, OH^{-} and $^{\cdot}O^{-}_{2}$ cannot penetrate the bacterial membrane (Xie *et al.*, 2011) and therefore stay in direct contact with the bacteria's exterior surface. In contrast, H_2O_2 penetrates the bacterial cell wall and causes lipid, DNA, and protein destruction (Dutta *et al.*, 2012). Interestingly, halogen adsorption on MgO NM surface induced higher antibacterial activity (Blecher, Nasir and Friedman, 2017). The rough surface of NM, the oxidative action of adsorbed halogens and strong electrostatic interaction with the negatively charged bacterial membrane is a major reason for their excellent antimicrobial activity (He *et al.*, 2016)(Chen *et al.*, 2014).

Protein dysfunction and loss of enzyme activity

Several studies have shown that the FeS family of bacteria are susceptible to site-specific inactivation by toxic metals, including dihydroxy acid dehydratases (DHAD) and isopropyl-malate isomerases (IPMIs) involved in branched-chain amino acid synthesizes (Xu and Imlay, 2012)(Booth, Weljie and Turner, 2015). Moreover, the reduced fumarase A and 6-phosphogluconate dehydratase activity are one of the most significant toxic effects of Cu, two enzymes that also depend upon Fe-S catalysts (Macomber and Imlay, 2009). It was reported that Ag, Hg, Cd, and Zn (but not Mn, Co, Ni or Pb) might harm FeS clusters *in vitro* and *in vivo*, which contains dehydrates independently of the ROS with bacteriostatic effect. Proteins that repair FeS clusters, such as cysteine desulphurase (IscS) or the SufA scaffold protein FeS cluster, may restore inactive bacterial enzymes (Xu and Imlay, 2012). In addition to the destruction of FeS clusters, metals are also able to use a route called the ionic simulation to inhibit the site's

enzyme. For example, Pb removes Zn from the δ aminolevulinic acid dehydratase (ALAD) active site, leading to enzymatic inhibition (Scinicariello *et al.*, 2007) and antimicrobial toxicity (Ogunseitan, Yang and Ericson, 2000). Further, Ni can substitute Zn at the non-catalytic Zn site of fructose-1,6-bisphosphate aldolase (FbaA) in *E. coli*, resulting in loss of activity (Macomber, Elsey and Hausinger, 2011).

Release of toxic ions

The antimicrobial efficacy of NM is directly commensurate with the release of ions. Metal ions accumulate and pass through cell membranes and intercalate with proteins and nucleic acids inhibiting bacterial function (Slavin *et al.*, 2017). For instance, Ag NM can be oxidised by O_2 and other cellular molecules leading to the release of Ag^+ ions. Ag NM can penetrate the bacterial membrane and release Ag^+ ions, which subsequently bind to amino acids (cysteine) affecting their functionality (Hu and Hong, 2017)(Sharma, Kwon and Chen, 2013)(Kanematsu and Barry, 2015). Yamanaka et al. (Yamanaka, Hara and Kudo, 2005) used two-dimensional electrophoresis to evaluate the influence of Ag^+ ions on specific proteins in *E. coli*. Reduced expression of 30S ribosomal subunit, succinyl CoA synthetase (SCS), maltose transporter (MalK), and fructose bisphosphate aldolase were observed after *E. coli* incubation with 900 ppb Ag^+ ions compared to the untreated group. The authors sulfurized Ag NM to Ag_2S NM and found out that sulfidation reduced the release of Ag^+ ions from Ag NM and reduced their toxicity towards *E. coli.* Similarly, bacterial toxicity of ZnO NM is associated with the dissolution of Zn^{2+} ions within the microbes (Chang *et al.*, 2012)(Ivask *et al.*, 2012). It has been reported that the Zn^{2+} ions are toxic towards *S. cerevisiae* (Kasemets *et al.*, 2009). Similarly, Cu NM exhibited bacterial toxicity in the same manner (Zhang, 2016).

Photocatalysis

Photocatalysis is the excitation of NM, such as Fe_2O_3, WO_3, ZnO, and TiO_2**,** by UV irradiation to generate ROS that first damage the lipopolysaccharide layer of the bacterial cell wall, followed by the inner peptidoglycan layer. Also, ROS induce peroxidation of lipids and proteins in the cell membrane, eventually resulting in organelles leaching from the plasma membrane (PM). TiO_2 has been shown to produce ROS under UV irradiation (Nowack, 2008). High photocatalytic-mediated antimicrobial activity of TiO_2 NM compared to ZnO NM under UV irradiation has been observed (Leung *et al.*, 2016). Saito *et al.* (Saito *et al.*, 1992) used TEM to study the effect of TiO_2 NM and observed disruption of PM of bacteria due to photocatalytic induced ROS. Carre *et al.* used proteomics data to conclude that TiO_2 NM can not only down-regulate but also impair membrane proteins under UV irradiation (Carré *et al.*, 2014). Wu et al. studied the photocatalytic activity of PdO/TiO_2 nanofibers against *E. coli* (Wu, Imlay and Shang, 2010). The authors reported changes in membrane permeability of *E. coli*, followed by DNA damage upon photocatalytic irradiation of PdO/ TiO_2.

2.2. NM against gram-positive and gram-negative bacteria

Difference in composition of the cell wall of gram-positive and gram-negative bacteria affects the NM activity. It has been shown that gram-positive bacteria are more resistant to Ag NM than gram-negative bacteria. The thick peptidoglycan layer of gram-positive bacteria restricts the entry of most of the NM. However, a study conducted by Ruparelia et al. (Ruparelia *et al.*, 2008) observed higher antimicrobial activity of CuO NM towards gram-positive *B. subtilis*, which may be due to strong affinity of CuO NM for amine and carboxyl groups. Antimicrobial activity of NM may be altered by other characteristics of the NM, like form, size,

coating/capping agent, microbial type, surface morphology, crystallinity, and pH (Agnihotri, Mukherji and Mukherji, 2014). In this section, will discuss the bacteriostatic and bactericidal role of various NM against gram-positive and gram-negative bacteria

Silver nanomaterials (Ag NM)

Ag has, for prolonged times, been used as an antimicrobial agent in medicine. It has several mechanisms of bactericidal/bacteriostatic effects. As a result, Ag NM are incorporated into various consumer goods such as surgical coatings, medical implants, food packaging, textiles, and cosmetics. A study, which investigated the size-dependent antimicrobial efficacy of Ag NM on gram-positive and gram-negative strains found a significant reduction in bacterial count when treated with 5 nm Ag NM on *E. coli*, *B. subtilis*, and *S. aureus* species for 90, 20 and 120 min, respectively. Similarly, the reduction of *E. coli*, *B. subtilis*, and *S. aureus* in 180 min was observed when treated with 7 and 10 nm Ag NM. The fastest bactericidal effect was observed for the smaller 5 nm sized NM compared to 7 and 10 nm sized NM, which may be attributed to the higher surface-area-to-volume ratio of smaller sized NM. The antimicrobial behavior was due to the same mechanisms as found by other researchers, such as membrane disruption and interferences. The negative charge of citrate-capped Ag NM tended to enable the electrostatic attraction reported in Figure 2 (Agnihotri, Mukherji and Mukherji, 2014).

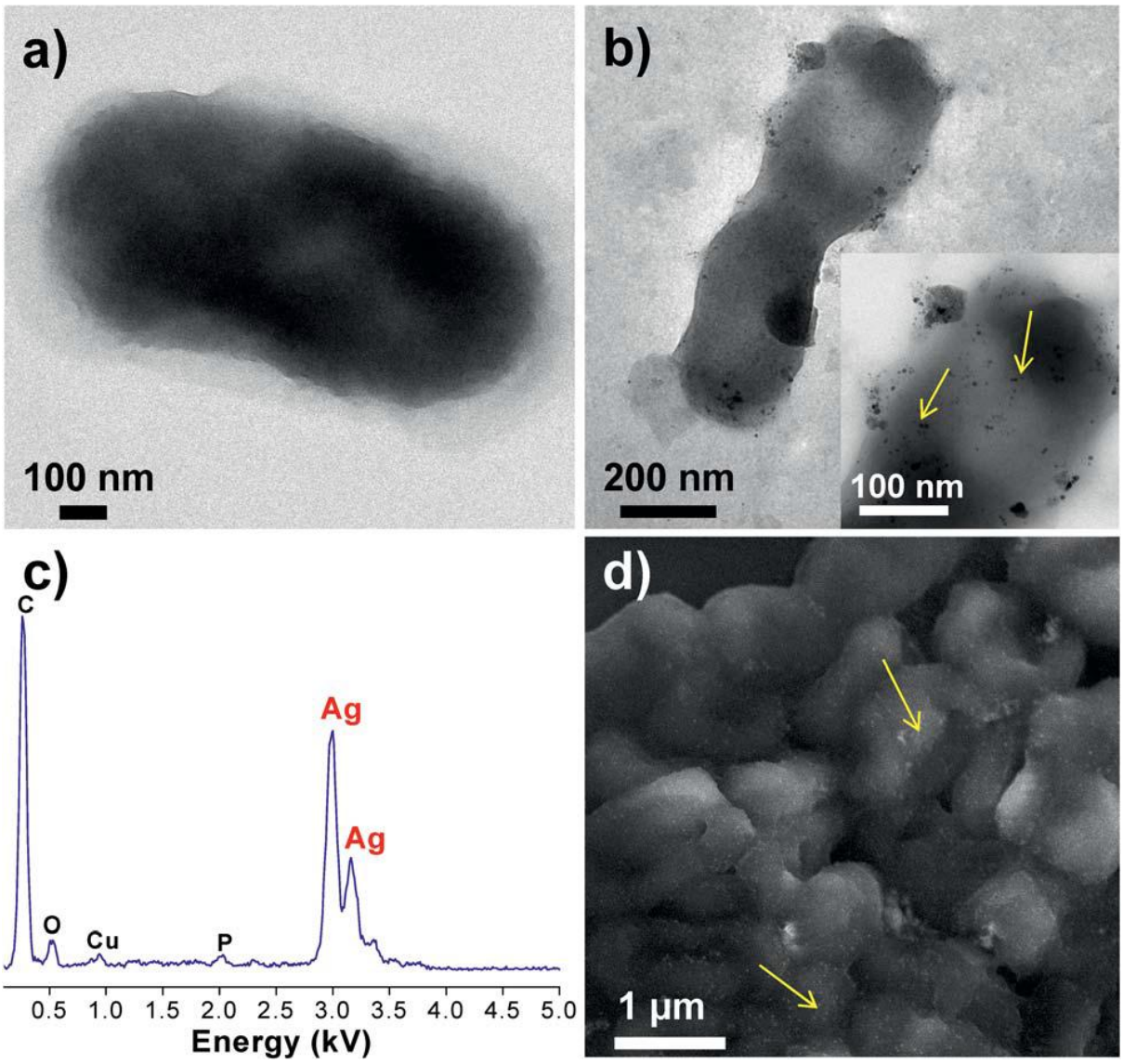

Fig.2 FEG-TEM images of *E. coli* (a) untreated and (b) treated with Ag NM. The inset, as indicated by arrows, shows the presence of Ag NM. EDX spectrum (c) demonstrates the presence of.Ag The FEG-SEM picture (d) confirms the Ag existence throughout the bacterial surface. Reproduced with the permission from (Agnihotri, Mukherji and Mukherji, 2014) Copyright © 2014 The Royal Society of Chemistry.

Another study comparing the antimicrobial efficacy of different shapes of Ag NM (triangular, spherical, and rod) incubated with *E.coli* of Ag concentrations 1, 12.5, 50, and 100 µg, concluded that triangular and nanosphere forms killed *E.coli* more efficiently than rods and ionic Ag (Pal, Tak and Song, 2015). Triangular Ag NM with an average width of 1 µm was found to produce a bacteriostatic effect, and inhibited *E. coli* 10^6 colony forming units (CFUs). Considering the effect of Ag spheres, almost 12.5 µg Ag was required to reduce *E. coli* CFUs. In contrast, rod-shaped and Ag^{3+} were unable to reduce *E. coli* viability even at 100 µg Ag concentration. This experiment further confirms the size and dose-dependent antimicrobial activity of Ag NM. Another similar *in vitro* study investigated Ag-spheres (Ag NM-sp) and Ag-rods (AgNR) on gram-positive and gram-negative bacteria using an optical density method. The study reported lower MIC values of Ag NM-sp (190,195,188,184,190 µg/ml) than AgNR (358,350,348,320,340 µg/ml) for *S. aureus*, *B. subtilis, P. aeruginosa*, *K. pneumonia,* and *E. coli*, respectively. When studied against *K. pneumonia,* different concentrations of Ag NM-sp (184,197,207 µg/ml) and Ag NR (320,560, 720 µg/ml), that were selected based on their MIC values, were incubated with 10^8–10^9 CFU/ml. Cellular viability was reduced to 71.0% and 42.63%, respectively, in the presence of 197 µg/ml of Ag NM-sp and 720 µg/ml of AgNR. The higher antimicrobial effect of Ag NM-sp over AgNR was attributed to its granular shape with the larger surface area and better distribution.

Green synthesis of Ag NM, which are produced from biologically derived moieties, have shown to be more toxic than traditionally synthesized Ag NM (Siddiqi, Husen and Rao, 2018). One approach of Ag NM synthesis from *Ricinus communisvar* plant extract has been reported by Bora et al. (Ojha, Sett and Bora, 2017). Leave extracts acted as reducing and capping agents to generate

spherical Ag NM with a particle size of 30-40 nm. Antimicrobial activity against *B. subtilis*, *S. aureus*, S. *zooepidemicus*, *E. coli*, and *E. aerogenes* was reported as Ag NM having maximum inhibitory activity (MIC 10 µg/ml) against *B. subtilis* and *S. aureus*. Ag NM also showed antimicrobial activity against *E. coli* and *S. zooepidemicus* at 20 µg/ml concentration, while showing no cytotoxicity towards mouse fibroblast cells. Another approach observed high antibacterial effect of Ag NM synthesized from flower extract of *Millettiapinnata* against *Proteus vulgaris, Staphylococcus aureus, Klebsiella pneumonia, E. coli*, and *Pseudomonas aeruginosa.* The mode of action for the antimicrobial activity of Ag NM is distinct. First, positively charged Ag NM electrostatically adsorbed to the negative bacteria. Subsequently, Ag NM interacts with cysteines in protein, which eventually deactivates the protein and releases ROS (Rajakumar *et al.*, 2017).

Gold nanomaterials (Au NM)

The bactericidal activity of Au NM is related to their increased penetration into the bacterial cell wall, inducing vacuole formation as an indication of the elevated oxidative stress within the cytoplasm. For instance, Au NM with an average size of 25 ± 5 nm and surface charge of -39 mV were shown to reduce the viability of *C. pseudotuberculosis* at a concentration of 200 µg/ml (Mohamed *et al.*, 2017). Mixed charged (+/-) Au NM were non-toxic to mammalian cells while exhibiting selectivity towards different bacterial strains. For example, a positively charged NM surface strongly interacts with gram-negative bacteria, whereas negative surface charge has a preference for gram-positive bacteria. Wang et al. studied the selective photothermal ablation of *Salmonella* over *E. coli* using oval-shaped Au NM conjugated with an anti-*Salmonella* antibody.

The authors observed almost 97% reduction of the bacterial viability under irradiation of λ_{670} nm for 15 min, whereas *E. coli* bacteria survived under the same conditions (Wang *et al.*, 2010).

Titanium dioxide nanomaterials (TiO_2 NM)

The evaluation of ROS from the TiO_2 NM surface under UV irradiation have shown to exhibit a linear correlation between the viability of *E. coli* and ROS concentration induced after UV irradiation (Li *et al.*, 2012). Interactions between superoxide radicals with the unsaturated phosphate lipids in *E. coli* membrane, followed by its lipid peroxidation, was believed to interrupt the cell membrane integrity; ultimately reducing bacterial viability (Cai, Strømme and Welch, 2013)(Figure 3).

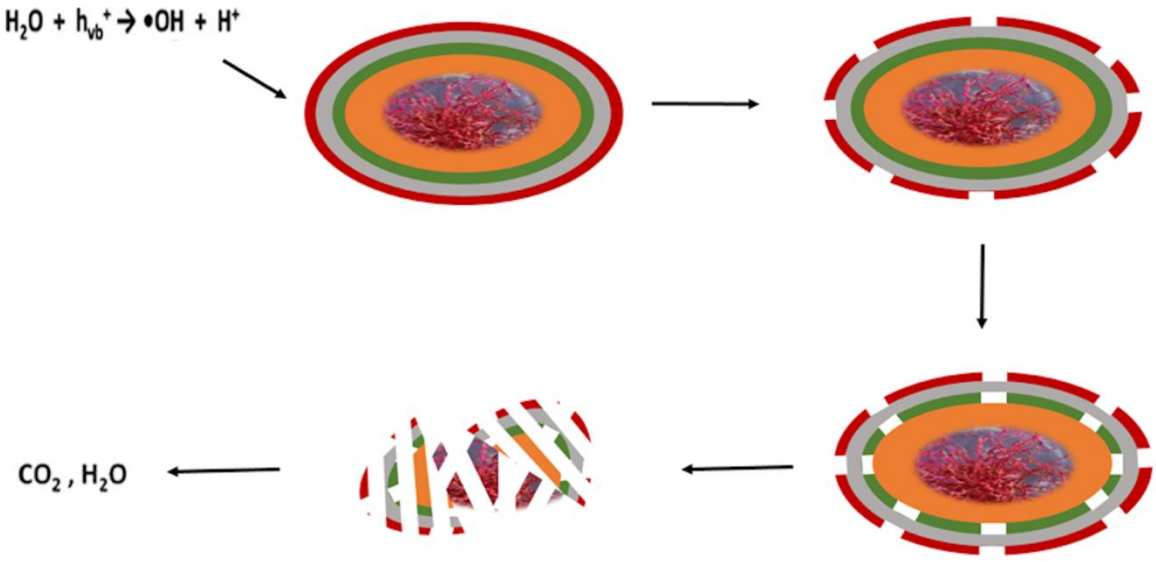

Fig.3 Photocatalytic bactericidal activity of TiO_2 based photocatalyst.

However, TiO_2NM induce adverse effects on human cells and tissue; hence, their use as antibacterial agents remains under limitation (Shah *et al.*, 2017). Doping with Au, Ag, Pt, or Ag,

can narrow the bandgap of TiO_2 NM and enhance its photocatalytic effect (Ahamed *et al.*, 2017). Various reports have described the visible-light-induced antimicrobial activity of Fe, Cu, Ni, and Ag-doped TiO_2NM against *S. aureus* and *E.coli* bacteria (Yadav *et al.*, 2016)(Moongraksathum and Chen, 2018).

Copper and copper oxide nanomaterials (Cu and CuO NM)

In different composite forms of Cu and CuO NM, such as SiO_2-Cu, Cu NM were proven to be efficient antimicrobial agents against different strains of bacteria (Muthukrishnan, 2015). Bacterial membrane destruction was found to be the primary source of bacterial death if subjected to such composites. In addition, CuO NM have recently been found to exert a pH-dependent anti-bacterial effect against *S. aureus*. CuO NM interact with *S. aureus* at acidic pH (pH=5) whereby significant bactericidal activity was observed due to their lower agglomeration, which facilitates solubility dependent release of Cu^{2+} ions compared to pH 6 and 7. The released Cu^{2+} ions induced the production of ROS (Hsueh, Tsai and Lin, 2017)(Hajipour *et al.*, 2012).

Cu substituted with hydroxyapatite and fluorapatite (a bone mimetic material) was studied against gram-positive and gram-negative bacteria, as well as fungi to overcome possible infection of artificial bone implant material after surgery (Shanmugam and Gopal, 2014). In this study, Cu-substituted hydroxyapatite displayed antimicrobial activity against gram-positive bacteria. In contrast, Cu-substituted fluorapatite showed antimicrobial activity not only against gram-positive and gram-negative bacteria but also fungi. A higher release of Cu from Cu-substituted-fluorapatite is the main reason for this intense antimicrobial action. Compared with Ag NM, Cu NM are much more potent, and a promising therapeutic with higher colloidal stability and

resistance for surface oxidation; being critical factors for Cu NM as antimicrobial agent (Khurana and Chudasama, 2018).

Zinc oxide nanomaterials (ZnO NM)

ZnO displays vigorous antimicrobial activity due to its electrostatic interaction and internalization, the release of Zn^{2+} ions, and ROS formation. ZnO NM was proven effective against *S. typhimurium, C. jejuni, Vibrio fischer, P. aeruginosa, P. alcaligenes, P. vulgaris, S. entericaserovar enteritidis,* and *E. coli* (Xie *et al.*, 2011)(Heinlaan *et al.*, 2008)(Jones *et al.*, 2008) (Nair *et al.*, 2011)(Nair *et al.*, 2011). The cytotoxic action of ZnO NM against prokaryotic and eukaryotic cells via flow cytometry viability assays concluded that ZnO NM more effectively reduced *S. aureus* and *E. coli* strains. In contrast, ZnO NM were least effective on human $CD4^+$ T cells. Significant reduction of *E. coli* and *S. aureus* colonies were observed when >3.4 and 1 mM of 13 nm ZnO was added to the agar plate. Dose and time-dependent inhibition of bactericidal activity was observed for ZnO NM, with entire colonies inhibition after 24 h of treatment. Alternatively, ZnO was tested against human T-lymphocytes, whereby no significant reduction of cell viability was observed. Overall, these findings display selective antimicrobial activity of ZnO/ZnO NM against prokaryotic cells without harming eukaryotic cells (Reddy *et al.*, 2007).

In a study comparing inhibition produced by ZnO, CuO, and Fe_2O_3 NM against gram-positive (*S. aureus and B. subtilis*) and gram-negative (*P. aeruginosa and E. coli*), ZnO NM was reported as more potent antibacterial agents in comparison to those of Fe_2O_3 and CuO NM (Yemmireddy and Hung, 2017). The antimicrobial mechanism of ZnO NM is believed to be related to particle size, which facilitates their bacterial penetration and generation of ROS, being more effective against

gram-positive than gram-negative bacteria (Seil and Webster, 2012) (Premanathan *et al.*, 2011). For *E. coli* and *S. aureus*, the viability was reduced upon the incubation overnight with three nm-sized ZnO NM at a concentration of 3.1 mg/ml and 1.5 mg/ml, respectively. Gram-positive bacteria were more impacted due to the structural differences of the cell wall composition. Smaller ZnO NM were able to interact and increase abrasiveness on the bacterial cell wall (Nair *et al.*, 2009) (Yuan *et al.*, 2018).

ZnO NM photoconductivity was reported following UV illumination (390 nm, 1.8 W cm^{-2}) ZnO-rods and ZnO-plates reduced viability of *E. coli* by 18% (ZnO-rod) and 13% (ZnO-plate), whereas the viability of *S. aureus* was reduced by 22% when exposed to ZnO-rod and by 21% with ZnO-plate compared to control. ZnO NM illumination lead to the desorption of loosely bound oxygen molecules, thereby increasing its concentration on the ZnO surface, which ultimately generated oxygen species such as H_2O_2, O_2^- and OH^-. These ROS inactivate proteins, enzymes, and DNA (Ann *et al.*, 2014). In a similar study by Zhou et al. (Zhou *et al.*, 2008) a strong antibacterial rate of ZnO complex was obtained in *S. aureus* (99.45%) due to higher permeability of OH^- ions generated under UV light through the membrane of *S. aureus* compared to *E. coli* (95.65%), whose outer lipopolysaccharide (LPS) membrane restricts OH^- ions inside *E. coli*. For microbes, OH^- ions interact with nuclear acids or respiratory classes of sulfhydryl and stop breathing for bacteria. The Zn-CuO-coated fabrics benefit from injection into Cu, giving different benefits in contrast to ZnO and CuO NM. For example, Zn-CuO NM display 10000 times more antibiotic activity within a short time. The potency of antimicrobial bandages, which were prepared by depositing Zn-CuO NM on cotton fabric using ultrasound irradiation to exert activity, was evaluated by using four microbial models (*E. coli, S. aureus*, MRSA, and MDR *E. coli*). Zn-CuO coated fabrics were incubated with 10^8 CFUs for 30 min. 5 and 6 log reduction of *E. coli* and *S. aureus* were observed

after 10 min of treatment. In contrast, inhibition of only 1 and 2 orders of magnitude was detected for *S. aureus* after ZnO and CuO treatment and negligible effect was observed for *E. coli.* An elevated amount of OH^-, O^-_2, and singlet oxygen formation by Zn-CuO composites resulted in higher bactericidal activity compared to ZnO and CuO NM (Malka *et al.*, 2013).

Mesoporous silica nanoparticles (MSNs)

Silicon dioxide NM, especially so the type of mesoporous silica nanoparticles (MSNs), have attracted significant attention as an ideal antibacterial platform (Şen Karaman, Manner and Rosenholm, 2018) (Martínez-Carmona, Gun'ko and Vallet-Regí, 2018). Their size, matrix, and surface functionality can be adjusted to improve their interaction with bacteria and improve biofilm penetration (Camporotondia *et al.*, 2013). Besides, MSNs may also interfere with bacterial cell-to-cell communication (quorum sensing) to avoid the development of biofilms. For just over a decade, the use of MSNs as effective drug delivery systems, particularly for anticancer therapies, has been thoroughly documented. The unique physical features of MSNs (e.g. high specific surface areas, large pore volumes and tunable pore sizes), two distinct (external and internal) surfaces that can be independently functionalized and further utilised for incorporating controlled drug release strategies, and the ability of MSNs to penetrate through biological barriers make them compelling candidates for the design of sophisticated antibacterial delivery systems (Gounani *et al.*, 2019). Recent studies have reported the usefulness of MSNs for efficient antibiotic supply and the preparation of hybrid materials by incorporating MSNs with antibacterial enzymes (Li and Wang, 2013), peptides (Braun *et al.*, 2016), metal ions/particles (Tian *et al.*, 2014) and polymers (surface modifiers) (Şen Karaman *et al.*, 2016). Moreover, MSNs have been developed for dual antibiotic delivery. For instance, recently, Gounani et al. (Gounani *et*

al., 2019) performed the loading of two different antibiotics into MSNs to increase the therapeutic efficiency on both gram-positive and gram-negative bacteria. Thus, combinatory therapy with dual antibiotic-loaded MSNs could be provided with better treatment results for diseases requiring elevated levels of various drugs.

In another study, hollow structured, well-defined mesoporous shells for sustained release of entrapped antimicrobial agents were prepared. Such hollow, mesoporous shells not only confers stability to the entrapped biological moieties but also acts as a reservoir. For example, amine-functionalized hollow MSN (HMSN) have shown to act as an efficient carrier for antimicrobial agents. When loaded with antituberculosis drug isoniazid, HMSN could release isoniazid in a sustained manner (released 60% after 72 h). Isoniazid loaded HMSN exhibited potent antimicrobial activity against isoniazid resistant *M. smegmatis* stain mc^2 651 (MIC 640 and 320 ug/mL) and lowered the half inhibitory concentration (IC_{50}) by 3.3- and 4.1-fold compared to free isoniazid (MIC 1280 ug/mL) after 24 and 72 h treatment, respectively. The enhanced bactericidal activity of isoniazid loaded HMSN may be attributed to increased intra-bacterial accumulation of isoniazid in a sustained manner from the well-defined mesoporous shell, conjointly with a strong interaction of amine moieties on the HMSN surface with bacteria (Hao *et al.*, 2015).

2.3 NM against multidrug-resistant (MDR) bacterial strains

Multidrug resistance (MDR) developed by certain microorganisms against multiple drugs is a leading cause of hospital-acquired infections. It is being assessed that MDR causes 40-60% of nosocomial infections in the United States and the United Kingdom (Haque *et al.*, 2018). Metal

oxides, metals, doped metals, and metal halides play a vital role in the selective and non-selective photothermal killing of MDR (Khlebtsov and Dykman, 2011)(Dizaj *et al.*, 2014). Several metallic NM have superior antibacterial activity against MDR bacteria over traditional antibiotics (Blair *et al.*, 2015). Bacteria can develop resistance towards metal NM through different mechanisms: 1) reduction of metal ions to non-toxic neutral oxidation, 2) increase in efflux of metal ions through chemiosmotic antiporters or P-type adenosine triphosphatases, and 3) production of flagellin, a bacterial adhesive protein from gram-negative strains, which aggregate metal NM on the bacterial surface and reduces antimicrobial efficacy (Nies, 2003)(Li, Nikaido and Williams, 1997)(Gupta *et al.*, 1999)(Panáček *et al.*, 2018). Recently, Graves et al. (Siddiqi, Husen and Rao, 2018) observed a genetic mutation in *E.coli* for 225 generations after regular exposure of Ag NM. To date, there have been no studies demonstrating the resistance of bacteria towards ROS species. However, most of the photosensitisers are water-insoluble and aggregate in water, which ultimately reduces their ROS generation capacity. To overcome this issue, a new hydrophobic photosensitiser based on amphiphilic block copolymer containing Chlorin e6 (Ce6) conjugated to Au NM surface, have shown effectiveness against *Staphylococcus aureus* (MRSA) (Wijesiri *et al.*, 2017). Table 1 summarises other metal and metal oxide designs that have been investigated against MDR bacteria.

Table 1 Antimicrobial activity of metallic NM and metal oxide NM against multi-drug resistant bacteria

Metal NMs	Test MDR Bacteria	Mechanism of antimicrobial activity	Formulation Type	References
Ag	MRSA VRE	Investigation under process	Ag containing dressing	(Percival, Bowler and Dolman, 2007)
	MRSA	Reduce glucose uptake and ATP synthesis, production of ROS, alter membrane permeability	Ag supported silicate platelets	(Su *et al.*, 2011)
	Erythromycinresistant *S. pyogenes* Ampicillin resistant *E. coli* Multidrug-resistant *P. aeruginosa*	Inhibit respiratory enzymes, binds to DNA and RNA and inhibit its replication, denature 30S ribosome subunit, alter membrane permeability	NM	(Lara *et al.*, 2010)

Metal NMs	Test MDR Bacteria	Mechanism of antimicrobial activity	Formulation Type	References
Ag	Erythromycin resistant *Bacillus cereus* Erythromycin resistant *S. typhimurium* Erythromycinresistant *Enterococcus faecalis*	Cell membrane disruption	Ag-Alginate (Ag-Alg) biohydrogel	(Otari *et al.*, 2013)
	Extended-spectrum beta-lactamases (ESBL) positive *E. coli* Teicoplanin resistant *S. pneumoniae* MRSA	Generation and uptake of Ag+ inside the bacteria membrane	AgNMs coated surgical suture	(Thapa *et al.*, 2017)
	MDR *P. aeruginosa*	Thermal destruction of the membrane, ROS generation, Penetration of Ag^{+} inside the membrane	AgNMs with blue light	(El Din *et al.*, 2016)

Metal NMs	Test MDR Bacteria	Mechanism of antimicrobial activity	Formulation Type	References
Au	MRSA	Photothermal abilation and ROS production	Au Nanorod (Au NR)	(Kuo *et al.*, 2009)
	MDR *E. Coli* MDR *E. Cloacae* MDR *K. pneunoniae*	Membrane disruption, singlet oxygen generation, DNA degradation	MB@GNM_{DEX}-ConA	(Khan *et al.*, 2017)
Cu	MRSA	Cu+ release that damage bacterial DNA Size-dependent antimicrobial activity of CuONMs	NM	(Kruk *et al.*, 2015)
ZnO	MRSA	Inhibition of ß-galactosidase (GAL)	ZnO nanopyramids	(Cha *et al.*, 2015)

Metal NMs	Test MDR Bacteria	Mechanism of antimicrobial activity	Formulation Type	References
ZnO	Meticillin resistant *S. agalactiae* and *S. aureus*	penetration and disorganization of cell membranes	NM	(Huang *et al.*, 2008)
CuO	MRSA	Cu^{2+} ions released from the NMs permeate through the bacterial membrane and disturb enzyme function	NM	(Ren *et al.*, 2009)
Fe3O4 (Iron Oxide)	MDR *E. Coli* MDR *S. aureus*	Magnetic core under radiofrequency (RF) current alter bacterial membrane potential	NM	(Chaurasia *et al.*, 2016)
Al_2O_3	MRSA, MSSA MSCoNS (methicillin-sensitive Coagulasenegative Staphylococcus)	Damage of membrane, leakage of cellular content, and interacts with macromolecules	NM	(Ansari *et al.*, 2013)

Metal NMs	Test MDR Bacteria	Mechanism of antimicrobial activity	Formulation Type	References
Al_2O_3	ESBL positive *E. coli*	uptake of NMs inside the membrane and damage the biomolecules	NM	(Ansari *et al.*, 2014)
TiO_2	MRSA	Interact and inactivate the bacterial surface proteins	NM	(S. Roy *et al.*, 2010)
		UV light-induced ROS generation and physical damage of the membrane	Biphasic brookite-anatase TiO_2NMs	(Shah *et al.*, 2008)
NO (Nitric Oxide)	MRSA	Induce immune response	NM	(Han *et al.*, 2009)

2.3.1 Antibiotic conjugated NM against MDR

Metallic NM conjugated with antibiotics can exhibit synergistic antimicrobial activity. As stated above in section 2.3, metallic NM may effectively inhibit the viability of MDR bacteria. As a result, the potency of antibiotics increases, thereby reducing the side effects towards mammalian cells as well as antibacterial resistance (Allahverdiyev *et al.*, 2011). Moreover, metallic NM are suitable carriers for the delivery of antibiotics. For example, tetracycline conjugated Ag NM increased antibacterial action of tetracycline, due to enhanced accumulation of the Ag^{+} around the bacterial cell membranes (Kumar, Curtis and Hoskins, 2018). Similarly, when Au NM was conjugated to a fluoroquinolone antibiotic, the antibacterial effects of fluoroquinolone was boosted lowering the MIC against MDR bacteria by 8–16 folds compared to free fluoroquinolone; which was due to their capacity of conjugates to behave as Tolc-AcrAB efflux pumps (Gupta *et al.*, 2017). Recently, Katya et al. (Katva *et al.*, 2018) pointed out the synergistic antimicrobial activity of Ag NM with gentamicin and chloramphenicol against MDR *E. faecalis* compared to antibiotics alone. Several studies involving the antibacterial activity of NM are listed in Table 2.

Antibiotic conjugated Nanometals	Test MDR strain	Mechanism of antimicrobial action	References
Ampicillin-Ag NMs	Ampicillin resistance *E. coli* Ampicillin resistance *P. aeruginosa*	Blockage of the efflux pump	(Brown *et al.*, 2012)
Clindamycin- Ag NMs	MRSA	Synergistic antimicrobial activity, inhibition of protein synthesis, an altercation in the respiratory chain	(Rahim and Mohamed, 2015)
Vancomycin- Ag NMs	MRSA	Synergistic antimicrobial activity	(Saeb *et al.*, 2014)
	MDR *E. faecalis* MDR *S. epidermidis*	An altercation of bacterial permeability	(Esmaeillou *et al.*, 2017)(Panácek *et al.*, 2016)
Ofloxacin- Ag NMs	MDR *P. aeruginosa*	Inhibition of multidrug efflux pump activity	(Ding *et al.*, 2018)
Tetracycline- Ag NMs	Tetracycline resistance *E. coli* Tetracycline resistance *S. aureus*	Cytotoxic effect of Ag^{+}	(Djafari *et al.*, 2016)

Antibiotic conjugated Nanometals	Test MDR strain	Mechanism of antimicrobial action	References
Tetracycline- Ag NMs Neomycin- Ag NMs	MDR *S. typhimurium*	Antibiotics facilitate binding of Ag NM to the bacteria membrane, increase in the concentration of Ag^+ on bacteria membrane	(McShan *et al.*, 2015)
Anti-*S. aureus*- Au NMs	MRSA	Antibiotics facilitate binding of Au NM to the bacteria membrane, photothermal destruction of bacteria cells	(Millenbaugh *et al.*, 2015)
Levofloxacin, ceftriaxone, cefotaxime, and ciprofloxacin- Au NMs	MDR *E. coli* MDR *K. Pneumoniae* MDR *S. Aureus*	Disorganization and disruption of the bacterial membrane, loss of intracellular cytoplasmic content	(Pradeepa *et al.*, 2016)
Cefotaxime-Au NMs	Cefotaxime resistance *E. coli*, *K. Pneumoniae*	Altercation in the bacterial cell wall, DNA damage	(Shaikh *et al.*, 2017)
Meropenem-Au NMs	Carbapenem resistance *K. Pneumoniae*, *P. Mirabilis*, *A. Baumanii*	Alter osmatic balance and membrane integrity, damage of membrane, inhibition of protein synthesis	(Shaker and Shaaban, 2017)

Antibiotic conjugated Nanometals	Test MDR strain	Mechanism of antimicrobial action	References
Kanamycin-Au NMs	Kanamycin resistance *S. Bovis*, *S. Epidermidis*, *E. Aerogenes* *P. Aeruginosa PA01* MDR *P. Aeruginosa*	Alter cell membrane integrity, lysis of cell wall, leakage of cellular content, inhibition of protein synthesis	(Payne *et al.*, 2016)
Vancozycin-Au NMs	vancomycin-resistant *E. faecium* (VRE 4), *E. faecalis* (VRE1) MRSA Pandrug-resistant *A. baumannii* (PDRAB)	Bacteriostatic effect	(Lai *et al.*, 2015)
Antibiotics-TiO_2	MRSA	Synergistic antimicrobial activity	(S. Roy *et al.*, 2010)

Antibiotic conjugated Nanometals	Test MDR strain	Mechanism of antimicrobial action	References
Vancomycin-Silica NMs	MRSA	MRSA sensitive near-infrared fluorescence (NIRF) nanoprobe for imaging and photothermal antibacterial therapy	(Zhao *et al.*, 2017)

2.4 NM against biofilms

Biofilm is a bacterial cell community that adheres to metals, plastics, and human or animal tissues with the aid of highly hydrated extracellular polymeric substance (EPS) matrix (Wingender, Neu and Flemming, 1999)(Donlan, 2002). Secreted EPS is responsible for the maintenance of the three-dimensional biofilm structure (Flemming and Wingender, 2010)(Markowska, Grudniak and Wolska, 2013). In a biofilm environment, bacteria can propagate quickly with efficient protection and consequently create 100-1000 times more resistance of the cells towards the phagocytic process (Aaron *et al.*, 2002)(Khan and Khan, 2016). Several studies have shown that biofilm-grown microorganisms acquire resistance by a variety of mechanisms as listed below:

A) EPS in biofilm acts as a physicochemical barrier and restricts the penetration of antimicrobial drugs (Billings *et al.*, 2013)(Tseng *et al.*, 2013). Additionally, an enzymatic substance in biofilm matrix hydrolyzes antimicrobial agents and reduces their activity. For example, β-lactamase present in *P. aeruginosa* degrades β-lactam antibiotics (Mah and O'Toole, 2001)(Ciofu *et al.*, 2000)(Schooling and Beveridge, 2006).

B) High-density bacterial growth within a biofilm promotes stress response, which induces the production of antimicrobial degrading enzymes (Schembri, Kjaergaard and Klemm, 2003).

C) Increased DNA exchange between bacteria, which facilitates resistance-gene transmission (Qayyum *et al.*, 2016).

D) By quorum sensing, bacteria are capable of controlling gene transcription (Husain *et al.*, 2016).

E) The slow growth of bacteria in biofilms is another mechanism of resistance (Mah and O'Toole, 2001).

NM are increasingly regarded as an alternative to standard antibiotics to eliminate biofilms or limit their development on biomedical devices (Iannitelli *et al.*, 2011)(Lellouche *et al.*, 2012).

Metal NM have a benefit over other frequently used antimicrobials because they do not differentiate between pathogenic and drug-resistant microbes with no specific target (Campoccia, Montanaro and Arciola, 2013)(Rai, Yadav and Gade, 2009). A diagrammatic representation of the antibiofilm mode of action of NM is shown in Figures 4 and 5.

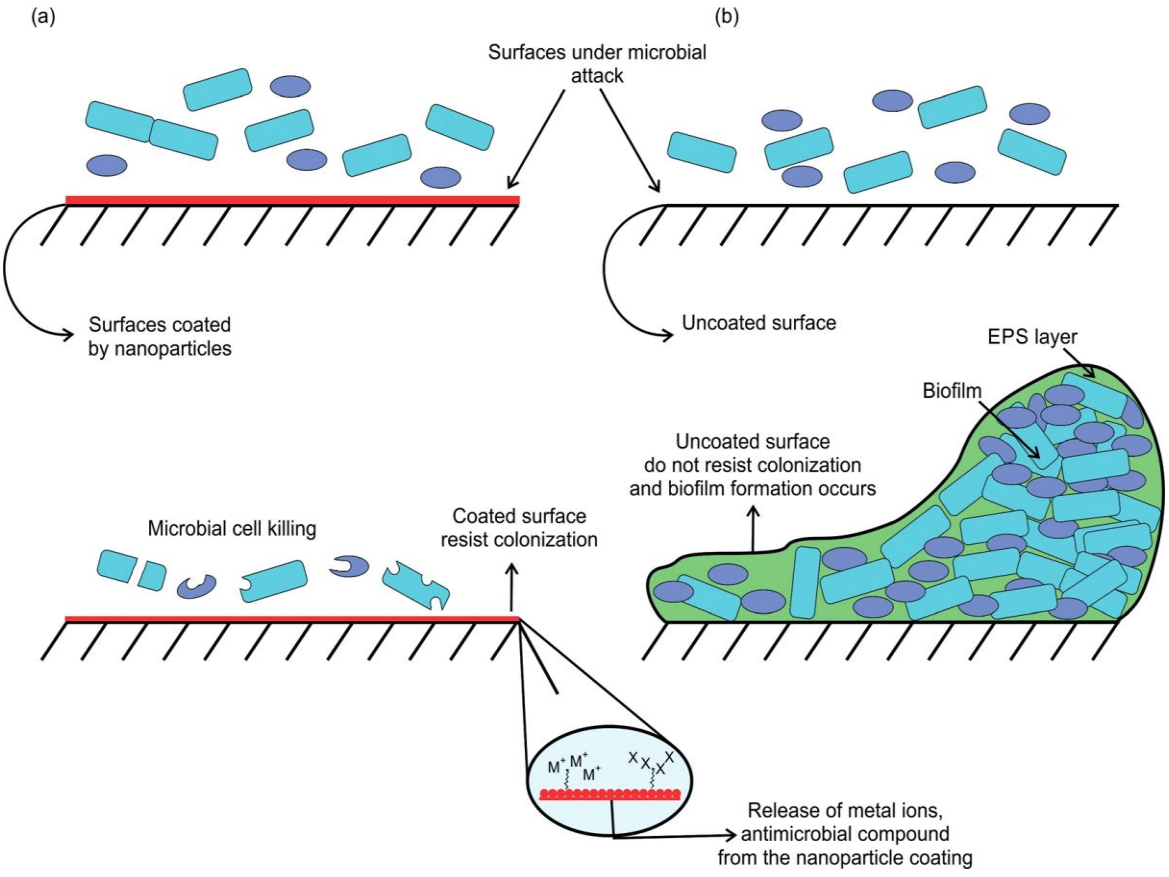

Fig. 4. Illustration of the inhibition of biofilm formation on surfaces coated by metal NM. Reproduced with permission (Qayyum *et al.*, 2016) Copyright © 2016 RSC Publishing

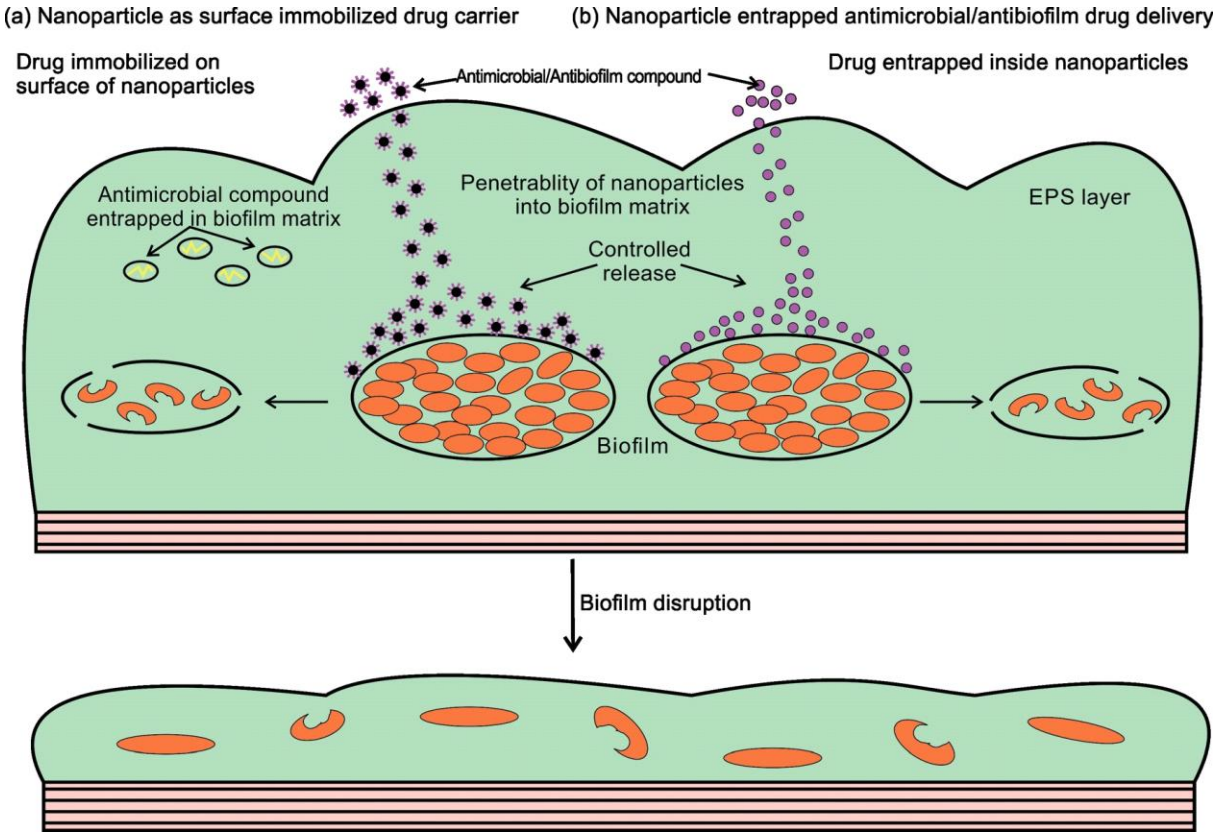

Fig. 5 Biofilm disrupting the action of metal NM on the pre-formed biofilms. Reproduced with permission (Qayyum et al., 2016) Copyright © 2016 RSC Publishing

Several approaches have been developed to eradicate biofilms, bactericidal, and bacteriostatic anti-biofilm formation (Chen, Yu and Sun, 2013)(Dos Santos Ramos *et al.*, 2018). For example, Ag NM have been widely used to prevent biofilm formation for various applications such as catheters, dental materials, medical devices, implants, and wound dressings (Wang, Shen and Haapasalo, 2014)(Thiwawong, Onlaor and Tunhoo, 2013). Secinti et al. (Secinti *et al.*, 2011) studied the anti-biofilm properties of Ag^{+} ion coated titanium implants against *S. aureus* biofilm in 20 New Zealand rabbits; the result showed that no bacteria or biofilm layer formed on the coated implants, whereas biofilm was detected on uncoated implants. Additionally, no Ag^{+} accumulation was observed in host tissues (cornea, kidney, liver, and brain) after 28 days post-implantation. However, coating

medical devices with Ag^+ ions or Ag NM sometimes have disappointing results, probably due to dose-dependent cytotoxicity (Huang *et al.,* 2016). At optimal concentration, Ag NM is non-toxic with low bactericidal effects in mammalian cells (Ewald *et al.,* 2006) (Burd *et al.,* 2007). Han et al. (Han *et al.,* 2014) studied the potential toxicity of 20 nm Ag NM in male and female mice *in vivo,* and found a negative impact of Ag NM on the reproduction of mice. Catheters coated with the Ag NM reported inducing thrombin formation and platelet activation, resulting in thrombosis (Stevens et al., 2009). Recently, Lee et al. (Ramasamy, Lee and Lee, 2017) studied the antibiofilm activity of Au NM linked cinnamaldehyde (CNMA-Au NM) and reported significant biofilm inhibition of MSSA, MRSA (gram-positive) as well as *E.coli* (gram-negative) compared to non-conjugated Au NM. The smaller size of Au NM and lipophilic nature of cinnamaldehyde facilitated attraction between CNMA-Au NM and bacterial membrane within biofilms, which can lead CNMA-Au NM to penetrate the biofilm architecture and inhibit biofilm formation by reducing metabolic activity and bacterial motility. In a subsequent study, the results demonstrated that cinnamaldehyde conjugated with silica (SiO_2) coated Au NM (CNMA-Si-Au NM) led to disintegration and disorganization of the bacterial membrane, while preserving its integrity when treated with SiO_2-Au NM (silica coated-Au NM). The authors also reported that CNMA-SiO_2-Au NM hydrolyzed in the acidic pH environment of the biofilm (Mohankandhasamy *et al.,* 2017).

3. Antimicrobial peptides and their antimicrobial potential

Antimicrobial peptides (AMP) are components of the immune system of many organisms, such as bacteria, plants, fish, amphibians, insects, mammals, and even viruses; which not only protect them against infections but also display remarkable ability to tune the innate immune responses for microbial clearance (Papo and Shai, 2003)(Hancock and Sahl, 2006)(Malmsten,

2014)(Etayash *et al.*, 2013). AMP are amphipathic arrangements of 12-50 amino acids, categorised into α-helical, β-sheet, extended, and mixed (α & β) with different secondary structure configurations (Figure 6) (Wang and Wang, 2004)(Wang, Li and Wang, 2009).

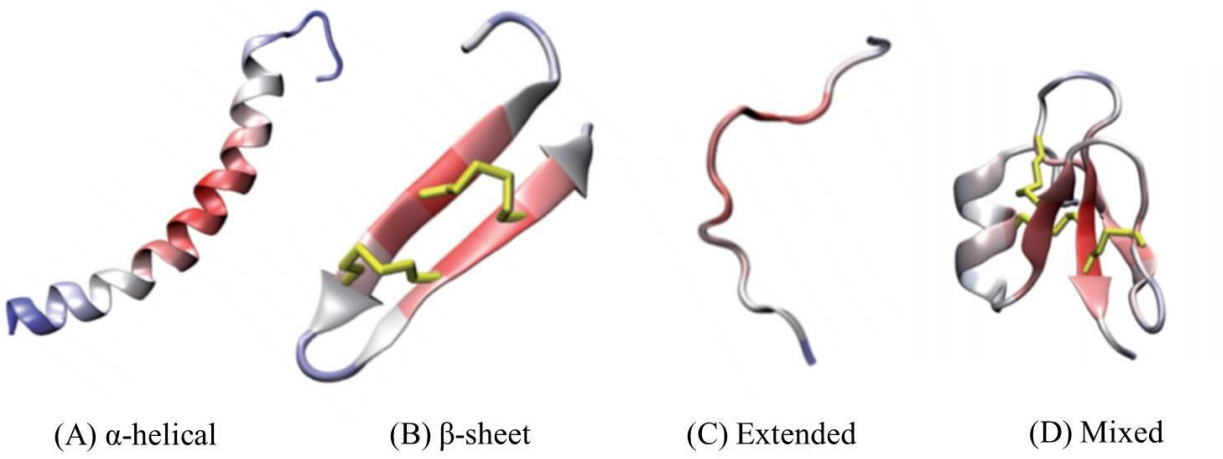

Figure 6. Structural classification of AMP. (A) α-helical, (B) β-sheet, (C) extended, and (D) mixed (α & β) peptides. Reproduced with the permission from (Rajchakit and Sarojini, 2017a) Copyright © 2017 ACS Publication.

AMP can be aromatic, non-cationic, and anionic peptides; the largest group belonging to cationic AMP (Marshall and Arenas, 2003). The α-helical class of cationic AMP has two separate characteristics: first, they have a polycationic sequence with a net positive charge (arginine and/or lysine) (Wang, Li and Wang, 2009)(Dennison *et al.*, 2005). Positively charged residues are the primary driving force for AMP to target anionic membranes of gram-positive and gram–negative bacteria. Negatively charged moieties (phospholipids, phosphatidylglycerol, cardiolipin, phosphatidylserine, and phosphatidylethanolamine) present on the membrane of gram-positive and gram–negative bacteria confer electronegativity to the bacterial surface, whereas eukaryotic

cells have a neutral net charge on their surface. Cationic AMP are, therefore, ideal for prokaryotic cell targeting.

Additionally, cholesterol in mammalian cell membranes reduces the activity of AMP. AMP retain their antibacterial activity in prokaryotic cells, as cells have lower cholesterol levels. In essence, negative surface charge and lack of cholesterol content of prokaryotic membrane attribute for the particular bactericidal activity of AMP (Ebenhan *et al.*, 2014)(Zasloff, 2002). Second, the common characteristic of all AMP is hydrophobicity (alanine, leucine, isoleucine, valine, methionine, phenylalanine, tyrosine, and tryptophan), which is an essential requirement for membrane internalization and selective antimicrobial activity. It has been observed that excessive hydrophobicity is not only cytotoxic to mammalian cells, but also induces non-selective antimicrobial activity. For instance, the increased hydrophobicity of α helical AMP (V13KL) resulted in RBC hemolysis, which may have been due to the penetration of AMP deep inside the hydrophobic membrane of RBCs. Additionally, excessive hydrophobicity increased the dimerization of α-helical AMP (V13KL) and restricted AMP access to through the pathogen membrane, which decreased its antimicrobial activity (Chen *et al.*, 2007). In addition to being polycationic and hydrophobic, AMP are amphiphilic, with segregated hydrophobic and hydrophilic residues, which allows them to be inserted into a pathogen plasma membrane (Cornup *et al.*, 1994).

3.1. Antimicrobial action of peptides

AMP can affect bacteria by various mechanisms, which are divided into three major classes: membrane disruption, intracellular targeting, and activation of immune responses.

Membrane disruption

Bacterial cell wall (CW) provides cellular integrity and stress-bearing ability, and as a result, maintains higher osmotic pressure and prevents cell lysis. Due to the bacterial CW composition compared to eukaryotic cells, these are a viable drug targeting choice. Among the potential targeting ligands are AMP, which display combinatory activity of cell membrane disorganization and inhibition of CW formation. AMP self-assembles on the prokaryotic membrane by hydrophobic/electrostatic interactions followed by cell membrane disintegration and disorganization. Three different significant models explain the action of AMP: Barrel-Stave Model, toroidal pore, and carpet model.

In the Barrel-Stave Model, parallel orientation of α-AMP on the PM is achieved through electrostatic interactions (Huang, 2009), leading to formation of transmembrane pores, which leads to cell death through the leakage of ribosome and mitochondrial organelles (Brogden, 2005)(Yang *et al.*, 2001)(Vedovato and Rispoli, 2007). For example, intestinal C-type lectin binds to the peptidoglycan carbohydrate of bacteria and kills it by forming membrane-penetrating pores (Mukherjee *et al.*, 2014)(Miki, Holsts and Hardt, 2012).

According to the toroidal pore model, AMP accumulates at specific concentrations on the PM surface and bends it by increasing the distance between phospholipid moieties, which eventually results in a toroidal pore. Subsequently, phospholipids disturb with PM forming pores. In this model, unlike Barrel-Stave Model, the lipophilic and hydrophilic arrangement of PM bilayer is disorganised. AMP such as magainin-2 (Lee and Aguilar, 2016), lacticin Q (Lee and Aguilar, 2016), aurein 2.2 (Cheng *et al.*, 2009), and melittin (Lee and Aguilar, 2016) can self-assemble around bacteria in toroidal pore fashion.

In the carpet model, AMP are oriented on the PM to disturb the bilayer in a detergent-like manner resulting in micelle formation, causing cell death. Human peptides such as cathelicidin LL-37 (Shai, 2002), cecropin (Sitaram and Nagaraj, 1999), indolicidin (Rozek, Friedrich and Hancock, 2000), and aurein 1.2 (Fernandez *et al.*, 2012) can kill different bacteria by carpet mechanism (Gable *et al.*, 2009).

AMP often binds to various precursors, which are engaged in CW synthesis. *uppP* (*bacA*) genes of UppP enzyme, a membrane protein engaged in CW synthesis, is one example of such precursors. AMP such as Lactococcin-G and Enterocin-1071 interact with *uppP* (*bacA*) genes and inhibit CW synthesis (Kjos *et al.*, 2014)(Belguesmia *et al.*, 2017). Likewise, AMP (class I&II) bacteriocins bind to the lipid-II, essential for the synthesis of peptidoglycan in gram-positive and gram–negative bacteria, and inhibit the formation of CW through pores formation (Islam *et al.*, 2012)(Yount and Yeaman, 2013). Some AMP can induce CW production of a lytic enzyme called N-acetylmuramoyl-L-alanine amidase, responsible for CW wall disintegration and disorganization (Wilmes *et al.*, 2014)(Bierbaum and Sahl, 1987). Pep5, nisin, Θ-defensin are examples of AMP that induce the activity of N-acetylmuramoyl-L-alanine amidase.

Intracellular targets

Recently, it has been reported that some AMP produce a bactericidal effect by cellular accumulation inside the PM targeting intracellular organelles. AMP induce activities such as inactivation of bacterial ribosomes, inhibition of protein synthesis, and interference in enzyme activity. For example, $Bac7_{1-35}$, oncocin, and apidaecins rich in proline residues that bind to the 70S ribosome and block its exit tunnel, eventually inhibits protein synthesis (Gagnon *et al.*, 2016).

Buforin-II, a cationic peptide, accumulates and interacts with nucleic acids without interfering with the *E. coli* PM (Park, Kim and Kim, 1998). Interestingly, buforin-II displays anti-endotoxin activity, reducing endotoxin generated in gram–negative bacteria (Giacometti *et al.*, 2002). Because of the inhibitory effect on endotoxin level, buforin-II prevents multiple organ failure and septic shock associated with the endotoxin-induced cytokines production. Microcins, antimicrobial peptides from gram-negative enterobacteria, can target intracellular and extracellular pathogens. For example, Microcin C (McC) translocates into sensitive cells to reach the target site via external membrane porins and internal ABC membrane transporters. In the cytoplasm, McC releases non-hydrolyzable aminoacyl adenylate, which obstructs a crucial aminoacyl-tRNA synthetase, enzyme for biosynthesis of protein (Fang and Guo, 2015)(Nocek *et al.*, 2012)(Rebuffat, 2012). Microcidin B17 is a peptide that enables the J25 (MccJ25) micron to cross the envelope of the cells, which inhibits DNA replication by inhibiting bacterial RNA polymerase (Mukhopadhyay *et al.*, 2004) (Hassan *et al.*, 2014). Haney et al. studied the antimicrobial effect of puroindoline derived Pur-B peptide against gram-positive and gram–negative bacteria (Haney *et al.*, 2013). They found that the positive charge of peptides leads to electrostatic attachment to negatively charged membranes. Pur-B peptide further penetrates PM and binds to nucleic acids, which ultimately inhibits the transcription and translation process. Gosh et al. found that Indolicidin, an antimicrobial peptide from cathelicidin family, binds and wraps around duplex DNA, which leads to transcription inhibition (Ghosh *et al.*, 2014). PR39 is another family of cathelicidin peptides with potent antimicrobial activity. This AMP obstructs bacterial nucleic acid replication (Bals and Wilson, 2003). This peptide has also been discovered to play a crucial role in innate immunity (Veldhuizen *et al.*, 2014). Some studies have observed that PR-39 inhibits the 20S proteasome in a non-competitive and reversible manner and blocks degradation

of NF-kB inhibitor. As a result, NF-kB dependent pro-inflammatory gene expression is suppressed in mouse myocardial infarction model and cell culture, thereby reducing inflammatory responses (Gao *et al.*, 2000)(Anbanandam *et al.*, 2008). Two bovine bactenecins, Bac5 and Bac7, exhibited potent bactericidal activity by obstructing the production of nucleic acid and proteins in *E. coli* and *K. pneumonia* (Skerlavaj, Romeo and Gennaro, 1990). Interestingly, some antimicrobial peptides produced from bacteria, such as bacteriocins, kill the pathogens through a receptor-mediated mechanism.

Modulation of Immune Responses

Apart from direct bactericidal activity, AMP generate different innate immune responses. They induce the modulation and expression of multiple cytokines and chemokines, as well as reduce inflammation by neutralizing cytokines released from macrophages and monocytes, promoting wound repair, modulating the responses of T-cells, and dendritic cells inducing angiogenesis (Diamond *et al.*, 2009). Such responses further modulate the innate immunity protecting the host against microbial infection. For example, human defensins bind to the CCR6, a protein-coupled receptor, and raise the amount of dendritic and T cells at the site of microbial infection. A very low MIC (> 2 µg/ml) of LL37 *in vivo* compared to *in vitro* (32 µg/ml) against *E. coli*, confirmed the indirect antimicrobial activity of AMP *in vivo* through modulating immune responses (Jenssen, Hamill and Hancock, 2006). AMP also recruit phagocytes cells at bacteria-infected sites and modulate immune responses against microbial infections. As-CATH2-6, out of six novel cathelicidins (As-CATH1-6) from Chinese alligator (*A. sinensis)*, showed antimicrobial and immunomodulatory activity in a bacteria-infected murine mouse model. As-CATH2-6 generate chemokines and recruit neutrophils, monocytes, and macrophages at the microbial infected sites; these intracellular granules invade and kill bacteria through phagocytosis (Chen *et al.*, 2017). Likewise,

the immunomodulatory role of mammalian host defense peptides (HDP) in combating *Leishmania* infection has also been studied by Rafati et al. (Abdossamadi, Seyed and Rafati, 2016). Upon stimulation by pathogens at microbial infected sites, LL-37 promotes the expression of TNF-β and IFN-γ from macrophages, promoting pathogen phagocytosis through CD32 and CD64 expression (Soehnlein *et al.*, 2008). All these activities promote immune hemostasis. Curiously, AMP stored at a sufficient concentration in immune cells, such as NK-cells, mast cells, neutrophils, monocytes, and macrophages, are the leading molecules that firstly deal with the pathogens and their expression at the infected site regulated by the vitamin-D receptor. Vitamin-D receptor promotes the production of vitamins 1,25 D3, which not only induces the expression of AMP but also enables the host to acknowledge and react to microbes by CD14 and TLR2 expression (Schauber *et al.*, 2007)(Liu *et al.*, 2006). Even though the expression of AMP can kill microbes, continuous expression leads to autoimmune disorders, such as rosacea and psoriasis (Zhang and Gallo, 2016).

3.2. Resistance to AMP

Bacteria can develop various types of resistance mechanisms to AMP (Figure 7). Resistance can be expressed, for example, by surface remodeling (Hankins *et al.*, 2012)(Malanovic and Lohner, 2016), modulation of gene expression of AMP (Sperandio *et al.*, 2008) biofilm formation (Yeaman and Yount, 2003), proteolytic degradation (Kooi and Sokol, 2009) efflux pumps (Shafer *et al.*, 1998)(Piddock, 2006)(Bengoechea and Skurnik, 2000), and by lipopolysaccharide (LPS) modification (Gunn, 2001). Recently, Cullen et al. (Cullen *et al.*, 2015) reported a gene to induce dephosphorylation of LPS, which reduces the net negative charge on the bacterial membrane; as a result, the pathogen resists AMP by decreasing their electrostatic interaction with PM. It is worth mentioning that the anionic lipid bilayer structure of bacterial outer membranes interacts with the positive charge of AMP, resulting in rapid destruction of the bacterial membrane

(Diehnelt, 2013). Additionally, reduced propensity to develop resistance against AMP compared to common antibiotics have been reported to date (Bahar and Ren, 2013). Figure 7 illustrates the overall resistance mechanisms to AMP.

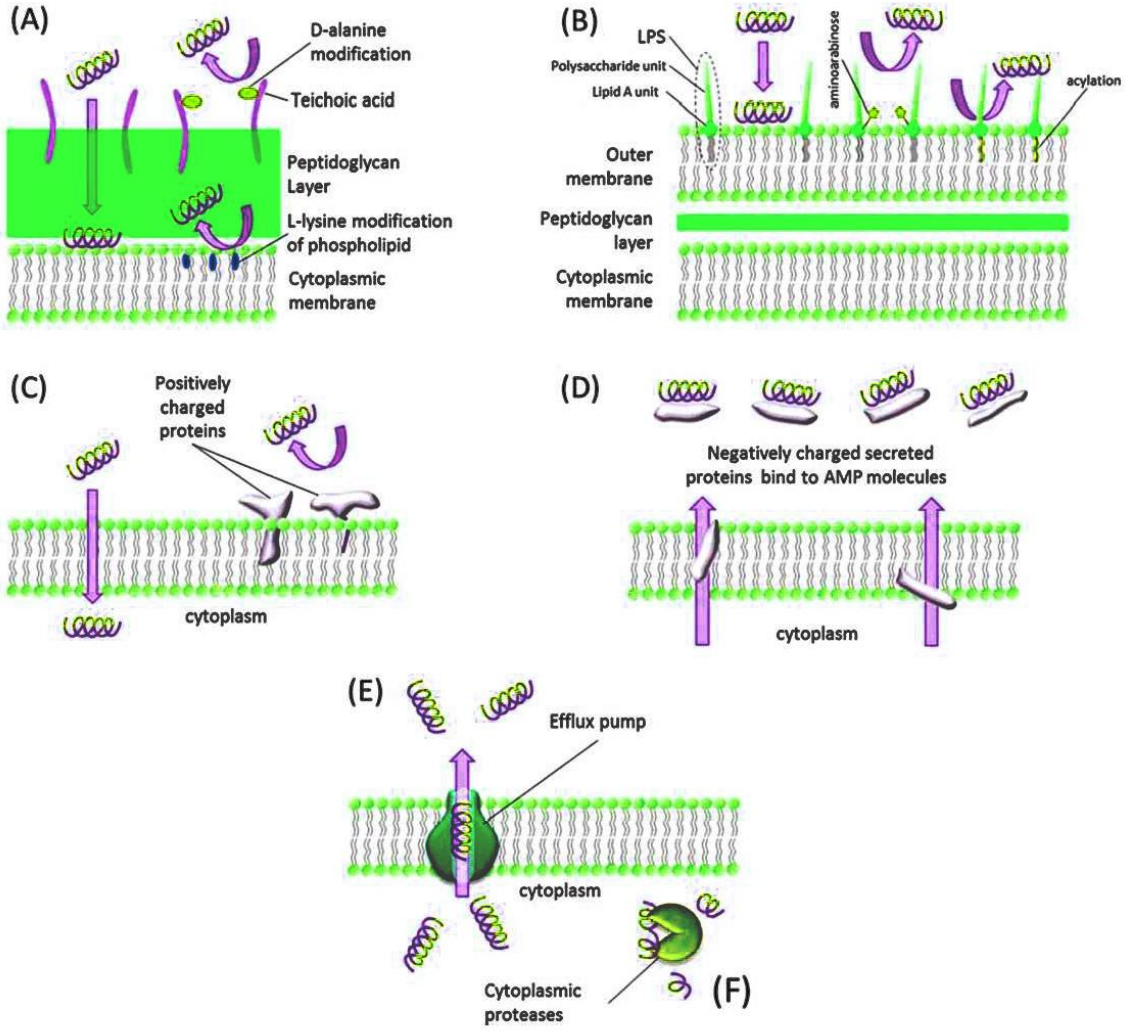

Fig. 7. Diagrammatic illustration of AMP resistance mechanisms: (**A**) Gram-positive bacteria, resistance to AMP occurs by D-alanine and L-lysine alteration of phospholipids by teichoic acids. (**B**) Gram-negative strains exhibits resistance towards AMP via altering lipopolysaccharide molecules with aminoarabinose or acylation of Lipid A unit of lipopolysaccharide molecules. (**C**) Bacteria release proteins (+ charged) that integrate with the membrane, and positively charged bacterial membrane can regulate cationic AMP. (**D**) Bacteria induce the formation of negatively charged proteins secreted to bind and block AMP in the

extracellular environment. (**E**) The intracellular AMP are squeezed out by efflux pumps. (**F**) The AMP within the cell are susceptible to degradation by proteases.

4. Inorganic NM as carriers for AMP

Although AMP display broad-spectrum antimicrobial activity, they are rapidly degraded by proteases of bacteria and human defense cells, resulting in the loss of their antimicrobial efficacy. When administered intravenously, AMP are rapidly cleared from the circulatory system and deposition in the reticuloendothelial system (RES). AMP in the RES display loss in antimicrobial activity and increase in systemic toxicity (Singh *et al.*, 2014)(VanderVen *et al.*, 2015). Consequently, especially inorganic NM have gained significant interest lately as potential carriers of AMP, which confer protection against chemical and enzymatic degradation, controlled release possibilities, enhanced potency and reduced systemic toxicity of encapsulated or surface-immobilised AMP (Almaaytah *et al.*, 2017).

4.1. Inorganic NM for the delivery of loaded AMP

The well-defined nanometer pores of MSNs allow active packing of AMP and further control of their release at the target site. Loading and release kinetics of entrapped drug/biological moieties inside MSNs can be regulated by varying surface properties and pore size (Bharti *et al.*, 2015). Adsorption of AMP to mesoporous silica was found to be regulated by the pore size and AMP concentration in solution. At low AMP concentration, AMP's dimer form strongly adsorbed into mesoporous silica with the same pore size as that of the AMP dimer through multivalent peptide-silica interactions (Braun *et al.*, 2017). However, at higher AMP concentration, formation of trimer and tetramer forms of peptides showed higher affinity for the pore walls and outer surface of larger sized MSNs.

The adsorption of AMP did not occur due to the low peptide-silica interaction that demands larger peptide aggregates to enhance adsorption. Peptides adsorbed onto small-sized MSN exhibited a faster release rate compared to that of peptides absorbed onto larger MSNs (Braun *et al.*, 2017). Likewise, Lzquierdo-Barba et al (Izquierdo-Barba *et al.*, 2009) compared the release behavior of high molecular weight LL-37 with low molecular weight chlorhexidine from silica monoliths and reported slow-release (~200 h) of both antimicrobial compounds. The release rate of such antimicrobials can be altered through the conjugation of the thiol group to the pore wall of silica monoliths. Furthermore, both LL-37 and chlorhexidine containing mesoporous silica monoliths exhibited antimicrobial activity against both *S. aureus* and *E.coli* , and the monolith containing LL-37 displayed low cytotoxicity against human keratinocytes and erythrocytes (Izquierdo-Barba *et al.*, 2009). Linden et al. compared anionic MSN(-ve), cationic MSN(+ve), and anionic non-porous silica nanoparticles (NSP) for loading and discharge of the cationic LL-37(LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES) AMP, along with its impacts on the antimicrobial effect. MSN(-ve) were found to carry a higher amount of LL-37 compared to MSN(+ve) and NSP, and conferred protection to AMP against degradation by proteases. Interestingly, though less in quantity, cationic LL-37 non-electrostatically adsorbed onto MSN(+ve), which might happen due to the reduction of positive surface charge. When studied adsorption of these NM on anionic DOPE/DOPG (dioleoylphosphatidylethnolamine/ dioleylphosphatidylglycerol) bilayers, a bacteria-mimicking PM, it was found that MSN(-ve) neither adsorbed nor disturbed DOPE/DOPG bilayers unless it was loaded with LL-37 AMP. At the same time, NSP because of high –ve charge on their surface obtains a resilient LL-37 coating,

exhibited low AMP release (Jacob, 2016). Like NSP, MSN(+ve) displayed particle mediated membrane disruption but also exhibited toxicity against erythrocytes (Braun *et al.*, 2016).

Further addressing SiO_2 NM as sustained delivery vehicles of AMP, Johnson et al. studied the diffusion of AMP from bioinorganic Si-ANM. They were reported to release the entrapped cationic decapeptide, KSL (KKVVFKVKFK), in a sustained manner over 5 days. Interestingly, released KSL was found to be identical to its free form and retained antimicrobial activity. After 5 days of diffusion study, when Si-ANM had undergone antimicrobial assay against *S.aureus* and *S. epidemidis,* no antimicrobial effects were observed (MIC> 225 µg/mL). Furthermore, the trypsin digestion assay revealed that free KSL was degraded entirely, whereas KSL recovered from Si-ANM after trypsin treatment was intact and active. These results show that SiO_2NM not only confer protection to KSL against proteolytic degradation, but also facilitate sustained release of AMP. Sustained release of AMP over extended periods is an essential requirement for implant-associated infection, i.e. inhibition of bacterial growth or biofilm formation on the device surface (Eby, Farrington and Johnson, 2008).

4.2. Inorganic NM for the delivery of the surface conjugated AMP

The biomimetic mimicry, a novel approach for precise drug delivery, where biomolecules are conjugated on NM surfaces is one of the new frontiers in material research to solve the limitations associated with metal NM (Lee, Ashe and Laurencin, 2014). Surface conjugation of biomolecules to NM confers stability, prevent agglomeration, attenuate toxicity, prolong the circulation time, and increase biocompatibility (Baptista *et al.*, 2013). For example, lysozyme incorporated with TiO_2 NM protect it from denaturation and confer functional properties to the bio-nanocomposite (Luckarift *et al.*, 2006). Magnetic FeO NM were also used as carriers for delivery of significant biomolecules as depicted in Figure 8, such as peptides (Veiseh *et al.*, 2009),

antibodies (Tsourkas *et al.*, 2005), aptamers, DNA (Josephson, Manuel Perez and Weissleder, 2001), and RNA (Reimhult, 2013) according to five different strategies:

A] Ligand binding through chemisorption of thiol groups onto the exterior surface

B] Electrostatic adsorption of opposite charges

C] Covalent binding by compatible functional groups

D] Non-covalent bonding, e.g. biotinylated oligonucleotides conjugated to streptavidin-Au NM

E] Encapsulation, i.e., incorporation of the biomolecules inside within the NM matrix.

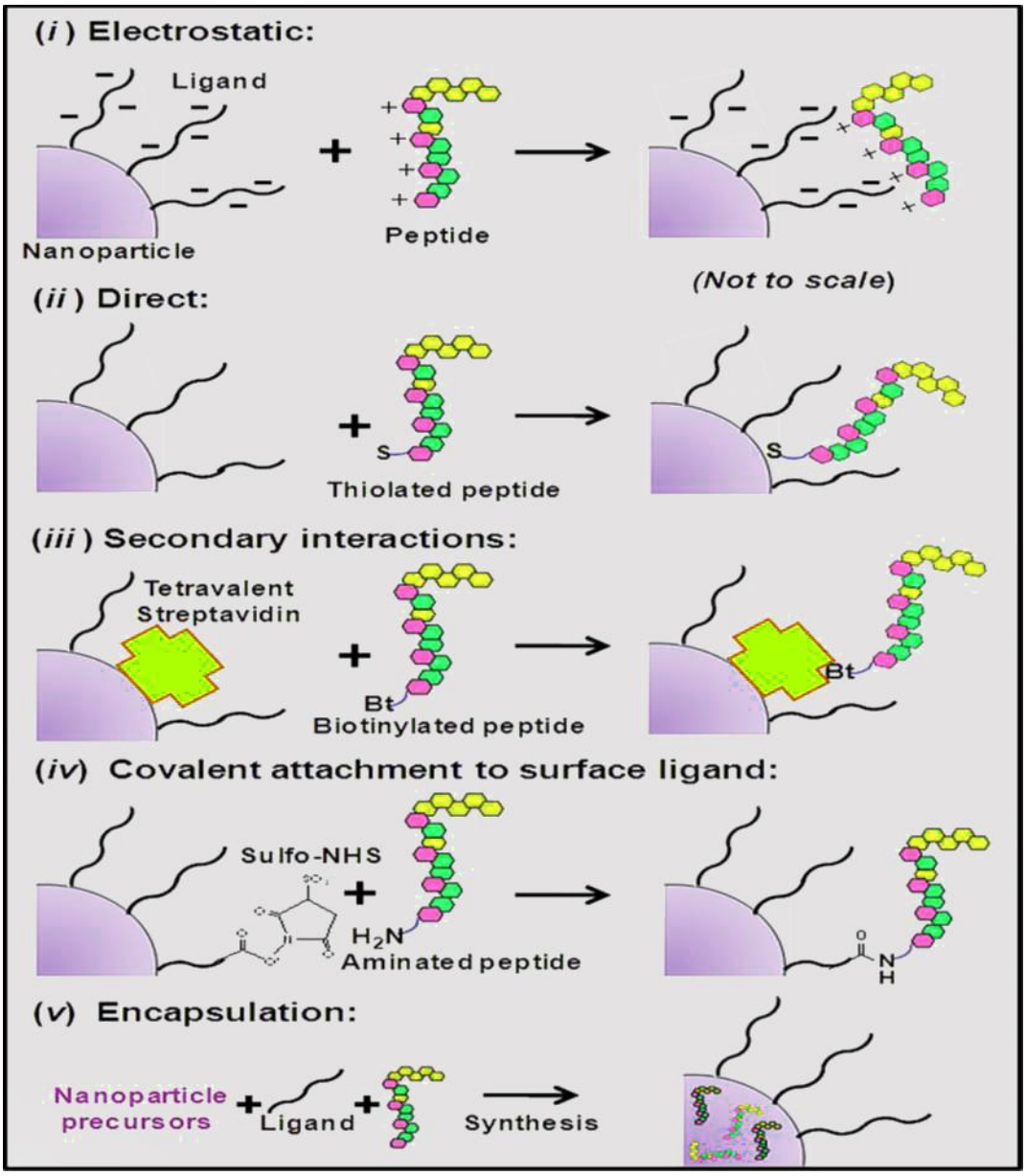

Fig.8. Five general strategies of peptides-NM generation: (i) Electrostatic interaction between the surface of the NM and the peptide to induce peptide assembly; (ii) Direct binding to NM surface; for example, direct binding of free thiols with the surface of Au NM. (iii) These strategies can modify the peptide surface so that it can specifically interact to form biotin−streptavidin complexes. (iv) Direct covalent attachment, by utilizing chemistry such as 1-ethyl-3-(3-dimethyl aminopropyl)-carbodiimide (EDC)-based coupling of amines to carboxyls; (v) Encapsulation of peptide inside the NM matrix.

4.3. Antimicrobial applications of AMP-conjugated inorganic NM

In this section, examples of recent research on AMP conjugated NM as antimicrobial agents are reviewed. In general, peptides exhibit a lower degree of toxicity and immune response than to high-molecular-weight polymers, such as polyethyleneimine (PEIs), which are easily degraded in the body by enzymes (Xue, Liu and Wong, 2014). Few of these peptides have been proven to have a specific binding affinity for cells. Conjugation of NM with these peptides not only elevate their cell uptake but also enhance their cellular localization (Liu *et al.*, 2007)(Nativo, Prior and Brust, 2008). For example, chemical functionalization of the TAT-derived peptide (Chem, 1997) on other biomolecules such as proteins (Fawell *et al.*, 1994), small interfering RNA (siRNA)(Turner *et al.*, 2007), and liposomes (Pappalardo *et al.*, 2009) facilitates their cellular uptake. Furthermore, Tat-derived peptides act as carriers to facilitate delivery of proteins (Schwarze *et al.*, 1999), QDs (Stroh *et al.*, 2005) and polymeric micelles (Liu *et al.*, 2008) to the central nervous system (CNS), because of their capability to cross the blood-brain barrier (BBB)(Alyautdin *et al.*, 2014). Moreover, as described in section 3.1., peptide-NM are involved in cellular uptake in phagocytic immune cells and innate immune responses (Yang *et al.*, 2016). Recent literature has also indicated that antimicrobial activity against gram-positive bacteria is exerted by cationic NM created by amphiphilic peptide self-assembly (Niño-Martínez *et al.*, 2019). This amphiphilic peptide, C17H35GR7RGDS NM, contained seven arginine residues that facilitated membrane translocation and reduced bacterial adhesion to fibronectin.

AMP functionalized Au NM

Ferreira et al. observed high antimicrobial activity by peptide-conjugated Au NM (CM-SH-Au NM) against *E. coli* (Ferreira *et al.*, 2016). The *in vivo* antimicrobial potency of CM-SH-Au NM was tested in murine wounds, topically infected with gram-positive and gram–negative bacteria. Animals treated with CM-SH-Au NM were found to have a significant attenuation in bacterial count. Furthermore, *in vivo* biodistribution study revealed that less than 5% of Au NM was deposited in the spleen, liver, lung, and kidney. The researchers concluded from the *in vitro* and *in vivo* results that CM-SH-Au NM has promising antibacterial potency. A disadvantage of AMP, when decorated onto Au NM, is induction of precipitation of the Au NM. Consequently, in this case, AMP on the Au NM surface is insufficient for antimicrobial activity. In contrast, AMP conjugation onto Au NM surfaces via the reduction of Au(III) in the presence of side-chain protected N-terminus Cys containing peptide and $NaBH_4$, confer secondary α-helical structure upon AMP-conjugated Au NM interacting with the bacterial membrane. As a consequence, the desired antimicrobial effect is preserved, as shown in Figure 9.

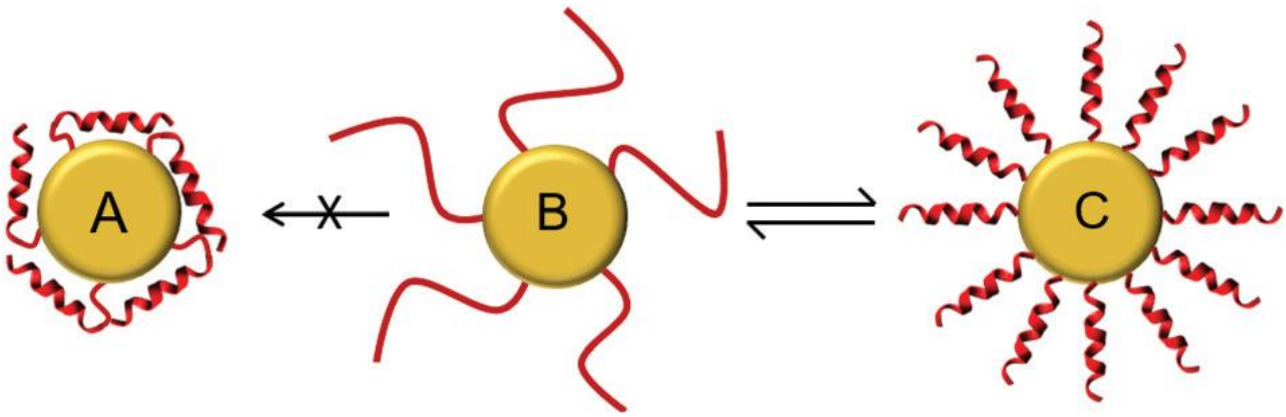

Fig. 9. Illustration of AMP conjugated onto the Au NM surface (B). Conjugates adopt secondary α-helical structure upon contact with bacterial membranes to exhibit antimicrobial property (C). Any attachment of Lys with the Au NM leads to precipitation of Au NM (A). Reproduced with permission from (Wadhwani *et al.*, 2017) Copyright © 2017 RSC Publishing

AMP-Au NM conjugates confers AMP stability against protease degradation and enhances the AMP half-life for up to 24 h. Interestingly, it was reported that AMP-Au NM retained similar activity as the free AMP in an aqueous buffer against both gram-positive and gram–negative bacteria (Wadhwani *et al.*, 2017). Chang et al. evaluated the antimicrobial efficacy of 1-dodecanethiol (DT) and Surfactin (SFT) immobilised gold nanodots (SFT-DT-AuNDs). They found >80-fold lower MIC compared to SFT and DT-AuNDs against MRSA stains. These effects were correlated to synergistic bacterial membrane damage by SFT and DT-AuNDs. Besides, hemolysis and cytotoxicity assays revealed higher biocompatibility of SFT-DT-AuNDs than free SFT. Lee *et al.* studied the efficacy of Au NM-Apt (Au NM conjugated with DNA aptamer) for efficient delivery of C-terminally hexahistidine tagged A3-APO^{His} peptide into *S. typhimurium* infected mammalian cells (Yeom *et al.*, 2016). Changes in the morphology of *S. typhimurium* cells were observed when the cells were treated with A3-APO and A3-APO^{His}peptide. SEM analysis showed disruption of PM when incubated with A3-APO^{His} whereas A3-APO did not affect the membrane. Besides, the SYTOX assay disclosed that A3APOH caused *S. Typhimurium* internal PM disturbance resulting in increased SYTOX green fluorescence intensity due to binding of the cationic dye to the nucleic acid. *S. typhimurium*-infected HeLa cells were used to test the antimicrobial potency of Au NM-AptHis-A3-APO^{His}. The result showed that the number of intracellular *S. typhimurium* cells was found to be 30-50% less when treated with Au NM-AptHis-A3-APO^{His} than those treated with Au NM-Apt^{His}, which exhibits negligible effects on *S. typhimurium* viability. Because all the *S. typhimurium* infected mice after intravenous administration of Au NM-AptHis-A3-APO^{His} survived, it was concluded that Au NM-Apt is more efficient in removing intracellular *S. typhimurium* cells both *in vitro* and *in vivo* without inducing toxicity to healthy mammalian cells.

AMP functionalized Ag NM

Metal/metal oxide NM can impart new functionality and reactivity to the new compounds, which can lead to promising bactericidal effects against multidrug-resistant bacteria (Chaloupka, Malam and Seifalian, 2010)(Kalishwaralal *et al.*, 2010). For example, bivalent Ag NM conjugated with histatin-1, an AMP that is involved in re-epithelialization processes (Oudhoff *et al.*, 2008), has shown promising MIC values (around 1 mg/L) against gram-positive and gram–negative bacteria with potent wound healing properties (Pal *et al.*, 2014)(Pal *et al.*, 2014). Ag NM at a certain concentration is known to be toxic to mammalian cell lines (Skalska and Strużyńska, 2015). Surface modification of Ag NM with AMP reduces the toxicity of Ag NM and enhances stability and antimicrobial activity. For instance, the strong interaction of positively charged non-cysteine containing AMP with Ag NM (Liu *et al.*, 2013) can disturb the conformation of AMP, resulting in decreased antimicrobial efficacy. Therefore, the main parameter in the design of new conjugates is the balance of stability/strong interaction for achieving optimal antimicrobial activity. The Ag NM and resultant AMP-Ag NM conjugate have shown enhanced stability and antimicrobial efficacy in comparison to the Ag NM and AMP alone. When the antibacterial mechanism of AMP-Ag NM was studied, conjugates disturb bacterial PM more significantly compared to AMP alone.

Furthermore, fluorescence-activated cell sorting (FACS) assay has been used to measure bactericidal potency of AMP, AMP-Ag NM, and Ag NM. Results of bacterial cell death revealed synergistic antimicrobial activity AMP-Ag NM (60% dead cell population) compared to Ag NM (31%) and AMP (33%), respectively. This study concluded that Ag NM, due to its negative charge, was unable to interact with PM and confirmed that AMP on the Ag NM surface is responsible for bacterial PM, damage followed by the arrest of transcription and translation (Pal *et al.*, 2016). Similarly, Liu et. al. showed that conjugation of AMP with Ag NM elevate their bactericidal effect compared to

unbound AMP, and enhanced the biocompatibility of Ag NM compared to using the Ag NM alone (Liu *et al.*, 2013). Navani et. al. (Lambadi *et al.*, 2015) found ~3- fold antibiofilm activity increase of Polymyxin-B conjugated Au NM (PBAu NM) compared to citrate-capped Au NM. The result revealed that the antimicrobial activity of PBAu NM coated surgical blades was enhanced compared to citrate-capped Au NM and uncoated surgical blades. This was due to highly cationic Polymyxin-B on the Au NM surface. The result of *in vitro* antimicrobial assay, live/dead staining, and flow cytometry are also in agreement with the elevated antimicrobial activity of PBAu NM against *P. aeruginosa*.

Table 4: Examples of various AMP-conjugated metal NM.

AMP	Metal NM	Tested microorganism(s)	Effect(s) of nano-formulation	Ref.
Bacteriocin				(Thirumurugan, Ramachandran and Shiamala Gowri, 2013)
Bacteriocin produced by *Lactobacillus plantarum* ATM 11 and nisin	Au	*B cereus*, *E. coli*, *S. aureus*, and *M luteus*	Enhanced the AMA against some food spoiling microorganisms	
24 AA LeuA	Au	*Lmonoytogenes*	24 AA LeuA peptide exhibited higher propensity towards Gram +bacteria whereas 14 AA did not show bacterial attachments	(Etayash *et al.*, 2013)
Enterocin	Ag	A group of Gram +and Gram - bacteria	Demonstrated broad-spectrum inhibition against a group of food pathogens without any detectable toxicity to red blood cell	(Sharma *et al.*, 2012)
Bacteriocin produced by *Lactobacillus acidophilus* CH1	Au	*E bieneusi* spores	Increased the anti-microsporidial effect without significant cell toxicity	(Mossallam, Amer and Diab, 2014)

Nisin	Nanofibers with Ag	*S. aureus, P. aeruginosa, K. pneumonia, E. coli, S. typhimurium*	Provided a broad-spectrum AMA	(Ahire, Neveling and Dicks, 2015)
PEP	Au	*S. aureus* *E. coli*	Enhanced transfection efficiency and synergetic AMA	(L. H. Peng *et al.*, 2016)
EEEEAAAVVVK-C14H26	Ag	*E. coli*	Exhibited low toxicity toward eukaryotic cells	(Pazos *et al.*, 2016)(Yu, Wang and Wei, 2017)
Esculentin-1a(1-21)NH_2	Au	*P. aeruginosa*	A 15fold increase in antimicrobial potency compared to free peptide alone without toxic to human keratinocytes	(Casciaro *et al.*, 2017)

5. **Conclusion and perspectives**

The bactericidal efficiency of various NM, antimicrobial peptides, and peptide conjugated NM against different bacterial strains have been provided in this review. In general, the antimicrobial activity of nanometals, AMP, and antimicrobial peptide conjugated NM depends on the bacterial cell surfaces and NM conjugation characteristics. The biomimetic properties of AMP assist the targeted delivery of peptide conjugated NM, whereas ions released from NM kill microbial pathogens. However, further studies should elucidate the precise mechanism of metal ions, whether membrane disturbance or cytoskeleton detachment from the plasma membrane is causing cell death. Most of the antimicrobial properties have to date been tested against *E. coli* and *S. aureus*. To demonstrate the wide variety of bactericidal/bacteriostatic characteristics of peptide conjugated NM, it will be essential to explore other pathogenic species and phenotypes.

In comparison to bacteria, there are very few reports on the antifungal properties of antimicrobial peptides. Some studies have demonstrated that covalent surface modification with exposure to oxygen or the presence of UV can create rapid cytotoxicity. Therefore, toxicity should also be studied under circumstances of UV and air exposure. It has also been found that different physical and chemical parameters of NM, such as size, shape, surface-to-volume ratio, the surface charge of the particles, and their synthesis methods influence their antibacterial efficiency. For example, Ag NM with the same surface areas but with distinct shapes may have different bactericidal activity against pathogens. Thus, the antimicrobial effects of AMP conjugated to NM with different physicochemical characteristics against different bacterial strains should be the next line of research. One major limitation for advancements of peptide conjugated metal NM is that *in vivo* and *in vitro* antimicrobial activity do not discriminate

whether peptide conjugated NM can kill pathogenic microorganisms selectively without influencing healthy mammalian cells. In this scenario, the study of the interaction of various shapes of NM on mammalian cells should be carried out. Moreover, non-metals such as Ag and Cu non-specifically kill pathogens and, therefore, toxicity against mammalian cells should also be investigated.

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List of abbreviations

NM	Nanomaterial
NO	Nitric oxide
PSi	Mesoporous silicon
THC	Thermal Hydrocarbonisation
AMP	Antimicrobial Peptides
SPION	Superparamagnetic iron oxide
APTES	3-aminopropyltriethoxysilane
Ln-ZnO	Laurusnobilis-Zn oxide
CLSM	Confocal laser scanning microscopy
FE-SEM	Field emission scanning electron microscopy
CNT	Carbon Nanotube
Ag	Silver
Cu	Copper
Au	Gold
Zn	Zinc
Ga	Gallium
Al	Aluminum
As	Arsenic
Pt	Platinum
Hg	Mercury

TiO_2	Titanium Dioxide
pG	pristine graphene
GO	graphene oxide
rGO	reduced graphene oxide
G-QDs	graphene quantum dots
SWCNTs-Ag	Ag coated SWCNTs-Ag
Zn	Zinc
ZnO	Zinc Oxide
CuO	Copper Oxide
LDH	Layered double hydroxide
CNFs	Carbon nanofibers
ACFs	Activated carbon fiber
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
AA	Amino Acid
Ag-NM-L leaves	Ag NM synthesised from Caltropisprocera
CM-SH	Cecropinmelittin with cysteine
ICP-MS	Inductively coupled plasma mass spectrometry
$A3\text{-}APO^{His}$	C-terminally hexahistidine-tagged A3-APO
i.v	intravenous
Au NM-Apt	Au NM-DNA aptamer
$Au\ NM\text{-}Apt^{His}$	Au NM conjugated with His-tag DNA aptamer
PEP	AMP (PEP) from lactoferrin
NQR	NADH: ubiquinone oxidoreductase
TBARS	Thiobarbituric acid-reactive substances
ONMG	*o*-nitrophenol β-d–galactopyranosideside
Ag NM-SiNW	Si nanowire composite with Ag NM
Cu NM- SiNW	Si nanowire composite with CuNM
Ag-MESs	Ag encapsulated mesoporous silica nanocrystals
DNA	Deoxyribonucleic acid,

RNA	Ribonucleic acid
MSSA	Methicillin-sensitive *Staphylococcus aureus*
MRSA	Methicillin-resistant *Staphylococcus aureus*
MRSE	Methicillin-resistant *Staphylococcus epidermidis*
V_2O_5	Vanadium pentoxide V_2O_5
OM	Outer membrane
PM	Plasma membrane
PGN	Peptidoglycan
LTA	Lipoteichoic acid
MLT	Maltose transporter

6. References

Aaron, S. D. *et al.* (2002) 'Single and combination antibiotic susceptibilities of planktonic adherent, and biofilm-grown Pseudomonas aeruginosa isolates cultured from sputa of adults with cystic fibrosis', *Journal of Clinical Microbiology*, 40(11), pp. 4172–4179. doi: 10.1128/JCM.40.11.4172-4179.2002.

Abdossamadi, Z., Seyed, N. and Rafati, S. (2016) 'Mammalian host defense peptides and their implication on combating Leishmania infection', *Cellular Immunology*, 309, pp. 23–31. doi: 10.1016/j.cellimm.2016.10.001.

Acker, H. Van and Coenye, T. (2016) 'The Role of Reactive Oxygen Species in Antibiotic-Mediated Killing of Bacteria', *Trends in Microbiology*. Elsevier Ltd, xx, pp. 1–11. doi: 10.1016/j.tim.2016.12.008.

Agnihotri, S., Mukherji, Soumyo and Mukherji, Suparna (2014) 'Size-controlled silver nanoparticles synthesized over the range 5-100 nm using the same protocol and their antibacterial efficacy', *RSC Advances*, 4(8), pp. 3974–3983. doi: 10.1039/c3ra44507k.

Ahamed, M. *et al.* (2017) 'Ag-doping regulates the cytotoxicity of TiO<inf>2</inf>nanoparticles via oxidative stress in human cancer cells', *Scientific Reports*, 7(1). doi: 10.1038/s41598-017-17559-9.

Ahire, J. J., Neveling, D. P. and Dicks, L. M. T. (2015) 'Co-spinning of Silver Nanoparticles with Nisin Increases the Antimicrobial Spectrum of PDLLA : PEO Nanofibers', *Current Microbiology*. Springer US, pp. 24–30. doi: 10.1007/s00284-015-0813-y.

Ahmed, K. B. A., Raman, T. and Veerappan, A. (2016) 'Future prospects of antibacterial metal nanoparticles as enzyme inhibitor', *Materials Science and Engineering: C*. Elsevier B.V., 68, pp. 939–947. doi: 10.1016/j.msec.2016.06.034.

Alekshun, M. N. and Levy, S. B. (2007) 'Molecular Mechanisms of Antibacterial Multidrug Resistance', *Cell*, 128(6), pp. 1037–1050. doi: 10.1016/j.cell.2007.03.004.

Allahverdiyev, A. M. *et al.* (2011) 'Coping with antibiotic resistance: combining nanoparticles with antibiotics and other antimicrobial agents', *Expert Review of Anti-infective Therapy*, 9(11), pp. 1035–1052. doi: 10.1586/eri.11.121.

Almaaytah, A. *et al.* (2017) 'Development of novel ultrashort antimicrobial peptide nanoparticles with potent antimicrobial and antibiofilm activities against multidrug-resistant bacteria', *Drug Design, Development and Therapy*. doi: 10.2147/DDDT.S147450.

Alyautdin, R. *et al.* (2014) 'Nanoscale drug delivery systems and the blood-brain barrier', *International Journal of Nanomedicine*, pp. 795–811. doi: 10.2147/IJN.S52236.

Anbanandam, A. *et al.* (2008) 'Molecular Basis for Proline- and Arginine-Rich Peptide Inhibition of Proteasome', *Journal of Molecular Biology*. Elsevier Ltd, 384(1), pp. 219–227. doi: 10.1016/j.jmb.2008.09.021.

Andersson, D. I., Hughes, D. and Kubicek-Sutherland, J. Z. (2016) 'Mechanisms and consequences of bacterial resistance to antimicrobial peptides', *Drug Resistance Updates*, pp. 43–57. doi: 10.1016/j.drup.2016.04.002.

Ann, L. C. *et al.* (2014) 'Effect of surface modification and UVA photoactivation on antibacterial bioactivity of zinc oxide powder', *Applied Surface Science*. Elsevier B.V., 292, pp. 405–412. doi: 10.1016/j.apsusc.2013.11.152.

Ansari, M. A. *et al.* (2013) 'Antibacterial potential of Al2O3 nanoparticles against multidrug resistance strains of Staphylococcus aureus isolated from skin exudates', *Journal of Nanoparticle Research*, 15(10), p. 1970. doi: 10.1007/s11051-013-1970-1.

Ansari, M. A. *et al.* (2014) 'Interaction of Al2O3 nanoparticles with Escherichia coli and their cell envelope biomolecules', *Journal of Applied Microbiology*, 116(4), pp. 772–783. doi: 10.1111/jam.12423.

Arakha, M. *et al.* (2015) 'Antimicrobial activity of iron oxide nanoparticle upon modulation of nanoparticle-bacteria interface.', *Scientific reports*, 5, p. 14813. doi: 10.1038/srep14813.

Bahar, A. A. and Ren, D. (2013) 'Antimicrobial peptides', *Pharmaceuticals*, 6(12), pp. 1543–1575. doi: 10.3390/ph6121543.

Bals, R. and Wilson, J. M. (2003) 'Cellular and Molecular Life Sciences Cathelicidins – a family of multifunctional antimicrobial peptides', 60, pp. 711–720. doi: 10.1007/s00018-003-2186-9.

Baltzer, S. a. and Brown, M. H. (2011) 'Antimicrobial Peptides – Promising Alternatives to Conventional Antibiotics', *Journal of Molecular Microbiology and Biotechnology*, 20(4), pp. 228–235. doi: 10.1159/000331009.

Baptista, A. *et al.* (2013) 'Nanofibers and Nanoparticles in Biomedical Applications', in *Bioengineered Nanomaterials*. CRC Press, pp. 93–124. doi: doi:10.1201/b15403-5.

Belguesmia, Y. *et al.* (2017) 'Chapter 1 - Resistance to Antibiotics and Antimicrobial Peptides: A Need of Novel Technology to Tackle This Phenomenon BT - Functionalized Nanomaterials for the Management of Microbial Infection', in *Micro and Nano Technologies*. Boston: Elsevier,

pp. 1–22. doi: http://doi.org/10.1016/B978-0-323-41625-2.00001-6.

Bengoechea, J. A. and Skurnik, M. (2000) 'Temperature-regulated efflux pump/potassium antiporter system mediates resistance to cationic antimicrobial peptides in Yersinia', *Molecular Microbiology*, 37(1), pp. 67–80. doi: 10.1046/j.1365-2958.2000.01956.x.

Bharti, C. *et al.* (2015) 'Mesoporous silica nanoparticles in target drug delivery system: A review', *International Journal of Pharmaceutical Investigation*. doi: 10.4103/2230-973X.160844.

Bhattacharya, R. and Mukherjee, P. (2008) 'Biological properties of " naked " metal nanoparticles ☆', 60, pp. 1289–1306. doi: 10.1016/j.addr.2008.03.013.

Bierbaum, G. and Sahl, H. G. (1987) 'Autolytic system of Staphylococcus simulans 22: Influence of cationic peptides on activity of N-acetylmuramoyl-L-alanine amidase', *Journal of Bacteriology*, 169(12), pp. 5452–5458. doi: 10.1128/jb.169.12.5452-5458.1987.

Billings, N. *et al.* (2013) 'The Extracellular Matrix Component Psl Provides Fast-Acting Antibiotic Defense in Pseudomonas aeruginosa Biofilms', *PLoS Pathogens*, 9(8). doi: 10.1371/journal.ppat.1003526.

Blair, J. M. A. *et al.* (2015) 'Molecular mechanisms of antibiotic resistance', *Nature Reviews Microbiology*, 13, pp. 42–51. doi: 10.1039/c0cc05111j.

Blecher, K., Nasir, A. and Friedman, A. (2017) 'infectious disease in combating infectious disease a n d e s i o s c i e n c e o n o t d i s t r i b u t e', 5594(March). doi: 10.4161/viru.2.5.17035.

Booth, S. C., Weljie, A. M. and Turner, R. J. (2015) 'Metabolomics reveals differences of metal toxicity in cultures of Pseudomonas pseudoalcaligenes KF707 grown on different carbon', *Frontiers in Microbiology*, 6(JUL). doi: 10.3389/fmicb.2015.00827.

Braun, K. *et al.* (2016) 'Membrane interactions of mesoporous silica nanoparticles as carriers of antimicrobial peptides', *Journal of Colloid and Interface Science*, 475, pp. 161–170. doi: 10.1016/j.jcis.2016.05.002.

Braun, K. *et al.* (2017) 'Influence of mesopore size and peptide aggregation on the adsorption and release of a model antimicrobial peptide onto/from mesoporous silica nanoparticles: In vitro', *Molecular Systems Design and Engineering*. Royal Society of Chemistry, 2(4), pp. 393–400. doi: 10.1039/c7me00059f.

Brogden, K. A. (2005) 'Antimicrobial peptides: pore formers or metabolic inhibitors in

bacteria?', *Nature reviews. Microbiology*, 3(3), pp. 238–50. doi: 10.1038/nrmicro1098.

Brown, A. N. *et al.* (2012) 'Nanoparticles functionalized with ampicillin destroy multiple-antibiotic-resistant isolates of Pseudomonas aeruginosa and Enterobacter aerogenes and methicillin-resistant Staphylococcus aureus', *Applied and Environmental Microbiology*, 78(8), pp. 2768–2774. doi: 10.1128/AEM.06513-11.

Burd, A. *et al.* (2007) 'A comparative study of the cytotoxicity of silver-based dressings in monolayer cell, tissue explant, and animal models', *Wound Repair and Regeneration*, 15(1), pp. 94–104. doi: 10.1111/j.1524-475X.2006.00190.x.

Cai, Y., Strømme, M. and Welch, K. (2013) 'Photocatalytic Antibacterial Effects Are Maintained on Resin-Based TiO2 Nanocomposites after Cessation of UV Irradiation', *PLoS ONE*, 8(10). doi: 10.1371/journal.pone.0075929.

Campoccia, D., Montanaro, L. and Arciola, C. R. (2013) 'A review of the biomaterials technologies for infection-resistant surfaces', *Biomaterials*, pp. 8533–8554. doi: 10.1016/j.biomaterials.2013.07.089.

Camporotondia, D. E. *et al.* (2013) 'Antimicrobial properties of silica modified nanoparticles', *Microbial pathogens and strategies for combating them: science, technology and education*.

Carré, G. *et al.* (2014) 'TiO 2 Photocatalysis Damages Lipids and Proteins in', 80(8), pp. 2573–2581. doi: 10.1128/AEM.03995-13.

Casals, E. *et al.* (2019) 'Biodistribution, Excretion, and Toxicity of Inorganic Nanoparticles', *Theranostic Bionanomaterials*. Elsevier, pp. 3–26. doi: 10.1016/B978-0-12-815341-3.00001-8.

Casciaro, B. *et al.* (2017) 'Acta Biomaterialia Gold-nanoparticles coated with the antimicrobial peptide esculentin-1a (1-21) NH 2 as a reliable strategy for antipseudomonal drugs'. Acta Materialia Inc., 47, pp. 170–181. doi: 10.1016/j.actbio.2016.09.041.

Cha, S. H. *et al.* (2015) 'Shape-Dependent Biomimetic Inhibition of Enzyme by Nanoparticles and Their Antibacterial Activity', *ACS Nano*, 9(9), pp. 9097–9105. doi: 10.1021/acsnano.5b03247.

Chaloupka, K., Malam, Y. and Seifalian, A. M. (2010) 'Nanosilver as a new generation of nanoproduct in biomedical applications', *Trends in Biotechnology*. Elsevier Ltd, 28(11), pp. 580–588. doi: 10.1016/j.tibtech.2010.07.006.

Chang, Y. *et al.* (2012) 'The Toxic Effects and Mechanisms of CuO and ZnO Nanoparticles', pp. 2850–2871. doi: 10.3390/ma5122850.

Chaurasia, A. K. *et al.* (2016) 'Coupling of radiofrequency with magnetic nanoparticles treatment as an alternative physical antibacterial strategy against multiple drug resistant bacteria', *Scientific Reports*, 6. doi: 10.1038/srep33662.

Chem, J. B. (1997) 'CELL BIOLOGY AND METABOLISM : A Truncated HIV-1 Tat Protein Basic Domain Rapidly Translocates through the Plasma Membrane and Accumulates in the Cell Nucleus A Truncated HIV-1 Tat Protein Basic Domain Rapidly Translocates through the Plasma Membrane and A', 272(25), pp. 16010–16017.

Chen, C. W. *et al.* (2014) 'Metal nanobullets for multidrug resistant bacteria and biofilms', *Advanced Drug Delivery Reviews*, pp. 88–104. doi: 10.1016/j.addr.2014.08.004.

Chen, M., Yu, Q. and Sun, H. (2013) 'Novel strategies for the prevention and treatment of biofilm related infections', *International Journal of Molecular Sciences*, pp. 18488–18501. doi: 10.3390/ijms140918488.

Chen, Y. *et al.* (2007) 'Role of peptide hydrophobicity in the mechanism of action of alpha-helical antimicrobial peptides.', *Antimicrobial agents and chemotherapy*, 51(4), pp. 1398–406. doi: 10.1128/AAC.00925-06.

Chen, Y. *et al.* (2017) 'As-CATH1–6, novel cathelicidins with potent antimicrobial and immunomodulatory properties from *Alligator sinensis* , play pivotal roles in host antimicrobial immune responses', *Biochemical Journal*, 474(16), pp. 2861–2885. doi: 10.1042/BCJ20170334.

Cheng, J. T. J. *et al.* (2009) 'Effect of membrane composition on antimicrobial peptides aurein 2.2 and 2.3 from australian southern bell frogs', *Biophysical Journal*, 96(2), pp. 552–565. doi: 10.1016/j.bpj.2008.10.012.

Ciofu, O. *et al.* (2000) 'Chromosomal beta-lactamase is packaged into membrane vesicles and secreted from Pseudomonas aeruginosa.', *The Journal of antimicrobial chemotherapy*, 45(1), pp. 9–13. doi: 10.1093/jac/45.1.9.

Cornup, I. *et al.* (1994) 'The amphipathic a-helix concept Application to the de novo design of ideally amphipathic Leu , Lys peptides with hemolytic activity higher than that of melittin', 349, pp. 29–33.

Cullen, T. W. *et al.* (2015) 'Gut microbiota. Antimicrobial peptide resistance mediates resilience of prominent gut commensals during inflammation.', *Science (New York, N.Y.)*, 347(6218), pp. 170–5. doi: 10.1126/science.1260580.

Dennison, S. R. *et al.* (2005) 'Amphiphilic alpha-helical antimicrobial peptides and their

structure/function relationships.', *Protein and peptide letters*, 12, pp. 31–39. doi: 10.2174/0929866053406039.

Diamond, G. *et al.* (2009) 'The Roles of Antimicrobial Peptides in Innate Host Defense', *Current Pharmaceutical Design*. doi: 10.2174/138161209788682325.

Diehnelt, C. W. (2013) 'Peptide array based discovery of synthetic antimicrobial peptides', *Frontiers in Microbiology*, 4(DEC), pp. 1–3. doi: 10.3389/fmicb.2013.00402.

El Din, S. N. *et al.* (2016) 'In vitro and in vivo antimicrobial activity of combined therapy of silver nanoparticles and visible blue light against Pseudomonas aeruginosa', *International Journal of Nanomedicine*, 11, pp. 1749–1758. doi: 10.2147/IJN.S102398.

Ding, F. *et al.* (2018) 'Size-Dependent Inhibitory Effects of Antibiotic Drug Nanocarriers against *Pseudomonas aeruginosa*', *ACS Omega*, 3(1), pp. 1231–1243. doi: 10.1021/acsomega.7b01956.

Dizaj, S. M. *et al.* (2014) 'Antimicrobial activity of the metals and metal oxide nanoparticles', *Materials Science and Engineering C*, pp. 278–284. doi: 10.1016/j.msec.2014.08.031.

Djafari, J. *et al.* (2016) 'New Synthesis of Gold- and Silver-Based Nano-Tetracycline Composites', *ChemistryOpen*, p. 169. doi: 10.1002/open.201600055.

Donlan, R. M. (2002) 'Biofilms: Microbial life on surfaces', *Emerging Infectious Diseases*, pp. 881–890. doi: 10.3201/eid0809.020063.

Dutta, R. K. *et al.* (2012) 'Studies on antibacterial activity of ZnO nanoparticles by ROS induced lipid peroxidation', *Colloids and Surfaces B: Biointerfaces*. Elsevier B.V., 94, pp. 143–150. doi: 10.1016/j.colsurfb.2012.01.046.

Ebbensgaard, A. *et al.* (2015) 'Comparative evaluation of the antimicrobial activity of different antimicrobial peptides against a range of pathogenic Bacteria', *PLoS ONE*, 10(12). doi: 10.1371/journal.pone.0144611.

Ebenhan, T. *et al.* (2014) 'Antimicrobial Peptides: Their Role as Infection-Selective Tracers for Molecular Imaging', *BioMed Research International*. doi: 10.1155/2014/867381.

Eby, D. M., Farrington, K. E. and Johnson, G. R. (2008) 'Synthesis of bioinorganic antimicrobial peptide nanoparticles with potential therapeutic properties', *Biomacromolecules*. doi: 10.1021/bm800512e.

Esmaeillou, M. *et al.* (2017) 'Vancomycin capped with silver nanoparticles as an antibacterial agent against multi-drug resistance bacteria', *Advanced Pharmaceutical Bulletin*, 7(3), pp. 479–483. doi: 10.15171/apb.2017.058.

Etayash, H. *et al.* (2013) 'Peptide-bacteria interactions using engineered surface-immobilized peptides from class IIa bacteriocins', *Langmuir*, 29(12), pp. 4048–4056. doi: 10.1021/la3041743.

Ewald, A. *et al.* (2006) 'Antimicrobial titanium/silver PVD coatings on titanium', *BioMedical Engineering OnLine*, 5(1), p. 22. doi: 10.1186/1475-925X-5-22.

Fang, P. and Guo, M. (2015) 'Evolutionary Limitation and Opportunities for Developing tRNA Synthetase Inhibitors with 5-Binding-Mode Classification', pp. 1703–1725. doi: 10.3390/life5041703.

Fawell, S. *et al.* (1994) 'Tat-mediated delivery of heterologous proteins into cells.', *Proceedings of the National Academy of Sciences of the United States of America*, 91(2), pp. 664–668. doi: 10.1073/pnas.91.2.664.

Fernandez, D. I. *et al.* (2012) 'The antimicrobial peptide aurein 1.2 disrupts model membranes via the carpet mechanism', *Physical Chemistry Chemical Physics*, 14(45), pp. 15739–15751. doi: 10.1039/c2cp43099a.

Ferreira, F. *et al.* (2016) 'Biomaterials One-step synthesis of high-density peptide-conjugated gold nanoparticles with antimicrobial ef fi cacy in a systemic infection model', 85. doi: 10.1016/j.biomaterials.2016.01.051.

Fidler, D. P. and Fidler, D. P. (2016) 'Relations The Globalization of Public Health : Emerging Infectious Diseases and International', 5(1), pp. 11–51.

Flemming, H. and Wingender, J. (2010) 'The biofilm matrix', *Nature reviews. Microbiology*, 8(9), pp. 623–33. doi: 10.1038/nrmicro2415.

Gable, J. E. *et al.* (2009) 'Fluorescence and UV resonance Raman study of peptide-vesicle interactions of human cathelicidin LL-37 and its F6W and F17W mutants', *Biochemistry*, 48(47), pp. 11264–11272. doi: 10.1021/bi900996q.

Gagnon, M. G. *et al.* (2016) 'Structures of proline-rich peptides bound to the ribosome reveal a common mechanism of protein synthesis inhibition', *Nucleic Acids Research*, 44(5), pp. 2439–2450. doi: 10.1093/nar/gkw018.

Gao, Y. *et al.* (2000) 'Inhibition of ubiquitin-proteasome pathway – mediated I κ B α degradation by a naturally occurring antibacterial peptide', 106(3), pp. 439–448.

Ghosh, A. *et al.* (2014) 'Indolicidin Targets Duplex DNA : Structural and Mechanistic Insight through a Combination of Spectroscopy and Microscopy', pp. 2052–2058. doi: 10.1002/cmdc.201402215.

Giacometti, A. *et al.* (2002) 'Potential therapeutic role of cationic peptides in three experimental models of septic shock', *Antimicrobial Agents and Chemotherapy*, 46(7), pp. 2132–2136. doi: 10.1128/AAC.46.7.2132-2136.2002.

Gordon, O. *et al.* (2010) 'Silver coordination polymers for prevention of implant infection: Thiol interaction, impact on respiratory chain enzymes, and hydroxyl radical induction', *Antimicrobial Agents and Chemotherapy*, 54(10), pp. 4208–4218. doi: 10.1128/AAC.01830-09.

Gounani, Z. *et al.* (2019) 'Mesoporous silica nanoparticles carrying multiple antibiotics provide enhanced synergistic effect and improved biocompatibility', *Colloids and Surfaces B: Biointerfaces*. doi: 10.1016/j.colsurfb.2018.12.035.

Gunn, J. S. (2001) 'Bacterial modification of LPS and resistance to antimicrobial peptides', in *Journal of Endotoxin Research*. doi: 10.1179/096805101101532558.

Gupta, A. *et al.* (1999) 'Molecular basis for resistance to silver cations in Salmonella', *Nature Medicine*, 5(2), pp. 183–188. doi: 10.1038/5545.

Gupta, A. *et al.* (2017) 'Synergistic antimicrobial therapy using nanoparticles and antibiotics for the treatment of multidrug-resistant bacterial infection', *Nano Futures*, 1(1), p. 015004. doi: 10.1088/2399-1984/aa69fb.

Hajipour, M. J. *et al.* (2012) 'Antibacterial properties of nanoparticles', *Trends in Biotechnology*, pp. 499–511. doi: 10.1016/j.tibtech.2012.06.004.

Han, G. *et al.* (2009) 'Nitric oxide releasing nanoparticles are therapeutic for Staphylococcus aureus abscesses in a murine model of infection', *PLoS ONE*, 4(11), pp. 1–7. doi: 10.1371/journal.pone.0007804.

Han, J. W. *et al.* (2014) 'Oxidative stress mediated cytotoxicity of biologically synthesized silver nanoparticles in human lung epithelial adenocarcinoma cell line.', *Nanoscale research letters*, 9(1), p. 459. doi: 10.1186/1556-276X-9-459.

Hancock, R. E. W. and Sahl, H. G. (2006) 'Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies.', *Nature biotechnology*, 24(12), pp. 1551–7. doi: 10.1038/nbt1267.

Haney, E. F. *et al.* (2013) 'Biochimica et Biophysica Acta Mechanism of action of puroindoline derived tryptophan-rich antimicrobial peptides', *BBA - Biomembranes*. Elsevier B.V., 1828(8), pp. 1802–1813. doi: 10.1016/j.bbamem.2013.03.023.

Hankins, J. V *et al.* (2012) 'Amino acid addition to Vibrio cholerae LPS establishes a link

between surface remodeling in Gram-positive and Gram-negative bacteria'. doi: 10.1073/pnas.1201313109/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1201313109.

Hao, N. *et al.* (2015) 'One-step synthesis of amine-functionalized hollow mesoporous silica nanoparticles as efficient antibacterial and anticancer materials', *ACS Applied Materials and Interfaces*. doi: 10.1021/am508219g.

Haque, M. *et al.* (2018) 'Health care-associated infections – An overview', *Infection and Drug Resistance*. doi: 10.2147/IDR.S177247.

Hassan, M. *et al.* (2014) 'Antimicrobial Peptides from Prokaryotes', in *Novel Antimicrobial Agents and Strategies*, pp. 71–90. doi: 10.1002/9783527676132.ch5.

He, Y. *et al.* (2016) 'Study on the mechanism of antibacterial action of magnesium oxide nanoparticles against foodborne pathogens', *Journal of Nanobiotechnology*. BioMed Central, 14(1), p. 54. doi: 10.1186/s12951-016-0202-0.

Heinlaan, M. *et al.* (2008) 'Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria Vibrio fischeri and crustaceans Daphnia magna and Thamnocephalus platyurus', *Chemosphere*, 71(7), pp. 1308–1316. doi: 10.1016/j.chemosphere.2007.11.047.

Held, B. P. and Instruments, B. (2015) 'h i t e a p e r An Introduction to Reactive Oxygen Species Measurement of ROS in Cells', pp. 1–21.

Holban, A. M. and Andronescu, E. (2016) 'Chapter 10 – Inorganic nanoarchitectonics designed for drug delivery and anti-infective surfaces', in *Surface Chemistry of Nanobiomaterials*, pp. 301–327. doi: 10.1016/B978-0-323-42861-3.00010-8.

Hong, R. *et al.* (2012) 'Membrane Lipid Peroxidation in Copper Alloy-Mediated Contact Killing of Escherichia coli', pp. 1776–1784. doi: 10.1128/AEM.07068-11.

Hsueh, Y.-H., Tsai, P.-H. and Lin, K.-S. (2017) 'pH-Dependent Antimicrobial Properties of Copper Oxide Nanoparticles in Staphylococcus aureus', *International Journal of Molecular Sciences*, 18(4), p. 793. doi: 10.3390/ijms18040793.

Hu, Y. and Hong, X. (2017) 'Synthesis and Performance of Silver Photocatalytic Nanomaterials for Water Disinfection', in An, T., Zhao, H., and Wong, P. K. (eds) *Advances in Photocatalytic Disinfection*. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 85–127. doi: 10.1007/978-3-662-53496-0_5.

Huang, H. *et al.* (2016) 'An Evaluation of Blood Compatibility of Silver Nanoparticles', *Scientific Reports*, 6(April), p. 25518. doi: 10.1038/srep25518.

Huang, H. W. (2009) ‘Free energies of molecular bound states in lipid bilayers: Lethal concentrations of antimicrobial peptides’, *Biophysical Journal*, 96(8), pp. 3263–3272. doi: 10.1016/j.bpj.2009.01.030.

Huang, Q. *et al.* (2015) ‘Atomic force microscopy measurements of bacterial adhesion and biofilm formation onto clay-sized particles.’, *Scientific reports*. Nature Publishing Group, 5(October), p. 16857. doi: 10.1038/srep16857.

Huang, Y., Wu, C. and Aronstam, R. S. (2010) ‘Toxicity of Transition Metal Oxide Nanoparticles: Recent Insights from in vitro Studies’, pp. 4842–4859. doi: 10.3390/ma3104842.

Huang, Z. *et al.* (2008) ‘Toxicological effect of ZnO nanoparticles based on bacteria’, *Langmuir*, 24(8), pp. 4140–4144. doi: 10.1021/la7035949.

Husain, F. M. *et al.* (2016) ‘Quorum sensing and biofilm inhibition by lactonase producing Bacillus amyloliquefaciens SBF1 strain isolated from date palm rhizosphere of Saudi Arabia’, *Journal of Pure and Applied Microbiology*, 10(3), pp. 1745–1754.

Iannitelli, A. *et al.* (2011) ‘Potential antibacterial activity of carvacrol-loaded poly(DL-lactide-co-glycolide) (PLGA) nanoparticles against microbial biofilm’, *International Journal of Molecular Sciences*, 12(8), pp. 5039–5051. doi: 10.3390/ijms12085039.

Islam, M. R. *et al.* (2012) ‘Antimicrobial mechanism of lantibiotics’, *Biochemical Society Transactions*, 40(6), pp. 1528–1533. doi: 10.1042/BST20120190.

Ivask, A. *et al.* (2010) ‘Profiling of the reactive oxygen species-related ecotoxicity of CuO, ZnO, TiO2, silver and fullerene nanoparticles using a set of recombinant luminescent Escherichia coli strains: Differentiating the impact of particles and solubilised metals’, *Analytical and Bioanalytical Chemistry*, 398(2), pp. 701–716. doi: 10.1007/s00216-010-3962-7.

Ivask, A. *et al.* (2012) ‘Metal-Containing Nano-Antimicrobials: Differentiating the Impact of Solubilized Metals and Particles’, in Cioffi, N. and Rai, M. (eds) *Nano-Antimicrobials: Progress and Prospects*. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 253–290. doi: 10.1007/978-3-642-24428-5_9.

Izquierdo-Barba, I. *et al.* (2009) ‘Incorporation of antimicrobial compounds in mesoporous silica film monolith’, *Biomaterials*. doi: 10.1016/j.biomaterials.2009.07.003.

Jacob, R. (2016) ‘Lipid bilayers and their interactions with the antimicrobial peptide LL37 : a TIR Raman study’.

Jenssen, H., Hamill, P. and Hancock, R. E. W. (2006) ‘Peptide antimicrobial agents’, *Clinical*

Microbiology Reviews, pp. 491–511. doi: 10.1128/CMR.00056-05.

Jones, N. *et al.* (2008) 'Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms', *FEMS Microbiology Letters*, 279(1), pp. 71–76. doi: 10.1111/j.1574-6968.2007.01012.x.

Joo, H., Fu, C. and Otto, M. (2016) 'Bacterial Strategies of Resistance to Antimicrobial Peptides', *Phil. Trans. R. Soc. B*, 371, p. (this issue). doi: 10.1098/rstb.2015.0292.

Josephson, L., Manuel Perez, J. and Weissleder, R. (2001) 'Magnetic nanosensors for the detection of oligonucleotide sequences', *Angewandte Chemie - International Edition*, 40(17), pp. 3204–3206. doi: 10.1002/1521-3773(20010903)40:17<3204::AID-ANIE3204>3.0.CO;2-H.

Kalishwaralal, K. *et al.* (2010) 'Silver nanoparticles impede the biofilm formation by Pseudomonas aeruginosa and Staphylococcus epidermidis', *Colloids and Surfaces B: Biointerfaces*, 79(2), pp. 340–344. doi: 10.1016/j.colsurfb.2010.04.014.

Kanematsu, H. and Barry, D. M. (2015) 'Antibacterial Effect of Materials and Biofilm', in Kanematsu, H. and Barry, D. M. (eds) *Biofilm and Materials Science*. Cham: Springer International Publishing, pp. 169–174. doi: 10.1007/978-3-319-14565-5_21.

Karaman, D. Ş. *et al.* (2017) 'Current Approaches for Exploration of Nanoparticles as Antibacterial Agents', *Antibacterial Agents*. doi: 10.5772/68138.

Kasemets, K. *et al.* (2009) 'Toxicity of nanoparticles of ZnO, CuO and TiO2 to yeast Saccharomyces cerevisiae', *Toxicology in Vitro*, 23(6), pp. 1116–1122. doi: 10.1016/j.tiv.2009.05.015.

Katva, S. *et al.* (2018) 'Antibacterial Synergy of Silver Nanoparticles with Gentamicin and Chloramphenicol against Enterococcus faecalis.', *Pharmacognosy magazine*, 13(Suppl 4), pp. S828–S833. doi: 10.4103/pm.pm_120_17.

Khan, S. *et al.* (2017) 'Photoinactivation of multidrug resistant bacteria by monomeric methylene blue conjugated gold nanoparticles', *Journal of Photochemistry and Photobiology B: Biology*, 174, pp. 150–161. doi: 10.1016/j.jphotobiol.2017.07.011.

Khan, S. A. *et al.* (2011) 'Bio-conjugated popcorn shaped gold nanoparticles for targeted photothermal killing of multiple drug resistant Salmonella DT104', *Journal of Materials Chemistry*, 21(Atcc 700408), p. 17705. doi: 10.1039/c1jm13320a.

Khan, S. N. and Khan, A. U. (2016) 'Breaking the spell: Combating multidrug resistant "superbugs"', *Frontiers in Microbiology*. doi: 10.3389/fmicb.2016.00174.

Khlebtsov, N. and Dykman, L. (2011) 'Biodistribution and toxicity of engineered gold nanoparticles: A review of in vitro and in vivo studies', *Chemical Society Reviews*, pp. 1647–1671. doi: 10.1039/c0cs00018c.

Khurana, C. and Chudasama, B. (2018) 'Nanoantibiotics: strategic assets in the fight against drug-resistant superbugs', *International journal of nanomedicine*, 13, pp. 3–6. doi: 10.2147/IJN.S124698.

Kirisits, M. J. (2015) 'Mechanistic lessons learned from studies of planktonic bacteria with metallic nanomaterials : implications for interactions between nanomaterials and biofilm bacteria', 6(July), pp. 1–8. doi: 10.3389/fmicb.2015.00677.

Kjos, M. *et al.* (2014) 'Sensitivity to the two-peptide bacteriocin lactococcin G is dependent on UppP, an enzyme involved in cell-wall synthesis', *Molecular Microbiology*, 92(6), pp. 1177–1187. doi: 10.1111/mmi.12632.

Kooi, C. and Sokol, P. A. (2009) 'Burkholderia cenocepacia zinc metalloproteases influence resistance to antimicrobial peptides', *Microbiology*, 155(9), pp. 2818–2825. doi: 10.1099/mic.0.028969-0.

Kruk, T. *et al.* (2015) 'Synthesis and antimicrobial activity of monodisperse copper nanoparticles', *Colloids and Surfaces B: Biointerfaces*, 128, pp. 17–22. doi: 10.1016/j.colsurfb.2015.02.009.

Kumar, A. *et al.* (2012) 'Microorganisms: A Versatile Model for Toxicity Assessment of Engineered Nanoparticles', in Cioffi, N. and Rai, M. (eds) *Nano-Antimicrobials: Progress and Prospects*. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 497–524. doi: 10.1007/978-3-642-24428-5_17.

Kumar, M., Curtis, A. and Hoskins, C. (2018) 'Application of nanoparticle technologies in the combat against anti-microbial resistance', *Pharmaceutics*. doi: 10.3390/pharmaceutics10010011.

Kuo, W. S. *et al.* (2009) 'Antimicrobial gold nanorods with dual-modality photodynamic inactivation and hyperthermia', *Chemical Communications*, (32), pp. 4853–4855. doi: 10.1039/b907274h.

Lai, H. Z. *et al.* (2015) 'Potent antibacterial nanoparticles for pathogenic bacteria', *ACS Applied Materials and Interfaces*, 7(3), pp. 2046–2054. doi: 10.1021/am507919m.

Lambadi, P. R. *et al.* (2015) 'Facile biofunctionalization of silver nanoparticles for enhanced antibacterial properties, endotoxin removal, and biofilm control', *International Journal of*

Nanomedicine. doi: 10.2147/IJN.S72923.

Lara, H. H. *et al.* (2010) 'Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria', *World Journal of Microbiology & Biotechnology*, 26(4), pp. 615–621. doi: 10.1007/s11274-009-0211-3.

Lee, D., Ashe, K. and Laurencin, C. (2014) 'Surface Modification of Biomaterials at the Nanoscale: Biomimetic Scaffolds for Tissue Engineering Applications', in *Nanotechnology and Regenerative Engineering*. CRC Press, pp. 191–226. doi: doi:10.1201/b17444-9.

Lee, T.-H. and Aguilar, K. N. H. and M.-I. (2016) 'Antimicrobial Peptide Structure and Mechanism of Action: A Focus on the Role of Membrane Structure', *Current Topics in Medicinal Chemistry*, pp. 25–39. doi: http://dx.doi.org/10.2174/1568026615666150703121700.

Lellouche, J. *et al.* (2012) 'Antibiofilm surface functionalization of catheters by magnesium fluoride nanoparticles', *International Journal of Nanomedicine*, 7, pp. 1175–1188. doi: 10.2147/IJN.S26770.

Lemire, J. a, Harrison, J. J. and Turner, R. J. (2013) 'Antimicrobial activity of metals: mechanisms, molecular targets and applications.', *Nature reviews. Microbiology*. Nature Publishing Group, 11(6), pp. 371–84. doi: 10.1038/nrmicro3028.

Leung, Y. H. *et al.* (2016) 'Toxicity of ZnO and TiO 2 to Escherichia coli cells', (October), pp. 1–13. doi: 10.1038/srep35243.

Li, L. li and Wang, H. (2013) 'Enzyme-coated mesoporous silica nanoparticles as efficient antibacterial agents in vivo', *Advanced Healthcare Materials*. doi: 10.1002/adhm.201300051.

Li, W.-R. *et al.* (2010) 'Antibacterial activity and mechanism of silver nanoparticles on Escherichia coli.', *Applied microbiology and biotechnology*, 85(4), pp. 1115–22. doi: 10.1007/s00253-009-2159-5.

Li, X. Z., Nikaido, H. and Williams, K. E. (1997) 'Silver-resistant mutants of Escherichia coli display active efflux of Ag+and are deficient in porins', *Journal of Bacteriology*, 179(19), pp. 6127–6132. doi: 10.1128/jb.179.19.6127-6132.1997.

Li, Y. *et al.* (2012) 'Mechanism of photogenerated reactive oxygen species and correlation with the antibacterial properties of engineered metal-oxide nanoparticles', *ACS Nano*, 6(6), pp. 5164–5173. doi: 10.1021/nn300934k.

Liu, L. *et al.* (2008) 'Biologically active core/shell nanoparticles self-assembled from

cholesterol-terminated PEG-TAT for drug delivery across the blood-brain barrier', *Biomaterials*, 29(10), pp. 1509–1517. doi: 10.1016/j.biomaterials.2007.11.014.

Liu, L. *et al.* (2013) 'The potent antimicrobial properties of cell penetrating peptide-conjugated silver nanoparticles with excellent selectivity for Gram-positive bacteria over erythrocytes', *Nanoscale*. doi: 10.1039/c3nr34254a.

Liu, P. T. *et al.* (2006) 'Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response', *Science*, 311(5768), pp. 1770–1773. doi: 10.1126/science.1123933.

Liu, Y. *et al.* (2007) 'Synthesis , Stability , and Cellular Internalization of Gold Nanoparticles Containing Mixed Peptide − Poly (ethylene glycol) Monolayers Synthesis , Stability , and Cellular Internalization of Gold Nanoparticles Containing Mixed Peptide-Poly (ethylene g', *Analytical Chemistry*, 79(6), pp. 2221–2229. doi: 10.1021/ac061578f.

Luckarift, H. R. *et al.* (2006) 'Rapid, room-temperature synthesis of antibacterial bionanocomposites of lysozyme with amorphous silica or titania', *Small*, 2(5), pp. 640–643. doi: 10.1002/smll.200500376.

Macomber, L., Elsey, S. P. and Hausinger, R. P. (2011) 'Fructose-1,6-bisphosphate aldolase (class II) is the primary site of nickel toxicity in Escherichia coli', *Molecular Microbiology*, 82(5), pp. 1291–1300. doi: 10.1111/j.1365-2958.2011.07891.x.

Macomber, L. and Imlay, J. A. (2009) 'The iron-sulfur clusters of dehydratases are primary intracellular targets of copper toxicity.', *Proceedings of the National Academy of Sciences of the United States of America*, 106(20), pp. 8344–9. doi: 10.1073/pnas.0812808106.

Mah, T. F. C. and O'Toole, G. A. (2001) 'Mechanisms of biofilm resistance to antimicrobial agents', *Trends in Microbiology*, pp. 34–39. doi: 10.1016/S0966-842X(00)01913-2.

Malanovic, N. and Lohner, K. (2016) 'Gram-positive bacterial cell envelopes: The impact on the activity of antimicrobial peptides', *Biochimica et Biophysica Acta - Biomembranes*, 1858(5), pp. 936–946. doi: 10.1016/j.bbamem.2015.11.004.

Malka, E. *et al.* (2013) 'Eradication of multi-drug resistant bacteria by a novel Zn-doped CuO nanocomposite', *Small*, 9(23), pp. 4069–4076. doi: 10.1002/smll.201301081.

Malmsten, M. (2014) 'Antimicrobial peptides.', *Upsala journal of medical sciences*, 119(2), pp. 199–204. doi: 10.3109/03009734.2014.899278.

Manke, A., Wang, L. and Rojanasakul, Y. (2013) 'Mechanisms of Nanoparticle-Induced Oxidative Stress and Toxicity', 2013.

Marius, S. *et al.* (2011) 'Enhanced antibacterial effect of silver nanoparticles obtained by electrochemical synthesis in poly (amide-hydroxyurethane) media', pp. 789–796. doi: 10.1007/s10856-011-4281-z.

Markowska, K., Grudniak, A. M. and Wolska, K. I. (2013) 'Silver nanoparticles as an alternative strategy against bacterial biofilms', *Acta Biochimica Polonica*, pp. 523–530.

Marshall, S. H. and Arenas, G. (2003) 'Antimicrobial peptides: A natural alternative to chemical antibiotics andapotential for applied biotechnology', *Electronic Journal of Biotechnology*, pp. 96–109. doi: 10.2225/vol6-issue3-fulltext-1.

Martínez-Carmona, M., Gun'ko, Y. K. and Vallet-Regí, M. (2018) 'Mesoporous silica materials as drug delivery: "the nightmare" of bacterial infection', *Pharmaceutics*. doi: 10.3390/pharmaceutics10040279.

McShan, D. *et al.* (2015) 'Synergistic Antibacterial Effect of Silver Nanoparticles Combined with Ineffective Antibiotics on Drug Resistant Salmonella typhimurium DT104', *Journal of Environmental Science and Health, Part C*, 33(3), pp. 369–384. doi: 10.1080/10590501.2015.1055165.

Meghana, S. *et al.* (2015) 'Understanding the pathway of antibacterial activity of copper oxide nanoparticles', *RSC Adv.*, 5(16), pp. 12293–12299. doi: 10.1039/C4RA12163E.

Miki, T., Holsts, O. and Hardt, W. D. (2012) 'The bactericidal activity of the C-type lectin regIII?? against gram-negative bacteria involves binding to lipid A', *Journal of Biological Chemistry*, 287(41), pp. 34844–34855. doi: 10.1074/jbc.M112.399998.

Millenbaugh, N. J. *et al.* (2015) 'Photothermal killing of Staphylococcus aureus using antibody-targeted gold nanoparticles', *International Journal of Nanomedicine*, 10, pp. 1953–1960. doi: 10.2147/IJN.S76150.

Mohamed, M. M. *et al.* (2017) 'Antibacterial effect of gold nanoparticles against Corynebacterium pseudotuberculosis', *International Journal of Veterinary Science and Medicine*, 5(1), pp. 23–29. doi: 10.1016/j.ijvsm.2017.02.003.

Mohankandhasamy *et al.* (2017) 'Development of gold nanoparticles coated with silica containing the antibiofilm drug cinnamaldehyde and their effects on pathogenic bacteria', *International Journal of Nanomedicine*, 12, pp. 2813–2828. doi: 10.2147/IJN.S132784.

Moongraksathum, B. and Chen, Y. W. (2018) 'Anatase TiO2co-doped with silver and ceria for antibacterial application', *Catalysis Today*, 310, pp. 68–74. doi: 10.1016/j.cattod.2017.05.087.

Mossallam, S. F., Amer, E. I. and Diab, R. G. (2014) 'Potentiated anti-microsporidial activity of Lactobacillus acidophilus CH1 bacteriocin using gold nanoparticles', *Experimental Parasitology*, 144(1), pp. 14–21. doi: 10.1016/j.exppara.2014.06.002.

Mukherjee, S. *et al.* (2014) 'Antibacterial membrane attack by a pore-forming intestinal C-type lectin', *Nature*, 505(7481), pp. 103–107. doi: 10.1038/nature12729.

Mukhopadhyay, J. *et al.* (2004) 'Antibacterial peptide Microcin J25 inhibits transcription by binding within and obstructing the RNA polymerase secondary channel', *Molecular Cell*, 14(6), pp. 739–751. doi: 10.1016/j.molcel.2004.06.010.

Muthukrishnan, A. M. (2015) 'Green Synthesis of Copper-Chitosan Nanoparticles and Study of its Antibacterial Activity', *Journal of Nanomedicine & Nanotechnology*, 06(01). doi: 10.4172/2157-7439.1000251.

Nair, M. G. *et al.* (2011) 'Structural, optical, photo catalytic and antibacterial activity of ZnO and Co doped ZnO nanoparticles', *Materials Letters*. Elsevier B.V., 65(12), pp. 1797–1800. doi: 10.1016/j.matlet.2011.03.079.

Nair, Shantikumar *et al.* (2009) 'Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells', in *Journal of Materials Science: Materials in Medicine*. doi: 10.1007/s10856-008-3548-5.

Nativo, P., Prior, I. A. and Brust, M. (2008) 'Uptake and intracellular fate of surface-modified gold nanoparticles', *ACS Nano*, 2(8), pp. 1639–1644. doi: 10.1021/nn800330a.

Nies, D. H. (2003) 'Efflux-mediated heavy metal resistance in prokaryotes', *FEMS Microbiology Reviews*, pp. 313–339. doi: 10.1016/S0168-6445(03)00048-2.

Niño-Martínez, N. *et al.* (2019) 'Molecular Mechanisms of Bacterial Resistance to Metal and Metal Oxide Nanoparticles', *International journal of molecular sciences*, 20(11). doi: 10.3390/ijms20112808.

Nocek, B. *et al.* (2012) 'Structural and Functional Characterization of Microcin C Resistance Peptidase MccF from Bacillus anthracis', *Journal of Molecular Biology*. Elsevier Ltd, 420(4–5), pp. 366–383. doi: 10.1016/j.jmb.2012.04.011.

Nowack, B. (2008) 'Exposure Modeling of Engineered Nanoparticles in the Environment', 41(0), pp. 4447–4453.

O'Neill, J. (2014) 'Antimicrobial Resistance : Tackling a crisis for the health and wealth of nations', *Review on Antimicrobial Resistance*, (December), pp. 1–16.

Ogunseitan, O. A., Yang, S. and Ericson, J. (2000) ‘Microbial ??-aminolevulinate dehydratase as a biosensor of lead bioavailability in contaminated environments’, *Soil Biology and Biochemistry*, 32(13), pp. 1899–1906. doi: 10.1016/S0038-0717(00)00164-4.

Ojha, S., Sett, A. and Bora, U. (2017) ‘Green synthesis of silver nanoparticles by Ricinus communis var. carmencita leaf extract and its antibacterial study’, *Advances in Natural Sciences: Nanoscience and Nanotechnology*. IOP Publishing, 8(3). doi: 10.1088/2043-6254/aa724b.

Otari, S. V. *et al.* (2013) ‘A novel microbial synthesis of catalytically active Ag-alginate biohydrogel and its antimicrobial activity’, *Dalton Transactions*, 42(27), pp. 9966–9975. doi: 10.1039/c3dt51093j.

Oudhoff, M. J. *et al.* (2008) ‘Histatins are the major wound-closure stimulating factors in human saliva as identified in a cell culture assay.’, *The Federation of American Societies for Experimental Biology*, 22(11), pp. 3805–3812. doi: 10.1096/fj.08-112003.

Padmavathy, N. and Vijayaraghavan, R. (2008) ‘Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study’, *Science and Technology of Advanced Materials*, 9(3), p. 035004. doi: 10.1088/1468-6996/9/3/035004.

Pal, I. *et al.* (2016) ‘Enhanced stability and activity of an antimicrobial peptide in conjugation with silver nanoparticle’, *Journal of Colloid and Interface Science*. doi: 10.1016/j.jcis.2016.08.043.

Pal, S. *et al.* (2014) ‘A multifunctional composite of an antibacterial higher-valent silver metallopharmaceutical and a potent wound healing polypeptide: a combined killing and healing approach to wound care’, *New J. Chem. New J. Chem*, 38(38), pp. 3889–3898. doi: 10.1039/c4nj00160e.

Pal, S., Tak, Y. K. and Song, J. M. (2015) ‘Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli’, *Journal of Biological Chemistry*, 290(42), pp. 1712–1720. doi: 10.1128/AEM.02218-06.

Palza, H. (2015) ‘Antimicrobial polymers with metal nanoparticles’, *International Journal of Molecular Sciences*, pp. 2099–2116. doi: 10.3390/ijms16012099.

Panácek, A. *et al.* (2016) ‘Strong and nonspecific synergistic antibacterial efficiency of antibiotics combined with silver nanoparticles at very low concentrations showing no cytotoxic effect’, *Molecules*, 21(1). doi: 10.3390/molecules21010026.

Panáček, A. *et al.* (2018) ‘Bacterial resistance to silver nanoparticles and how to overcome it’,

Nature Nanotechnology, 13(1), pp. 65–71. doi: 10.1038/s41565-017-0013-y.

Papo, N. and Shai, Y. (2003) 'Can we predict biological activity of antimicrobial peptides from their interactions with model phospholipid membranes?', *Peptides*, pp. 1693–1703. doi: 10.1016/j.peptides.2003.09.013.

Pappalardo, J. S. *et al.* (2009) 'Improved transfection of spleen-derived antigen-presenting cells in culture using TATp-liposomes', *Journal of Controlled Release*, 134(1), pp. 41–46. doi: 10.1016/j.jconrel.2008.11.006.

Park, C. B., Kim, H. S. and Kim, S. C. (1998) 'Mechanism of action of the antimicrobial peptide buforin II: Buforin II kills microorganisms by penetrating the cell membrane and inhibiting cellular functions', *Biochemical and Biophysical Research Communications*, 244(1), pp. 253–257. doi: 10.1006/bbrc.1998.8159.

Payne, J. N. *et al.* (2016) 'Novel synthesis of kanamycin conjugated gold nanoparticles with potent antibacterial activity', *Frontiers in Microbiology*, 7(MAY), pp. 1–10. doi: 10.3389/fmicb.2016.00607.

Pazos, E. *et al.* (2016) 'Nucleation and Growth of Ordered Arrays of Silver Nanoparticles on Peptide Nano fi bers: Hybrid Nanostructures with Antimicrobial Properties', pp. 4–7. doi: 10.1021/jacs.6b01570.

Peng, L. *et al.* (2016) 'Biomaterials Integration of antimicrobial peptides with gold nanoparticles as unique non-viral vectors for gene delivery to mesenchymal stem cells with antibacterial activity'. Elsevier Ltd, 103, pp. 137–149. doi: 10.1016/j.biomaterials.2016.06.057.

Peng, L. H. *et al.* (2016) 'Integration of antimicrobial peptides with gold nanoparticles as unique non-viral vectors for gene delivery to mesenchymal stem cells with antibacterial activity', *Biomaterials*. Elsevier Ltd, 103, pp. 137–149. doi: 10.1016/j.biomaterials.2016.06.057.

Percival, S. L., Bowler, P. G. and Dolman, J. (2007) 'Antimicrobial activity of silver-containing dressings on wound microorganisms using an in vitro biofilm model', *International Wound Journal*, 4(2). doi: 10.1111/j.1742-481X.2007.00296.x.

Peters, B. M., Shirtliff, M. E. and Jabra-Rizk, M. A. (2010) 'Antimicrobial peptides: Primeval molecules or future drugs?', *PLoS Pathogens*, 6(10). doi: 10.1371/journal.ppat.1001067.

Piddock, L. J. V (2006) 'Multidrug-resistance efflux pumps - not just for resistance.', *Nature reviews. Microbiology*, 4(8), pp. 629–636. doi: 10.1038/nrmicro1464.

Pokhrel, S. *et al.* (2009) 'Comparison of the mechanism of toxicity of binary and mixed binary

metal oxide nanoparticles based on dissolution and oxidative stress properties', *Chemie Ingenieur Technik*, 81(8), pp. 1167–1167. doi: 10.1002/cite.200950629.

Prabhu, S. and Poulose, E. K. (2012) 'Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects', *International Nano Letters*, 2(1), p. 32. doi: 10.1186/2228-5326-2-32.

Pradeepa *et al.* (2016) 'Preparation of gold nanoparticles by novel bacterial exopolysaccharide for antibiotic delivery', *Life Sciences*, 153, pp. 171–179. doi: 10.1016/j.lfs.2016.04.022.

Premanathan, M. *et al.* (2011) 'Selective toxicity of ZnO nanoparticles toward Gram-positive bacteria and cancer cells by apoptosis through lipid peroxidation', *Nanomedicine: Nanotechnology, Biology, and Medicine*, 7(2), pp. 184–192. doi: 10.1016/j.nano.2010.10.001.

Qayyum, S. *et al.* (2016) 'Nanoparticles vs. biofilms: a battle against another paradigm of antibiotic resistance', *Med. Chem. Commun.*, 7(8), pp. 1479–1498. doi: 10.1039/C6MD00124F.

Rahim, K. A. A. and Mohamed, A. M. A. (2015) 'Bactericidal and antibiotic synergistic effect of nanosilver against methicillin-resistant staphylococcus aureus', *Jundishapur Journal of Microbiology*, 8(11), pp. 1–6. doi: 10.5812/jjm.25867.

Rai, M., Yadav, A. and Gade, A. (2009) 'Silver nanoparticles as a new generation of antimicrobials', *Biotechnology Advances*. Elsevier Inc., 27(1), pp. 76–83. doi: 10.1016/j.biotechadv.2008.09.002.

Rainnie, D. J. and Bragg, P. (1974) 'The effect of silver ions on the respiratory chain of Escherichia coli', *Can. J. Microbiol.*, 20, pp. 883–889. doi: 10.1139/m74-135.

Rajakumar, G. *et al.* (2017) 'Evaluation of anti-cholinesterase, antibacterial and cytotoxic activities of green synthesized silver nanoparticles using from Millettia pinnata flower extract', *Microbial Pathogenesis*, 103, pp. 123–128. doi: 10.1016/j.micpath.2016.12.019.

Rajchakit, U. and Sarojini, V. (2017a) 'Recent Developments in Antimicrobial-Peptide-Conjugated Gold Nanoparticles', *Bioconjugate Chemistry*, pp. 2673–2686. doi: 10.1021/acs.bioconjchem.7b00368.

Rajchakit, U. and Sarojini, V. (2017b) 'Recent Developments in Antimicrobial Peptide Conjugated Gold Nanoparticles', *Bioconjugate Chemistry*. American Chemical Society. doi: 10.1021/acs.bioconjchem.7b00368.

Ramasamy, M., Lee, J. H. and Lee, J. (2017) 'Direct one-pot synthesis of cinnamaldehyde immobilized on gold nanoparticles and their antibiofilm properties', *Colloids and Surfaces B:*

Biointerfaces, 160, pp. 639–648. doi: 10.1016/j.colsurfb.2017.10.018.

Rebuffat, S. (2012) 'Microcins in action : amazing defence strategies of Enterobacteria', 40, pp. 1456–1462. doi: 10.1042/BST20120183.

Reddy, K. M. *et al.* (2007) 'Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems', *Applied Physics Letters*, 90(21). doi: 10.1063/1.2742324.

Reimhult, E. (2013) 'Imaging and Triggered Release through Design of Ultrastable Core?Shell Iron Oxide Nanoparticles', in *Bioengineered Nanomaterials*. CRC Press, pp. 177–198. doi: doi:10.1201/b15403-9.

Ren, G. *et al.* (2009) 'Characterisation of copper oxide nanoparticles for antimicrobial applications', *International Journal of Antimicrobial Agents*, 33(6), pp. 587–590. doi: 10.1016/j.ijantimicag.2008.12.004.

Richards, R. *et al.* (2000) 'Consolidation of Metal Oxide Nanocrystals . Reactive Pellets with Controllable Pore Structure That Represent a New Family of Porous , Inorganic Materials', (11), pp. 4921–4925. doi: 10.1021/ja994383g.

Rizzo, L. *et al.* (2013) 'Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review', *Science of the Total Environment*. Elsevier B.V., 447, pp. 345–360. doi: 10.1016/j.scitotenv.2013.01.032.

Rozek, A., Friedrich, C. L. and Hancock, R. E. W. (2000) 'Structure of the bovine antimicrobial peptide indolicidin bound to dodecylphosphocholine and sodium dodecyl sulfate micelles', *Biochemistry*, 39(51), pp. 15765–15774. doi: 10.1021/bi000714m.

Ruparelia, J. P. *et al.* (2008) 'Strain specificity in antimicrobial activity of silver and copper nanoparticles', *Acta Biomaterialia*, 4(3), pp. 707–716. doi: 10.1016/j.actbio.2007.11.006.

S. Roy, A. *et al.* (2010) 'Effect of Nano - Titanium Dioxide with Different Antibiotics against Methicillin-Resistant Staphylococcus Aureus', *Journal of Biomaterials and Nanobiotechnology*, 01(01), pp. 37–41. doi: 10.4236/jbnb.2010.11005.

Saeb, A. T. M. *et al.* (2014) 'Production of Silver Nanoparticles with Strong and Stable Antimicrobial Activity against Highly Pathogenic and Multidrug Resistant Bacteria', 2014.

Saito, T. *et al.* (1992) 'Mode of photocatalytic bactericidal action of powdered semiconductor TiO2 on mutans streptococci', *Journal of Photochemistry and Photobiology, B: Biology*, 14(4), pp. 369–379. doi: 10.1016/1011-1344(92)85115-B.

Salouti, M. and Ahangari, A. (2014) 'Nanoparticle based Drug Delivery Systems for Treatment

of Infectious Diseases, Application of Nanotechnology in Drug Delivery', *Application of Nanotechnology in Drug Delivery*, pp. 1–20. doi: 10.5772/58423.

Dos Santos Ramos, M. A. *et al.* (2018) 'Nanotechnology-based drug delivery systems for control of microbial biofilms: A review', *International Journal of Nanomedicine*, 13, pp. 1179–1213. doi: 10.2147/IJN.S146195.

Sapsford, K. E. *et al.* (2013) 'Functionalizing nanoparticles with biological molecules: Developing chemistries that facilitate nanotechnology', *Chemical Reviews*, pp. 1904–2074. doi: 10.1021/cr300143v.

Schauber, J. *et al.* (2007) 'Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D-dependent mechanism', *Journal of Clinical Investigation*, 117(3), pp. 803–811. doi: 10.1172/JCI30142.

Schembri, M. A., Kjaergaard, K. and Klemm, P. (2003) 'Global gene expression in Escherichia coli biofilms', *Molecular Microbiology*, 48, pp. 253–267.

Schooling, S. R. and Beveridge, T. J. (2006) 'Membrane vesicles: An overlooked component of the matrices of biofilms', *Journal of Bacteriology*, 188(16), pp. 5945–5957. doi: 10.1128/JB.00257-06.

Schwarze, S. R. *et al.* (1999) 'In vivo protein transduction: delivery of a biologically active protein into the mouse.', *Science*, 285(5433), pp. 1569–1572. doi: 10.1126/science.285.5433.1569.

Scinicariello, F. *et al.* (2007) 'Lead and ??-aminolevulinic acid dehydratase polymorphism: Where does it lead? A meta-analysis', *Environmental Health Perspectives*, 115(1), pp. 35–41. doi: 10.1289/ehp.9448.

Secinti, K. D. *et al.* (2011) 'Nanoparticle silver ion coatings inhibit biofilm formation on titanium implants', *Journal of Clinical Neuroscience*, 18(3), pp. 391–395. doi: 10.1016/j.jocn.2010.06.022.

Seil, J. T. and Webster, T. J. (2012) 'Antimicrobial applications of nanotechnology: Methods and literature', *International Journal of Nanomedicine*, 7, pp. 2767–2781. doi: 10.2147/IJN.S24805.

Şen Karaman, D. *et al.* (2016) 'Shape engineering boosts antibacterial activity of chitosan coated mesoporous silica nanoparticle doped with silver: A mechanistic investigation', *Journal of Materials Chemistry B*. doi: 10.1039/c5tb02526e.

Şen Karaman, D., Manner, S. and Rosenholm, J. M. (2018) 'Mesoporous silica nanoparticles as

diagnostic and therapeutic tools: how can they combat bacterial infection?', *Therapeutic Delivery*. doi: 10.4155/tde-2017-0111.

Shafer, W. M. *et al.* (1998) 'Modulation of Neisseria gonorrhoeae susceptibility to vertebrate antibacterial peptides due to a member of the resistance/nodulation/division efflux pump family.', *Proceedings of the National Academy of Sciences of the United States of America*, 95(4), pp. 1829–1833. doi: 10.1073/pnas.95.4.1829.

Shah, R. R. *et al.* (2008) 'The antibacterial effects of biphasic brookite-anatase titanium dioxide nanoparticles on multiple-drug-resistant Staphylococcus aureus', in *Journal of Biomedical Nanotechnology*, pp. 339–348. doi: 10.1166/jbn.2008.324.

Shah, S. N. A. *et al.* (2017) 'Hazardous Effects of Titanium Dioxide Nanoparticles in Ecosystem', *Bioinorganic Chemistry and Applications*. doi: 10.1155/2017/4101735.

Shai, Y. (2002) 'Mode of action of membrane active antimicrobial peptides', *Biopolymers - Peptide Science Section*, pp. 236–248. doi: 10.1002/bip.10260.

Shaikh, S. *et al.* (2017) 'Synthesis and Characterization of Cefotaxime Conjugated Gold Nanoparticles and Their Use to Target Drug-Resistant CTX-M-Producing Bacterial Pathogens', *Journal of Cellular Biochemistry*, 118(9), pp. 2802–2808. doi: 10.1002/jcb.25929.

Shaker, M. A. and Shaaban, M. I. (2017) 'Formulation of carbapenems loaded gold nanoparticles to combat multi-antibiotic bacterial resistance: In vitro antibacterial study', *International Journal of Pharmaceutics*, 525(1), pp. 71–84. doi: 10.1016/j.ijpharm.2017.04.019.

Shanmugam, S. and Gopal, B. (2014) 'Copper substituted hydroxyapatite and fluorapatite: Synthesis, characterization and antimicrobial properties', *Ceramics International*, 40(10), pp. 15655–15662. doi: 10.1016/j.ceramint.2014.07.086.

Sharma, R., Kwon, S. and Chen, C. J. (2013) 'Silver Nanoparticles', in *Bioengineered Nanomaterials*. CRC Press, pp. 241–268. doi: doi:10.1201/b15403-12.

Sharma, T. K. *et al.* (2012) 'Interaction of Bacteriocin-Capped Silver Nanoparticles with Food Pathogens and Their Antibacterial Effect', *International Journal of Green Nanotechnology*, 4(2), pp. 93–110. doi: 10.1080/19430892.2012.678757.

Shimanovich, U. and Gedanken, A. (2016) 'Nanotechnology solutions to restore antibiotic activity', *J. Mater. Chem. B*. Royal Society of Chemistry, 4, pp. 824–833. doi: 10.1039/C5TB01527H.

Siddiqi, K. S., Husen, A. and Rao, R. A. K. (2018) 'A review on biosynthesis of silver

nanoparticles and their biocidal properties', *Journal of Nanobiotechnology*. doi: 10.1186/s12951-018-0334-5.

Singh, S. *et al.* (2014) 'Effects of PEGylation on membrane and lipopolysaccharide interactions of host defense peptides', *Biomacromolecules*, 15(4), pp. 1337–1345. doi: 10.1021/bm401884e.

Sitaram, N. and Nagaraj, R. (1999) 'Interaction of antimicrobial peptides with biological and model membranes: Structural and charge requirements for activity', *Biochimica et Biophysica Acta - Biomembranes*, pp. 29–54. doi: 10.1016/S0005-2736(99)00199-6.

Skalska, J. and Strużyńska, L. (2015) 'Toxic effects of silver nanoparticles in mammals – does a risk of neurotoxicity exist?', *Folia Neuropathologica*. doi: 10.5114/fn.2015.56543.

Skerlavaj, B., Romeo, D. and Gennaro, R. (1990) 'Rapid membrane permeabilization and inhibition of vital functions of gram-negative bacteria by bactenecins', *Infection and Immunity*, 58(11), pp. 3724–3730.

Slavin, Y. N. *et al.* (2017) 'Metal nanoparticles: Understanding the mechanisms behind antibacterial activity', *Journal of Nanobiotechnology*. doi: 10.1186/s12951-017-0308-z.

Soehnlein, O. *et al.* (2008) 'Neutrophil primary granule proteins HBP and HNP1-3 boost bacterial phagocytosis by human and murine macrophages', *Journal of Clinical Investigation*, 118(10), pp. 3491–3502. doi: 10.1172/JCI35740.

Sondi, I. and Salopek-Sondi, B. (2004) 'Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria', *J Colloid Interface Sci*, 275(1), pp. 177–182. doi: 10.1016/j.jcis.2004.02.012.

Sperandio, B. *et al.* (2008) 'Virulent Shigella flexneri subverts the host innate immune response through manipulation of antimicrobial peptide gene expression.', *The Journal of experimental medicine*, 205(5), pp. 1121–32. doi: 10.1084/jem.20071698.

Stevens, K. N. J. *et al.* (2009) 'The relationship between the antimicrobial effect of catheter coatings containing silver nanoparticles and the coagulation of contacting blood', *Biomaterials*, 30(22), pp. 3682–3690. doi: 10.1016/j.biomaterials.2009.03.054.

Stroh, M. *et al.* (2005) 'Quantum dots spectrally distinguish multiple species within the tumor milieu in vivo.', *Nature medicine*, 11(6), pp. 678–82. doi: 10.1038/nm1247.

Su, H. L. *et al.* (2011) 'Novel nanohybrids of silver particles on clay platelets for inhibiting silver-resistant bacteria', *PLoS ONE*, 6(6). doi: 10.1371/journal.pone.0021125.

Sutariya, V. *et al.* (2014) 'Introduction?Biointeractions of Nanomaterials: Challenges and

Solutions', in *Biointeractions of Nanomaterials*. CRC Press, pp. 1–48. doi: doi:10.1201/b17191-2.

Tee, J. K. *et al.* (2016) 'Oxidative stress by inorganic nanoparticles', *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, pp. 414–438. doi: 10.1002/wnan.1374.

Thannickal, V. J. and Fanburg, B. L. (2000) 'Reactive oxygen species in cell signaling.', *American journal of physiology. Lung cellular and molecular physiology*, 279(6), pp. L1005-28. doi: 10.1164/rccm.2206007.

Thapa, R. *et al.* (2017) 'Enzyme-mediated formulation of stable elliptical silver nanoparticles tested against clinical pathogens and MDR bacteria and development of antimicrobial surgical thread', *Annals of Clinical Microbiology and Antimicrobials*, 16(1). doi: 10.1186/s12941-017-0216-y.

Thirumurugan, A., Ramachandran, S. and Shiamala Gowri, A. (2013) 'Combined effect of bacteriocin with gold nanoparticles against food spoiling bacteria - an approach for food packaging material preparation', *International Food Research Journal*.

Thiwawong, T., Onlaor, K. and Tunhoo, B. (2013) 'A Humidity Sensor Based on Silver Nanoparticles Thin Film Prepared by Electrostatic Spray Deposition Process', 2013.

Tian, Y. *et al.* (2014) 'Facile, one-pot synthesis, and antibacterial activity of mesoporous silica nanoparticles decorated with well-dispersed silver nanoparticles', *ACS Applied Materials and Interfaces*. doi: 10.1021/am5026424.

Tillotson, G. S. and Theriault, N. (2013) 'New and alternative approaches to tackling antibiotic resistance.', *F1000prime reports*, 5(December), p. 51. doi: 10.12703/P5-51.

Travan, A. *et al.* (2007) 'Silver Nanocomposites and Their Biomedical Applications', in *Nanotechnologies for the Life Sciences*. Wiley-VCH Verlag GmbH & Co. KGaA. doi: 10.1002/9783527610419.ntls0219.

Tseng, B. S. *et al.* (2013) 'The extracellular matrix protects Pseudomonas aeruginosa biofilms by limiting the penetration of tobramycin', *Environmental Microbiology*, 15(10), pp. 2865–2878. doi: 10.1111/1462-2920.12155.

Tsourkas, A. *et al.* (2005) 'In vivo imaging of activated endothelium using an anti-VCAM-1 magnetooptical probe', *Bioconjugate Chemistry*, 16(3), pp. 576–581. doi: 10.1021/bc050002e.

Turner, J. J. *et al.* (2007) 'RNA targeting with peptide conjugates of oligonucleotides, siRNA and PNA', *Blood Cells, Molecules, and Diseases*, 38(1), pp. 1–7. doi:

10.1016/j.bcmd.2006.10.003.

Ubini, B. I. C. E. F. (2003) 'Serial Review : Role of Reactive Oxygen and Nitrogen Species (ROS / RNS) in Lung Injury and Diseases Guest Editor : Brook T . Mossman (RNS) GENERATION BY SILICA IN INFLAMMATION AND FIBROSIS', 34(12), pp. 1507–1516. doi: 10.1016/S0891-5849(03)00149-7.

VanderVen, B. C. *et al.* (2015) 'Novel Inhibitors of Cholesterol Degradation in Mycobacterium tuberculosis Reveal How the Bacterium's Metabolism Is Constrained by the Intracellular Environment', *PLoS Pathogens*. doi: 10.1371/journal.ppat.1004679.

Vedovato, N. and Rispoli, G. (2007) 'A novel technique to study pore-forming peptides in a natural membrane', *European Biophysics Journal*, 36(7), pp. 771–778. doi: 10.1007/s00249-007-0152-4.

Veiseh, O. *et al.* (2009) 'Inhibition of tumor-cell invasion with chlorotoxin-bound superparamagnetic nanoparticles', *Small*, 5(2), pp. 256–264. doi: 10.1002/smll.200800646.

Veldhuizen, E. J. A. *et al.* (2014) 'Antimicrobial and immunomodulatory activities of PR-39 derived peptides', *PLoS ONE*, 9(4). doi: 10.1371/journal.pone.0095939.

Wadhwani, P. *et al.* (2017) 'Antibiotic gold: Tethering of antimicrobial peptides to gold nanoparticles maintains conformational flexibility of peptides and improves trypsin susceptibility', *Biomaterials Science*. doi: 10.1039/c7bm00069c.

Wang, G., Li, X. and Wang, Z. (2009) 'APD2: The updated antimicrobial peptide database and its application in peptide design', *Nucleic Acids Research*, 37(SUPPL. 1). doi: 10.1093/nar/gkn823.

Wang, L., Hu, C. and Shao, L. (2017) 'The antimicrobial activity of nanoparticles: Present situation and prospects for the future', *International Journal of Nanomedicine*, pp. 1227–1249. doi: 10.2147/IJN.S121956.

Wang, S. *et al.* (2010) 'Rapid colorimetric identification and targeted photothermal lysis of Salmonella bacteria by using bioconjugated oval-shaped gold nanoparticles', *Chemistry - A European Journal*, 16(19), pp. 5600–5606. doi: 10.1002/chem.201000176.

Wang, S. *et al.* (2016) 'Antimicrobial peptides as potential alternatives to antibiotics in food animal industry', *International Journal of Molecular Sciences*. doi: 10.3390/ijms17050603.

Wang, Z., Shen, Y. and Haapasalo, M. (2014) 'Dental materials with antibiofilm properties', *Dental Materials*. doi: 10.1016/j.dental.2013.12.001.

Wang, Z. and Wang, G. (2004) ‘APD: the Antimicrobial Peptide Database.’, *Nucleic acids research*, 32(Database issue), pp. D590-2. doi: 10.1093/nar/gkh025.

Wijesiri, N. *et al.* (2017) ‘Photodynamic Inactivation of Multidrug-Resistant Staphylococcus aureus Using Hybrid Photosensitizers Based on Amphiphilic Block Copolymer-Functionalized Gold Nanoparticles’, pp. 2–7. doi: 10.1021/acsomega.7b00738.

Wilmes, M. *et al.* (2014) ‘Killing of staphylococci by θ-defensins involves membrane impairment and activation of autolytic enzymes’, *Antibiotics*, pp. 617–631. doi: 10.3390/antibiotics3040617.

Wingender, J., Neu, T. R. and Flemming, H.-C. (1999) ‘What are bacterial extracellular polymeric substances?’, in *Microbial Extracellular Polymeric Substances*, pp. 1–19. doi: 10.1007/978-3-642-60147-7_1.

Wu, P., Imlay, J. A. and Shang, J. K. (2010) ‘Mechanism of Escherichia coli inactivation on palladium-modified nitrogen-doped titanium dioxide’, *Biomaterials*, 31(29), pp. 7526–7533. doi: 10.1016/j.biomaterials.2010.06.032.

Xie, Y. *et al.* (2011) ‘Antibacterial activity and mechanism of action of zinc oxide nanoparticles against Campylobacter jejuni’, *Applied and Environmental Microbiology*, 77(7), pp. 2325–2331. doi: 10.1128/AEM.02149-10.

Xu, F. F. and Imlay, J. A. (2012) ‘Silver(I), mercury(II), cadmium(II), and zinc(II) target exposed enzymic iron-sulfur clusters when they toxify Escherichia coli’, *Applied and Environmental Microbiology*, 78(10), pp. 3614–3621. doi: 10.1128/AEM.07368-11.

Xue, H. Y., Liu, S. and Wong, H. L. (2014) ‘Nanotoxicity: a key obstacle to clinical translation of siRNA-based nanomedicine.’, *Nanomedicine (London, England)*, 9(2), pp. 295–312. doi: 10.2217/nnm.13.204.

Yadav, H. M. *et al.* (2016) ‘Enhanced photocatalytic inactivation of bacteria on Fe-containing TiO2 nanoparticles under fluorescent light’, *Journal of Materials Science: Materials in Medicine*, 27(3), pp. 1–9. doi: 10.1007/s10856-016-5675-8.

Yaganza, E. S. *et al.* (2004) ‘Ultrastructural alterations of Erwinia carotovora subsp. atroseptica caused by treatment with aluminum chloride and sodium metabisulfite’, *Applied and Environmental Microbiology*, 70(11), pp. 6800–6808. doi: 10.1128/AEM.70.11.6800-6808.2004.

Yamanaka, M., Hara, K. and Kudo, J. (2005) ‘Bactericidal actions of a silver ion solution on Escherichia coli, studied by energy-filtering transmission electron microscopy and proteomic

analysis', *Applied and Environmental Microbiology*, 71(11), pp. 7589–7593. doi: 10.1128/AEM.71.11.7589-7593.2005.

Yang, H. *et al.* (2016) 'Endosomal pH modulation by peptide-gold nanoparticle hybrids enables potent anti-inflammatory activity in phagocytic immune cells', *Biomaterials*, 111, pp. 90–102. doi: 10.1016/j.biomaterials.2016.09.032.

Yang, L. *et al.* (2001) 'Barrel-Stave Model or Toroidal Model? A Case Study on Melittin Pores', *Biophysical Journal*, 81(3), pp. 1475–1485. doi: 10.1016/S0006-3495(01)75802-X.

Yeaman, M. R. and Yount, N. Y. (2003) 'Mechanisms of antimicrobial peptide action and resistance.', *Pharmacological reviews*, 55(1), pp. 27–55. doi: 10.1124/pr.55.1.2.

Yemmireddy, V. K. and Hung, Y. C. (2017) 'Using Photocatalyst Metal Oxides as Antimicrobial Surface Coatings to Ensure Food Safety—Opportunities and Challenges', *Comprehensive Reviews in Food Science and Food Safety*. doi: 10.1111/1541-4337.12267.

Yen, H.-J., Hsu, S.-H. and Tsai, C.-L. (2009) 'Cytotoxicity and immunological response of gold and silver nanoparticles of different sizes.', *Small*, 5(13), pp. 1553–1561. doi: 10.1002/smll.200900126.

Yeom, J. H. *et al.* (2016) 'Gold nanoparticle-DNA aptamer conjugate-assisted delivery of antimicrobial peptide effectively eliminates intracellular Salmonella enterica serovar Typhimurium', *Biomaterials*, 104, pp. 43–51. doi: 10.1016/j.biomaterials.2016.07.009.

Yount, N. Y. *et al.* (2006) 'Advances in antimicrobial peptide immunobiology', *Biopolymers - Peptide Science Section*, pp. 435–458. doi: 10.1002/bip.20543.

Yount, N. Y. and Yeaman, M. R. (2013) 'Peptide antimicrobials: Cell wall as a bacterial target', *Annals of the New York Academy of Sciences*, 1277(1), pp. 127–138. doi: 10.1111/nyas.12005.

Yu, X., Wang, Z. and Wei, G. (2017) 'nanomaterial hybrids', *Journal of Materials Chemistry B*. Royal Society of Chemistry, 5, pp. 1130–1142. doi: 10.1039/C6TB02659A.

Yuan, P. *et al.* (2018) 'Metal Nanoparticles for Diagnosis and Therapy of Bacterial Infection', *Advanced Healthcare Materials*, 1701392, p. 1701392. doi: 10.1002/adhm.201701392.

Zasloff, M. (2002) 'Antimicrobial peptides of multicellular organisms', *Nature*, 415(0028-0836 (Print)), pp. 389–395. doi: 10.1038/415389a.

Zhang, L. and Gallo, R. L. (2016) 'Antimicrobial peptides', *Current Biology*, 26(1), pp. R14–R19. doi: 10.1016/j.cub.2015.11.017.

Zhang, P. (2016) 'Ecotoxicity Analyses of Nanomaterials', in *Toxicology of Nanomaterials*.

Wiley-VCH Verlag GmbH & Co. KGaA, pp. 367–392. doi: 10.1002/9783527689125.ch15.

Zhao, Z. *et al.* (2017) 'Bacteria-Activated Theranostic Nanoprobes against Methicillin-Resistant Staphylococcus aureus Infection', *ACS Nano*, 11(5), pp. 4428–4438. doi: 10.1021/acsnano.7b00041.

Zharov, V. P. *et al.* (2006) 'Photothermal Nanotherapeutics and Nanodiagnostics for Selective Killing of Bacteria Targeted with Gold Nanoparticles', *Biophysical Journal*. Elsevier, 90(2), pp. 619–627. doi: 10.1529/biophysj.105.061895.

Zhou, G. *et al.* (2008) 'Synthesis, characterization, and antibacterial activities of a novel nanohydroxyapatite/zinc oxide complex', *Journal of Biomedical Materials Research - Part A*, 85(4), pp. 929–937. doi: 10.1002/jbm.a.31527.

***Conflict of Interest**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Credit author statement

All authors have made substantial contributions to the design of the review paper at some stage during the manuscript preparation process; AND

Drafting the work / revising it critically for important intellectual content; AND

Final approval of the version to be published; AND

Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately presented.

On behalf of the authors,

in Turku, Finland 23.03.2020

Prof. Jessica Rosenholm
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