Nanomaterials as promising alternative in the infection treatment

María Vallet-Regí[1,2]*, Blanca González[1,2] and Isabel Izquierdo-Barba[1,2]

[1] Departamento de Química en Ciencias Farmacéuticas, Unidad de Química Inorgánica y Bioinorgánica, Universidad Complutense de Madrid. Instituto de Investigación Sanitaria Hospital 12 de Octubre i+12. Plaza Ramón y Cajal s/n, 28040 Madrid, Spain
[2] CIBER de Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain
* Correspondence: vallet@ucm.es; Tel.: (+34 913941861)

Abstract: Both the prevalence of antibiotic resistance and the increased biofilm-associated infections are boosting the demand for new advanced and more effective treatment for such infections. In this sense, nanotechnology offers a ground-breaking platform for addressing this challenge. This review shows the current progress in the field of antimicrobial inorganic-based nanomaterials and their activity against bacteria and bacterial biofilm. Herein, nanomaterials preventing the bacteria adhesion and nanomaterials treating the infection once formed are presented through a classification based on their functionality. To fight infection, nanoparticles with inherent antibacterial activity and nanoparticles acting as nanovehicles are described, emphasizing the design of the carrier nanosystems with targeting properties towards the bacteria and the biofilm.

1. Introduction

Currently, antimicrobial resistance (AMR) is pervasive across 22 countries with an estimated 500,000 people infected worldwide [1,2]. The macroeconomist, Peter O'Neill, has alerted all governments worldwide that deaths due to AMR will outpace cancer by 2050, estimating 10 million deaths in such date [3]. A consequence of uncontrolled bacterial growth is increased prevalence of biofilms, which are microorganisms' communities typically composed of multiple species coated in a self-produced protective extracellular matrix [4]. Biofilms protect the integral bacteria survive in hostile surroundings, as their physiology and behaviour are considerably different from their free-living counterparts (i.e. planktonic). In this way, biofilms confer resistance to antimicrobial agents and to the immune system causing persistent and chronic infections. Undoubtedly both AMR and biofilm formation constitute serious clinical problems and currently it is estimated that 60-80% of chronic persistent infections treated in hospitals today are produced by bacterial biofilms [5].

Nanotechnology offers potential opportunities in many fields, including infectious processes [6,7]. The term "nano", according to the FDA and IUPAC, is referred to any product with properties or phenomena attributable to its dimensions, when such dimensions are in the nanoscale range of 1-100 nm [8,9]. These nanomaterials have unique properties compared to their bulk chemical counterparts, such as large surface area to volume ratio and versatility, which could enhance their influence on a given microorganism and other diseases [10-12]. The advantage of these nanomaterial formulations over conventional systems is that they can increase treatment efficacy and decrease side effects through their precise targeting mode of action. In this sense, nanomedicine comprise the use of nanoparticles as therapeutic agents, drug delivery and diagnosis systems or the use of nanomaterials for medical devices [10]. In the last decade many billions of dollars have been invested in the global market of nanomaterials for medicine, with particular interest in the area of

46 <mark>drug delivery systems. Moreover, as consequence there are some concerns of the potential</mark>
47 <mark>toxicology of nanomaterials in human environment being diverse current literature on their possible</mark>
48 <mark>adverse health effects [13].</mark>
49 From the moment that nanomaterials were first described as drug delivery systems [14] much
50 research effort has been made, especially focused for cancer treatment [15,16]. Figure 1 displays how
51 the scientific research in the field of antimicrobial nanomaterials has grown exponentially over the
52 last decade with the expectancy of achieving an effective solution against infection. Among the
53 proposed mechanisms, the main approaches emphasize on two alternatives: i) preventing the
54 adhesion of bacteria to avoid biofilm formation or ii) destroying the formed biofilm and eliminating
55 bacteria without generation of AMR [17-30].
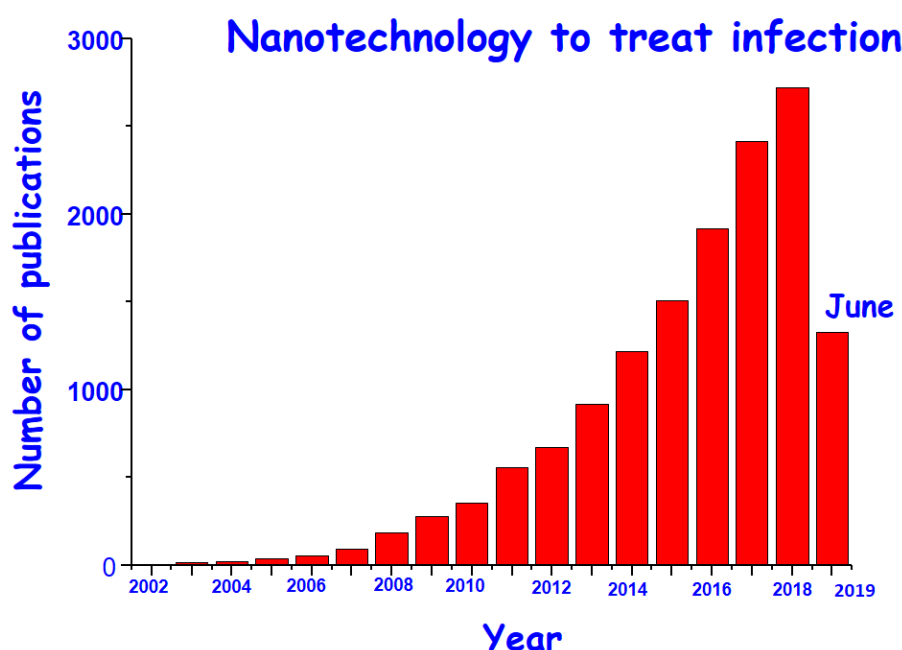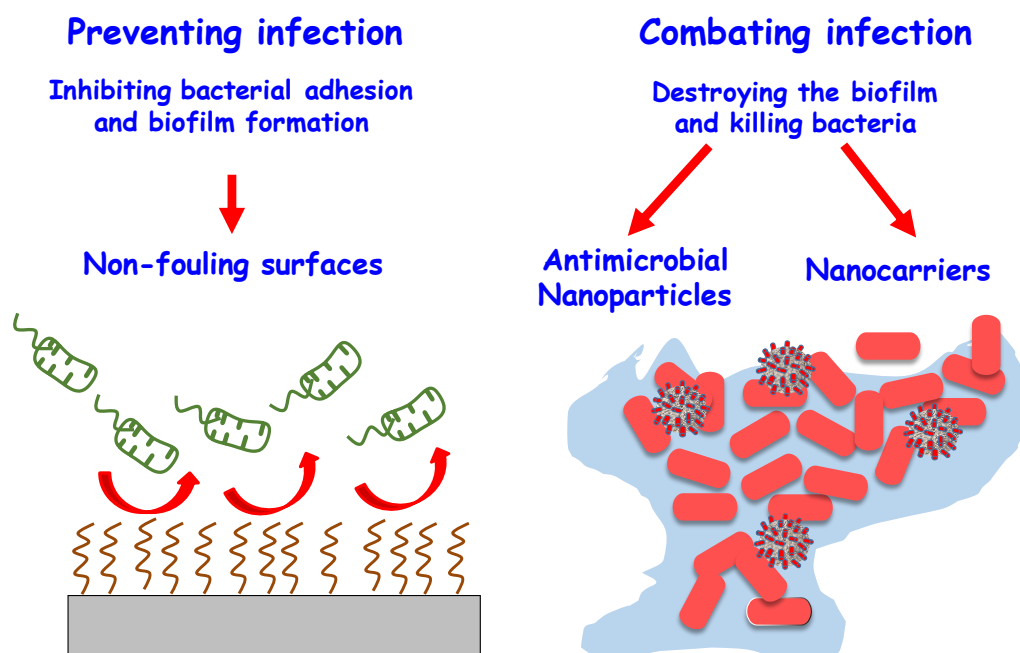56

58 **Figure 1.** Histogram showing the research in nanomaterials for infection treatment from 2002 until
59 today (June 2019). Database Web of Science (WOS) and keywords "nanomaterials" and "infection".

60 An example of the first approach is the design of antibiofouling surfaces made by altering their
61 chemical and/or physical properties to make them highly unfavorable for the bacteria attachment
62 and subsequent biofilm formation [31-35]. In this sense, the main requirement of this kind of
63 surfaces in biomedical applications, particularly in bone tissue regeneration, is that at the same time
64 the surface inhibits bacterial adhesion it also allows cell adhesion, which leads to integration of the
65 bone implant or regeneration of the bone tissue [33,36]. Regarding the second approach,
66 nanoparticles are successfully used to combat infection [10,20,21,25]. These nanomaterials have been
67 used from two flanks, i.e., those nanoparticles that intrinsically possess antimicrobial effect [37] or
68 those that are nanocarriers of antibiotics so a localized high concentration of the drug can be released
69 at the site of infection [38]. The purpose of this review is to summarize recently published work on
70 nanomaterials and their therapeutic potential for combating bacterial infection. Herein, we focus on
71 inorganic-based nanomaterials, which show advantages compared to their organic counterparts,
72 including their high thermal, chemical and mechanical stabilities under physiological conditions and
73 good biocompatibility. We provide a new viewpoint by dividing antibacterial nanomaterials into
74 two categories: materials to prevent the bacteria adhesion by the design of non-fouling surfaces and
75 materials to extinguish the infection because they bear antibacterial properties or nanoparticles
76 serving as vehicles for antimicrobial moieties. The activity of various inorganic-based nanomaterials
77 against planktonic bacteria and biofilms will be discussed as well as their mechanism of action and
78 potential toxicity. Figure 2 represents the challenge of this review manuscript.

79

The challenge

Preventing infection

Inhibiting bacterial adhesion and biofilm formation

Non-fouling surfaces

Combating infection

Destroying the biofilm and killing bacteria

Antimicrobial Nanoparticles

Nanocarriers

80

Figure 2. Two common infection-fighting strategies based on the design of nanostructured materials. The strategy to inhibit bacterial adhesion via surface modification is shown on the left. The use of nanosystems to destroy the formed biofilm using nanoparticles with intrinsic antimicrobial properties nanoparticles acting as nanocarriers of different of antimicrobial agents is shown on the right.

2. Preventing the bacterial adhesion

Implant-related infection encompasses a complex biological process, which encloses an initial step of bacteria adhesion and subsequent biofilm formation. Bacterial adhesion is divided into two stages. The first one is reversible and it is characterized by non-specific interactions between the bacteria wall and implant surface, while the second step involves specific and nonspecific interactions mediated by proteins, which guide the adhesion to an irreversible state. In fact, once the biofilm is formed, bacterial eradication becomes insurmountable, being a difficult task to treat due to biofilm impermeability to antimicrobial agents and immune system. Up to date, no treatment can guarantee the rapid and complete destruction of the biofilm, which constitutes a global challenge due to medical devices cannot yet actively resist to bacterial adhesion, colonization and biofilm formation. By consensus, inhibition of bacterial adhesion on the surface of an implant is one of the key strategies to prevent infection [39]. Different research groups work on the design of surfaces that inhibit the biofilm formation, which can be achieved by repelling bacterial adhesion or by killing approaching bacteria via direct impregnation with antibiotics, immobilization of bactericidal agents or coating with antimicrobial moieties as copper, silver, NO-releasing materials and titanium oxide films.

As it has been mentioned, for bone implant-devices, the osseointegration is decisive to achieve their long-term survival time. Thus, the efforts are addressed to the design of surfaces that inhibit the adhesion of bacteria at the same time that allow the adhesion of eukaryotic cells for an adequate osseointegration [40]. Within this surface design, chemical and structural modification are relatively simple methodologies that can be performed without altering the properties of the implant itself [32]. Between the different strategies, our research group has focused on both chemical modification

108 providing zwitterionic nature to the biomaterial surfaces and textural modification by tailoring the
109 nanostructure surface.

2.1. Chemical modifications to create zwitterionic surfaces

111 The zwitterionization consists in a simple method based on covalent grafting of different
112 moieties resulting in surfaces with equal number of positive and negative charges, therefore
113 maintaining overall electrical neutrality. It results in highly hydrophobic surfaces because a closely
114 bound water layer forms a physical and energetic obstacle that inhibits the bacterial adhesion. These
115 surfaces, which preparation has currently emerged as a groundbreaking strategy, are characterized
116 by high resistance to nonspecific protein adsorption, bacterial adhesion and biofilm formation.
117 Although there is great controversy in specifying the term zwitterion since by IUPAC definition
118 "zwitterionic surfaces are a subclass of polyampholites that have an equal number of non-ionizable
119 positive and negative charges in the same group of pendants", there are numerous strategies to
120 confer zwitterionic-like behavior to material surfaces [41]. In general, the efforts have been focused
121 in the covalent grafting to the surfaces of zwitterionic polymers or poly(sulfobetaine) and
122 poly(carboxybetaine) derivatives containing mixed positively and negatively charged moieties
123 within the same chain [42-45]. Another strategy would be the direct functionalization with
124 low-molecular weight moieties bearing the same number of negative and positive charges. In this
125 case some amino acids can be used due their biocompatibility but it cannot be rigorously considered
126 as zwitterions due to the presence of ionizable groups, providing a non-permanent or pH dependent
127 zwitterion-like action at the isoelectric point [46,47]. However, this could be taken as an advantage
128 since the infectious process (the environment) is enclosed in certain conditions of acidity compared
129 to normal physiological conditions. Thus, the lysine amino acid has been successfully used as
130 functional moiety to create zwitterionic surfaces on mesoporous bioactive glasses. The
131 functionalized materials successfully presented bacterial antiadhesive properties for *Staphylococcus*
132 *aureus* (*S. aureus*) and in vitro cytocompatibility behavior in MC3T3-E1 preosteoblast cells [48].
133 Another important strategy which has been developed is the design of zwitterionic-like surfaces on
134 nanobiomaterials by simultaneous direct grafting of two organosilanes positively and negatively
135 charged, respectively. In this case, it is possible to tailor the zwitterionic-like features by adjusting
136 the molar ratio of the different reactants. In this sense, zwitterionic silica-based mesoporous
137 bioceramics of SBA-15 type containing $-NH_3^+/COO^-$ groups have been reported [49,50]. The
138 zwitterionic nature was conferred by the co-condensation method using
139 3-aminopropyltriethoxysilane and carboxyethyl silanetriol sodium salt silanes, as $-NH_3^+$ and COO^-
140 sources respectively, during the SBA-15 synthesis. The zwitterionic nature was achieved at pH
141 values around 5.5 as determined through ζ potential studies of the isoelectric point. In this case, its
142 behavior against bacteria was determined in severe inflammation conditions, i.e., pH equal to 5.5.
143 The capability of inhibit the bacterial adhesion was tested by using *Escherichia coli* (*E. coli*) as
144 Gram-negative bacteria showing a reduced bacterial adhesion (around 93%) with respect to bare
145 pure silica SBA-15 material. Furthermore, human Saos-2 osteoblasts culture was used in order to
146 determine the biocompatibility at physiological pH, showing an adequate behavior in these
147 eukaryotic cells. Moreover, by taking advantage of the mesoporous structure, a zwitterionic SBA-15
148 type bioceramic with dual antibacterial ability has been prepared. In this case, in addition to having
149 created the zwitterionic surface, the pores have been loaded with a broad-spectrum antibiotic to
150 completely eradicate the biofilm. In this particular case, zwitterionic SBA-15 material was designed
151 by the co-condensation route using an alkoxysilane containing primary and secondary amine
152 groups, N-(2-am-inoethyl)-3-aminopropyl-trimethoxysilane. Thus, the zwitterionic features are
153 created from $-NH_3^+$/-SiO- and $=NH_2^+$/-SiO$^-$ zwitterionic pairs present on the material surface,
154 showing a 99.9% of inhibition of *S. aureus* after 90 minutes of incubation. At the same time, the
155 presence of cephalexin inside of the mesopores lead to a sustained and controlled release for 15 days
156 of incubation, which would help to eradicate the planktonic bacteria from the surroundings [51].

157 Additionally, metals widely used in clinic, such as Ti6Al4V alloy, also have been subjected to
158 the zwitterionization process [52]. Previously, the metal surface is coated with nanocrystalline

159 apatite layer to stimulate its bioactivity and to create an easier functionalizable surface. In this case
160 the zwitterionic nature is conferred by the post-grafting route using the silanes
161 3-aminopropyltriethoxysilane and carboxyethyl silanetriol sodium salt as $-NH_3^+$ and $-COO^-$ sources
162 respectively, in anhydrous conditions [53]. The in vitro bacteria test against *S. aureus* displayed a
163 notable inhibition of bacterial adhesion and no biofilm formation. At the same time, these metal
164 zwitterionic surfaces allowed a good osteoblast colonization and proliferation in preosteoblast
165 MC3T3-E1 culture.
166 Finally, up to date these zwitterionic surfaces on biomaterials have not been clinically tested
167 and just in vitro test have been reported. A more in-depth study including in vivo assays should be
168 reported to move these surfaces to the clinic.

169 *2.2. Textural modifications to tailor the nanostructure surface*

170 It is well known that both nanotopography and nanostructure of the surface play a crucial role
171 in bacterial adhesion and biofilm formation [54,55]. Consequently, different approaches have been
172 investigated to achieve artificial antibacterial surfaces based on the fabrication of different
173 nanopatterns as nanotubes, nanoparticles and nanopillars. In this sense, a TiO$_2$ nanotubes coating
174 has been performed onto titanium surfaces through the anodization route. Their antibacterial degree
175 is strongly associated with the nanotube size, crystallinity (rutile or anatase phase) and contact angle
176 [56-59]. Another example is the use of magnetron sputtering (MS-GLAD) on the surface of titanium
177 alloys which produces nanostructure coatings in a large area with a variety of morphologies [60].
178 Recently, a nanopattering coating onto Ti6Al4V substrates has been reported. The coating is formed
179 by almost vertically aligned nanocolumns with lengths of 250-350 nm and diameters of 40-60 nm,
180 separated from center to center by 100-200 nm [61]. These dense and highly packed nanotopography
181 confers a superhydrophobic behavior with a contact angle of 102° (from 56° for the uncoated
182 Ti6Al4V substrate). The antibacterial properties against different strains of *S. aureus* showed a
183 decrease of the 70% in bacterial adhesion after 90 min of incubation. As the most important result of
184 this study, it is demonstrated for the first time that these nanosurfaces inhibit the formation of
185 biofilm after 24 hours of incubation, i.e., no protective cover (characteristic of biofilm) is formed, and
186 only certain isolated bacteria appear on the surface of the biofilm. In this sense, the
187 mucopolysaccharide coverage characteristic of bacterial biofilm was not detected when stained by
188 calcofluor. Simultaneously, in vitro biocompatibility assays with HOS cell line culture were
189 performed. Osteoblast-like cells showed similar behaviour in both surfaces (nanopattering coated
190 and bare Ti alloy) with well-spreading of osteoblasts and adequate cell colonization, good adhesion
191 and appropriated cell proliferation and differentiation.

192 **3. Nanomaterials with unique features as potential weapons to fight infections**

193 At it has been commented in the introduction section, currently, cancer is the main area of
194 nanoparticle applications although research is also being carried out in other therapeutic areas such
195 as osteoporosis, cardiovascular diseases, Alzheimer's disease and infection [10,12,16,62]. In
196 particular, the use of nanoparticles for infection treatment is motivated because the conventional
197 antimicrobials fail due to AMR and the impenetrable biofilm formation as well as the absence of
198 novel drugs under expansion [15]. Furthermore, many bacteria are located intracellularly in an
199 active or latent state, making it difficult for antibiotics to access them.
200 Nanoparticles offer numerous advantages to overcome these problems. In this sense
201 nanoparticles act against bacteria through mechanisms which differ from the standard mechanisms
202 of action of antibiotics, making them extremely useful against bacterial infection avoiding the
203 dreaded AMR. The antibacterial mechanisms of nanoparticles are related to oxidative stress, metal
204 ion release, and non-oxidative mechanisms and generally trigger the formation of reactive oxygen
205 species (ROS), enzymatic inhibition, protein deactivation, DNA damage or changes in gene
206 expression as well as bacteria wall disruption [63-65]. Furthermore, the multiple modes of action of
207 nanoparticles significantly reduce the possibility of bacteria to gain resistances [66-69].

The production of ROS or oxygen free radicals, such as hydrogen peroxide (H_2O_2) or superoxide anions O_2^-, is indirectly induced by metal nanoparticles themselves. The excessive production of ROS by nanoparticles leads to a disturbed redox homeostasis and severe oxidative stress damaging cellular components, affecting membrane lipids and altering the structure of DNA and proteins [70-73]. Moreover, metal based nanoparticles gradually release metal ions that reach the intracellular compartment and interact with amino (–NH), mercapto (–SH), and carboxyl (–COOH) functional groups of proteins and nucleic acids [64,74,75]. As a result, several toxic effects can be produced such as protein coagulation, alteration in proteins related to electron transfer chain or deregulation of bacterial metabolic processes though impeded enzymatic activity. ROS production is also catalysed by metallic ions leading to bacterial lipids and DNA damage. Interaction of the nanoparticles with the bacteria wall and membrane lead to non-oxidative mechanisms [76]. Bacteria largely based upon the structure of their cell wall and membrane which are defensive barriers against environmental aggressions. Thus, damage of the cell wall leads to disrupted intracellular homeostasis and compromised bacterial function which causes mortality [64].

Gram-positive bacteria possess a rigid cell wall composed of a thin layer of peptidoglycans comprising carbohydrate polymers cross-linked through peptide residues [77]. Conversely, Gram-negative bacteria contain a thinner, more rigid peptidoglycan layer with much shorter cross-links, surrounded by a lipid membrane with lipopolysaccharides (LPS) forming a barrier presented on the surface [78]. The bacteria membrane components provide different adsorption pathways for the nanoparticles [79]. The negative charge on the surface of the bacteria wall can provide electrostatic interaction with positively charged nanoparticles that can accumulate disturbing metabolic processes or causing perforation and even membrane leakage [80]. Silver or gold nanoparticles specifically interact with sulphur-containing constituents within the cell membrane impeding cell wall synthesis [81-83].

3.1. Nanoparticles with inherent antibacterial properties

The intrinsic antibacterial properties of some metals, metallic oxides and metallic salts have been known for centuries, therefore being used to treat bacterial and fungal infections prior to the discovery of penicillin by Sir Alexander Fleming [84,85]. Although their medicinal utility was diminished with the antibiotic era, the actual emergence of AMR has led to the recovery of these earliest antimicrobial agents. Among metal and metal oxide nanoparticles, silver nanoparticles are probably the most promising of all the inorganic nanoparticles as a treatment for bacterial infections. Nevertheless, besides Ag other metal nanoparticles such as Au, and metal oxide nanoparticles such as zinc oxide, copper oxide, iron oxide and titanium dioxide, among others, are being intensively studied for antimicrobial treatment [85,86]. Some recent examples of this metal based nanoparticles NPs are described in this section.

3.1.1. Silver nanoparticles

Although silver nanoparticles (AgNPs) are the most intensely considered metal nanomaterial for antimicrobial treatment [87], the understanding of their precise mechanism of action upon microbes remains incomplete. Multiple mechanisms may be involved [88], such as direct interaction of AgNPs with the bacterial membrane inhibiting cell wall synthesis or causing pits leading to cell lysis [89-91]. Moreover, silver oxidation in the biological media releases Ag^+ ions continuously [92] which are bonded to thiol-containing proteins impairing their functions and also producing enhanced ROS generation [93-96]. While many attempts have been made to clarify the mode of action, the reported studies continue demonstrating their bactericidal efficacy. Hence, due to the potentiality of the AgNPs as bactericidal agents in clinical applications, another key point where many research efforts are being devoted is the synthetic methodology to prepare silver nanoparticles. Besides the traditional techniques based on the chemical reduction process, where a reducing agent for the Ag^+ ion is used in the presence of stabilizers in a suitable solvent, new alternative approaches based on green chemistry are booming. The eco-friendly techniques

incorporate the use of plants, biological or microbial agents as reducing and capping agents. Silver nanoparticles obtained by green biogenic synthesis offer a novel and potential alternative to chemically prepared nanoparticles [97].

Currently, AgNPs can be seen as an alternative treatment for some clinical situations due to their antimicrobial activity and also wound healing effects. For example, AgNPs have been in vivo evaluated as a post-surgical treatment for *Caseous lymphadenitis* in small ruminants. The etiological agent of this disease is *Corynebacterium pseudotuberculosis*, a Gram-positive and facultative intracellular bacterium. In the experiment twenty-nine goats and sheep with clinical signs of *Caseous lymphadenitis* were surgically operated to excise the caseous lesions that were treated with an ointment formulation based on AgNPs mixed with natural waxes and oils in the experimental group, or with the conventional treatment with 10% iodine in the control group. It could be concluded that post-surgical treatment of *Caseous lymphadenitis* using the AgNPs-based ointment led to faster healing, decreased wound contamination, and presented no apparent toxic effects [98]. Another field of clinical development of the AgNPs is related to orthopaedic implants. As above commented, for the treatment or prevention of implant-related infections, materials that exhibit antibacterial properties at the same time that promote osteogenesis are required. AgNPs coatings of implants must have into account the dose-dependent cytotoxicity of silver and its negative impact on bone implants. In view of this remark, a bioinspired hybrid coating containing polydopamine, hydroxyapatite, AgNPs, and chitosan has been prepared on the surface of titanium implants. The double chelating effect of polydopamine and chitosan significantly reduces silver ion release from the AgNPs in the hybrid coating. The coating exhibits excellent anti-biofilm efficiency of 91.7%, 89.5%, and 92.0% for *S. aureus*, *S. epidermidis*, and *E. coli*, respectively. In addition, the coating can significantly stimulate osteogenic differentiation of MC3T3-E1 cells and promotes bone-implant osseointegration in vivo. Therefore, the hybrid coating exhibits antibacterial properties as well as allow bone-implant osseointegration, thereby providing insights into the design of multifunctional implants for long-term orthopedic applications [99].

3.1.2. Gold nanoparticles

Metallic gold is stable against oxidation in biological medium, which makes it non-toxic and a biocompatible metal. However, gold nanoparticles (AuNPs) indeed exhibit antimicrobial effect via different mechanisms [64,69]. AuNPs and Au nanoclusters possess catalytic activity analogous to various enzymes such as peroxidase, glucose-oxidase and/or superoxide dismutase [100]. This enzyme-like activity led to an increased generation of ROS affecting bacteria through oxidative stress mechanism [101,102]. In addition, AuNPs can be irreversibly bound onto the thiol groups present on different proteins, for example in nicotinamide adenine dinucleotide (NADH) dehydrogenase, in this case affecting the reduction-oxidation balance within the bacterial respiratory chain and thus generating oxidative stress [103]. But perhaps one of the most recent and interesting applications of gold nanomaterials as bactericidal agents takes place when the physical properties of gold are exploited at the nanoscale. In this sense, AuNPs possess excellent photothermal properties since their plasmon resonance makes them to absorb light in the near infrared (NIR) window and in turn generates heat that can be used for ablation of bacteria or disruption of biofilms [104]. Recently, a non-antibiotics based nanoformulation containing Au nanorods has shown a remarkable antibacterial efficacy in treating drug-resistant pneumonia when applied in combination with NIR photothermal treatment. The 50-100 nm long gold nanorods are decorated with glycomimetic polymers to specifically block bacterial lectins which are essential for bacterial biofilm development. This novel formulation shows the most efficient bacteria inhabitation and killing against *Pseudomonas aeruginosa* infection, through lectin blocking and the NIR light-induced photothermal effect of gold nanorods [105].

3.1.3. Metal oxide nanoparticles

Metal oxide nanomaterials such as zinc oxide (ZnO), iron oxide (Fe_3O_4), copper oxide (CuO), magnesium oxide (MgO) and titanium dioxide (TiO_2) nanoparticles are known to possess

308 antimicrobial activity over a range of Gram-positive and Gram-negative bacteria, including resistant
309 bacterial strains [86,106]. Their antibacterial activity is usually related to generation of ROS
310 attributed to their intrinsic photocatalytic activity or to the release of the metallic ions [107,108].

311 Recently, amine functionalized ZnO nanocrystals have been designed as a highly biocompatible
312 and osteoinductive nanoantibiotic agent for bone tissue engineering. The ZnO nanocrystals of 20 nm
313 in diameter have been prepared with a novel, fast and reproducible microwave-assisted synthesis.
314 After chemical functionalization by anchoring aminopropyl groups onto the ZnO, surface the
315 ZnO-NH$_2$ nanocrystals were tested in terms of biocompatibility, promotion of cell proliferation and
316 differentiation towards preosteoblast cells, and also in terms of antimicrobial activity against
317 Gram-positive and Gram-negative bacteria, such as *E. coli* and *S. aureus*, respectively (see Figure 3).
318 The *in vitro* results suggest that ZnO-NH$_2$ nanocrystals are a promising candidate to solve infectious
319 diseases in bone implants and at the same time promote bone tissue proliferation [109].
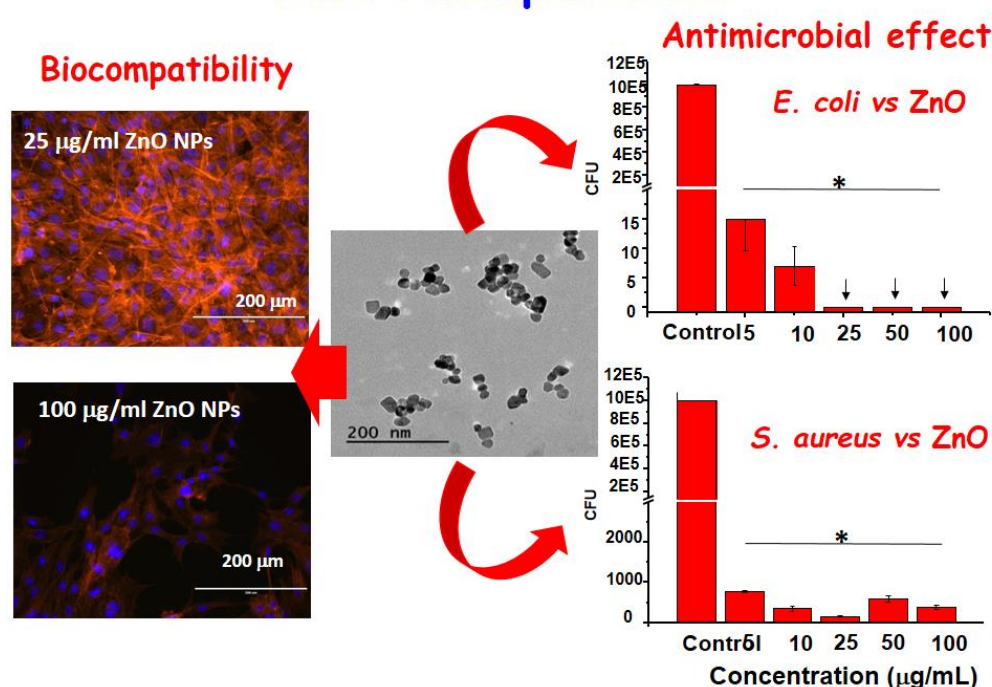320

321

322 *Figure 3.* Biocompatibility and antimicrobial effect of ZnO nanoparticles prepared by
323 microwave-assisted synthesis. A TEM image corresponding to the ZnO nanoparticles round-shaped
324 of 20 nm in diameter is shown in the center. (Left) Confocal images corresponding to preosteoblast
325 cultured up to 70% of confluence after incubation for 4 days with ZnO nanoparticles at different
326 concentrations, showing a good biocompatibility. (Right) Antimicrobial effect against *E. coli* and *S.*
327 *aureus* in planktonic stage incubated for 24 h in the presence of different concentrations of ZnO
328 nanoparticles. The reduction of colony forming units (CFU) is represented ($p < 0.05$, significant
329 differences compared to control denoted by an asterisk (*)). The arrows denote an absolute 100% of
330 efficacy.

331 Antimicrobial activity of iron oxide nanoparticles (IONPs) based nanosystems against different
332 microorganisms has been already recently reviewed. One of the main mechanisms of action by
333 which systems based on IONPs generate bacteria toxicity is ROS generation through the Fenton
334 reaction [110]. However, by taking advantage of the magnetic properties of IONPs, alternative
335 physical antibacterial strategies can be proposed to fight against AMRs. For example, multiple drug
336 resistant *S. aureus* and uropathogenic *E. coli* have been trapped into positively charged magnetic
337 core-shell nanoparticles by electrostatic interaction. All the trapped bacteria could be completely
338 killed within 30 minutes when exposed to a radiofrequency current owing to the loss of membrane

339   potential and dysfunction of membrane-associated complexes. This physical treatment kills
340   pathogenic bacteria and blocks biofilm formation without leading to antibiotic resistance [111].
341   Another research work used IONPs loaded with nisin, a known bacteriocin which is commonly
342   inefficient against Gram-negative bacteria. The IONPs were activated by high pulsed electric and
343   electromagnetic fields to induce additional permeabilization and local magnetic hyperthermia. The
344   results on the assays on the Gram-positive *Bacillus subtilis* and Gram-negative *E. coli* showed that the
345   high pulsed magnetic fields increase the antimicrobial efficiency of nisin loaded on the IONPs
346   similar to electroporation or magnetic hyperthermia methods, resulting in a synergistic treatment
347   [112].

348   *3.2. Nanomaterials as nanocarriers: Mesoporous silica nanoparticles*

349   An alternative strategy to fight infection with nanoparticles is to use them as vehicles to deliver
350   antimicrobial agents such as antibiotics or other bactericide nanoparticles. Nanocarriers of
351   antimicrobial agents should be able to shield the active compound from degradation and to enhance
352   the potency of the active compound or improve its bioavailability for treatment. The nanocarrier
353   may enable controlled and sustained release of the loaded antimicrobial drugs, which is useful for
354   maintaining an optimum level of drug concentration in the bloodstream for a period of time. They
355   also may offer the possibility to simultaneously deliver several antibiotics or to act in a combined
356   therapy if using other stimuli responsive nanoparticles in the same nanosystem. Moreover, the
357   nanocarriers may also provide a platform for surface modification that allows specific targeting to
358   the site of infection or only once an infection has occurred.
359   Among the different materials which can compose these nanocarriers, mesoporous silica
360   nanoparticles (MSNs) constitute one of the most promising due to their interesting properties of
361   advanced inorganic nanoplatforms as drug delivery systems. The main strengths of MSNs are high
362   loading capacity, biocompatibility, ease of production and high degree of tunability regarding size,
363   morphology and pore diameter. Furthermore, MSNs can be easily synthesized in a large scale
364   showing a great variety of morphologies and surface functionalities using different strategies [10].
365   Figure 4 shows a schematic representation of the versatility and functionality of MSNs regarding
366   their biomedical applications. Initially these nanocarriers have shown high interest in cancer
367   treatment due to the wide versatility in their functionalization, being able to design smart
368   nanomaterials with stimulus responsive components [10, 113], possessing also cancer cell targeting
369   capability [114] and penetrability towards the deepest areas of solid tumors [115]. Recently, this
370   technology has been also successfully applied to osteoporosis treatment in an animal model [12].
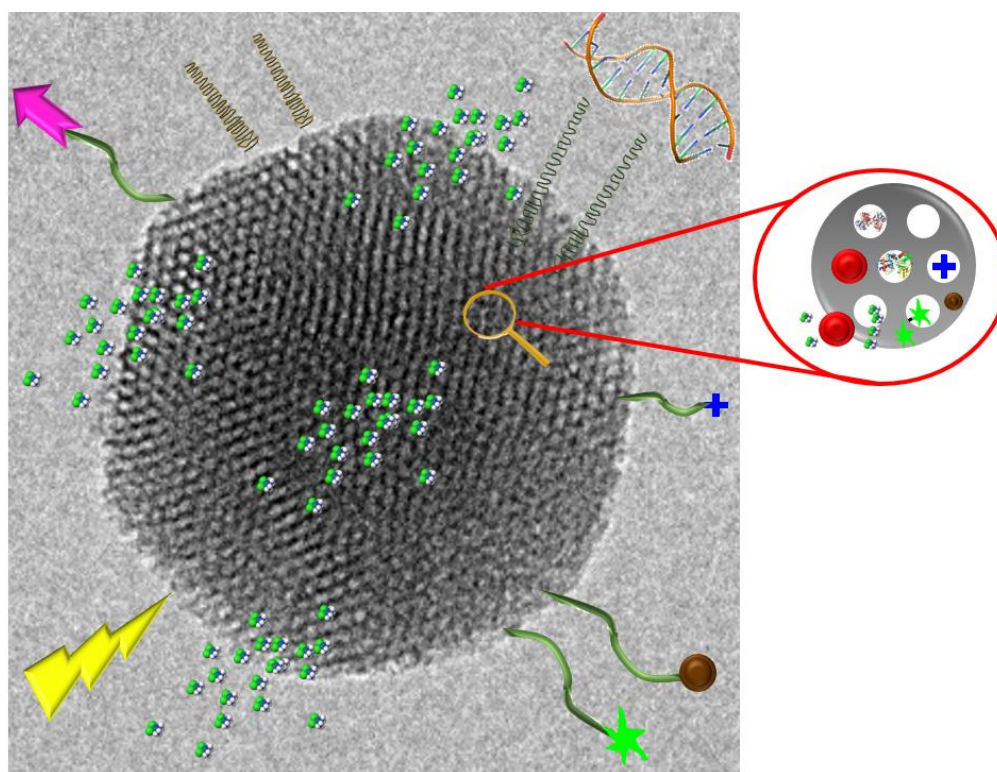371

Figure 4. Schematic representation of the versatility and functionality of MSNs. TEM image corresponding to a MSN of 150 nm in diameter showing the mesoporous arrangement in the 2D hexagonal structure (*p6mm* plain group). On the image, cartoons represent the drug loading capability, active targeting and stimuli-responsive possibilities of MSNs.

In this sense, effective new alternatives for the management of bone infection can be achieved through the development of antibiotic nanocarriers able to penetrate bacterial biofilm, thus enhancing antimicrobial effectiveness. An example of this kind of nanosystem, also denoted as "nanoantibiotic", it consists in MSNs loaded with levofloxacin (LEVO) as antimicrobial agent externally functionalized with N-(2-aminoethyl)-3-aminopropyltrimethoxysilane as targeting agent. This amine functionalization provides MSNs of positive charges, which improves the affinity towards the negatively charged bacteria wall and biofilm. After physicochemical characterization, "in vial" LEVO release profiles and the in vitro antimicrobial effectiveness of the different released doses were investigated. The efficacy of this nanoantibiotic against a *S. aureus* biofilm was also determined, showing the practically total destruction of the biofilm due to the high penetration ability of the developed nanosystem. These findings open up promising expectations in the field of bone infection treatment [116].

Another important strategy is to provide an effective and novel solution for the treatment of infection by using nanovehicles loaded with antibiotics capable of penetrating the bacterial wall, thus increasing the antimicrobial effectiveness. In this case these "nanoantibiotics" are composed of MSNs, which act as nanocarriers of LEVO localized inside the mesopores. To provide the nanosystem of bacterial membrane interaction capability, a polycationic poly(propyleneimine) dendrimer of third generation (G3) was covalently grafted to the external surface of the LEVO-loaded MSNs. After physicochemical characterization of this nanoantibiotic, the release kinetics of LEVO and the antimicrobial efficacy of each released dosage were evaluated. Besides, internalization studies of the MSNs functionalized with the G3 dendrimer were carried out, showing a high penetrability throughout Gram-negative bacterial membranes (see Figure 5). This work evidences that the synergistic combination of polycationic dendrimers as bacterial membrane permeabilization agents with LEVO-loaded MSNs triggers an efficient antimicrobial effect on Gram-negative bacterial biofilm. These positive results open up very promising expectations for their potential application in new infection therapies [117].
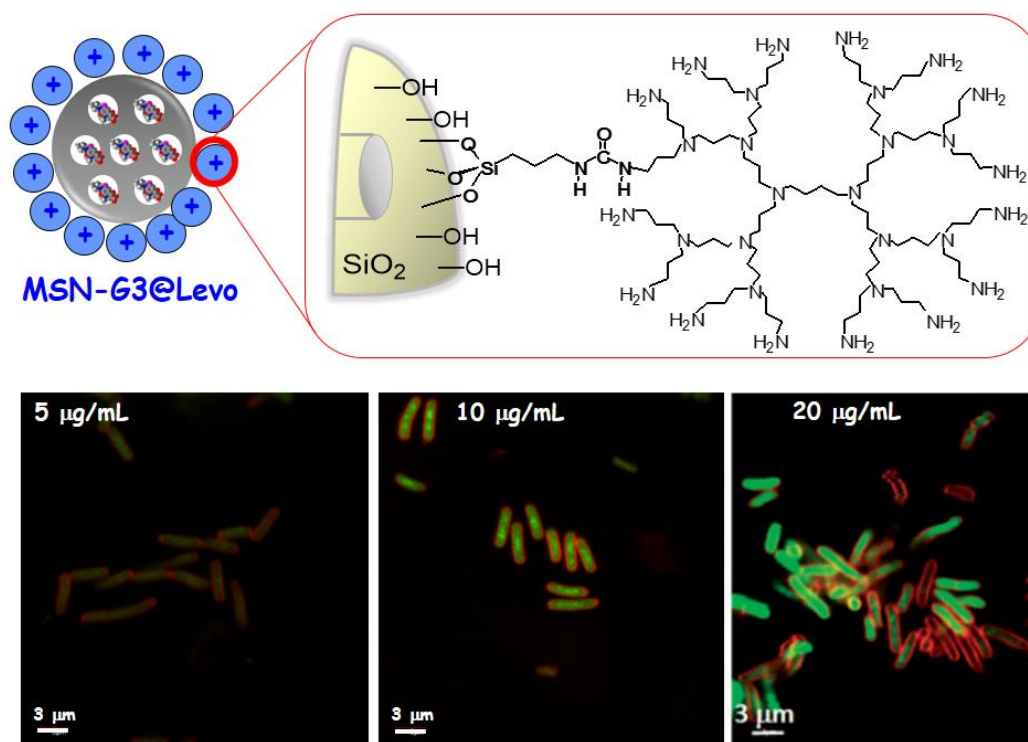
403

404

405 ***Figure 5.*** MSN as nanocarrier of antimicrobial agent (levofloxacin) and functionalized with a bacteria
406 membrane targeting agent (poly(propyleneimine) dendrimer of third generation, G3). In this case the
407 functionalization of MSNs with G3 macromolecules increase the internalization in bacteria Gram
408 negative (*E. coli*), which is dosage dependent as it can be observed in the confocal images. The
409 synergistic combination of polycationic dendrimers as bacterial membrane permeabilization agents
410 with LEVO-loaded MSNs triggers an efficient antimicrobial effect on *E. coli* biofilm. These results
411 open up very promising prospects for their potential application as new anti-infective therapies. In
412 the confocal images the red color represents the bacteria membrane and the green colour represents
413 the labelled-MSN materials.

414 Finally, the ability of bacteria to form biofilms hinders any conventional treatment for chronic
415 infections and has serious socio-economic implications. For this purpose, a nanocarrier capable of
416 overcoming the barrier of the mucopolysaccharide matrix of the biofilm and releasing its
417 loaded-antibiotic within this matrix would be highly desirable. Herein, we have developed a new
418 nanosystem based on LEVO-loaded MSNs decorated with the lectin concanavalin A (ConA). The
419 presence of ConA promotes the internalization of this nanosystem into the biofilm matrix, which
420 increases the antimicrobial efficacy of the antibiotic hosted within the mesopores (see Figure 6). This
421 nanodevice is envisioned as a promising alternative to conventional treatments for infection by
422 improving the antimicrobial efficacy and reducing side effects [118].
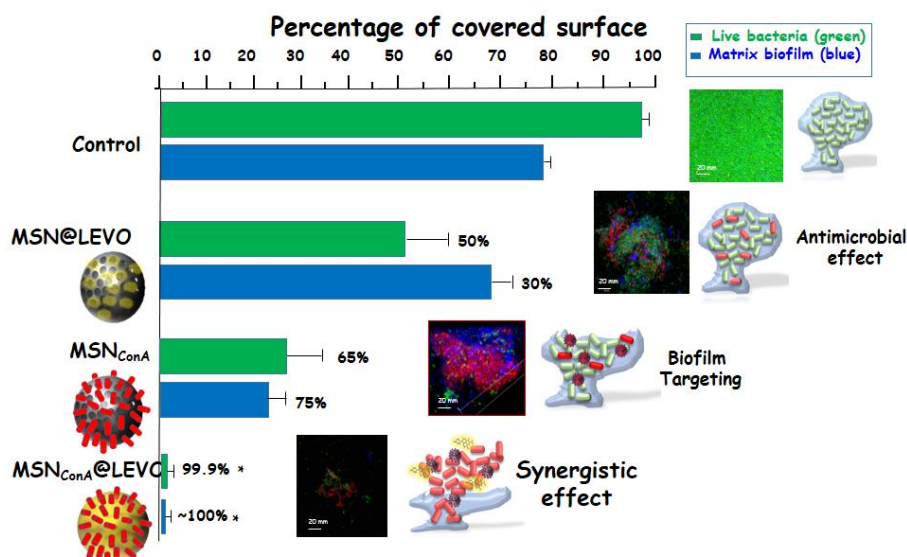
423

Figure 6. MSN as nanocarrier of antimicrobial agent (levofloxacin) and functionalized with a biofilm targeting agent (Concanavaline A, ConA). In this case the functionalization of MSNs with ConA favours its internalization in *E. coli* biofilms affording a synergistic combination with LEVO-loaded MSNs which triggers an efficient antimicrobial effect on *E. coli* biofilm. The image represents the percentage of covered surface by live bacteria (green) and mucopolysaccaride layer (blue) and the representative confocal images show a complete reduction after incubation with the nanosystems functionalized with ConA and loaded with levofloxacin (MSNConA@LEVO).

4. Conclusions and futures perspectives

The increased antibiotic resistance and consequently the formation of biofilms have been converted in a critical problem for the health industry, due to the ineffectiveness of the conventional antimicrobial therapies. Nanomaterials display a promising technology to solve these issues. This review tries to give an overview of the different solutions according to two different approaches: to prevent the infection via the modification of nanomaterial surfaces or to combat the infection by the accurate design of nanoparticles with inherent antimicrobial features or nanoparticles as carriers of antimicrobial agents. Both surface zwitterionization and nanostructured coatings are presented as highly powerful tools for the prevention of bacterial adhesion and biofilm formation. These surfaces show a good degree of biocompatibility, which is very important when bringing this type of technology to the clinic. Metal and metal oxide nanoparticles show effective antimicrobial activity with rapid-time-kill and evading of antibiotic resistance based on their specific properties at the nanoscale and their multiple mechanisms of action. Mesoporous silica nanoparticles used as nanocarriers offer extraordinary advantages by being able to functionalize their surface with targeting agents and considerably increase the activity of the loaded antimicrobial agent. This is certainly the starting point towards a considerable improvement in conventional treatments, where the tendency is to combine all the elements in order to effectively abolish the dreaded infections. In this point, for clinic translation is important to know about their safety and cytotoxicity which has been addressed, in the most of the cases, only in vitro in different cell cultures. However, in vivo models should be carried out to better understand the biological effect of the proposed nanosystems, comprising toxicity, metabolism, biodistribution, clearance and mechanism of action for a good practice towards the clinical application.

Author Contributions: Conceptualization, M.V.R.; B.G.; I.I.B, resources, B.G.; I.I.B, data curation, B.G.; I.I.B, writing—review and editing, M.V.R.; B.G.; I.I.B, visualization, M.V.R.; supervision, M.V.R.; project administration M.V.R.; funding acquisition, M.V.R.

459 **Conflicts of Interest:** The authors declare no conflict of interest.

460 **Abbreviations**

AMR	Antimicrobial resistance
ConA	concanavalin A
AuNPs	gold nanoparticles
IONPs	iron oxide nanoparticles
LEVO	levofloxacin
LPS	lipopolysaccharides
MS-GLAD	magnetron sputtering
MSNs	mesoporous silica nanoparticles
ROS	reactive oxygen species
AgNPs	Silver nanoparticles

461 **References**

462 1. WHO High Levels of Antibiotic Resistance Found Worldwide, New Data Shows. WHO, News Release.
463   Available online: https://www.who.int/mediacentre/news/releases/2018/antibiotic-resistancefound/en/
464   (accessed on 29 January 2018).
465 2. Magiorakos, A.P.; Srinivasan, A.; Carey, R.B.; Carmeli, Y.; Falagas, M.E.; Giske, C.G.; Harbarth, S.;
466   Hindler, J.F.; Kahlmeter, G.; Olsson-Liljequist, B.; Paterson, D.L.; Rice, L.B.; Stelling, J.; Struelens, M.J.;
467   Vatopoulos, A.; Weber, J.T.; Monnet, D.L. Multidrug-resistant, extensively drug-resistant and
468   pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired
469   resistance. Clin. Microbiol. Infect. 2012, 18, 268-281.
470 3. O'Neill, J. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. Rev.
471   Antimicrob. Resist. 2014, 1, 1-16.
472 4. Davies, D. Understanding biofilm resistance to antibacterial agents. Nat. Rev. Drug Discov. 2003, 2,
473   114-122.
474 5. Watnick, P.; Kolter, R. Biofilm, city of microbes. J. Bacteriol. 2000, 182, 2675-2679.
475 6. Beyth, N.; Houri-Haddad, Y.; Domb, A.; Khan, W.; Hazan, R. J. Evidence-Based Complementary Altern.
476   Med. 2015, 2015, NA 246012.
477 7. Kamaruzzaman, N.F.; Tan, L.P.; Hamdan, R.H.; Choong, S.S.; Wong, W.K.; Gibson, A.J.; Chivu A.; Pina,
478   M.F. Antimicrobial Polymers: The Potential Replacement of Existing Antibiotics?. Int. J. Mol. Sci. 2019, 20,
479   NA 2747.
480 8. Vert, M.; Doi, Y.; Hellwich, K.; Hess, M.; Hodge, P.; Kubisa, P.; Rinaudo, M.; Schué, F. Terminology for
481   biorelated polymers and applications (IUPAC recommendations). Pure Appl. Chem. 2012, 84, 377-410.
482 9. U.S. Department of Health and Human Services, Food and Drug Administration, Guidance for Industry,
483   Considering Whether an FDA-regulated Product Involves the Application of Nanotechnology, 2014.
484 10. Castillo, R.R.; Lozano, D.; González, B.; Manzano, M.; Izquierdo-Barba, I.; Vallet-Regí. M. Advances in
485   mesoporous silica nanoparticles for targeted stimuli-responsive drug delivery: an update. Expert Opin.
486   Drug Deliv. 2019, 16, 415-439.
487 11. Vallet-Regí, M.; Colilla, M.; Izquierdo-Barba, I. Chapter Three - Drug Delivery and Bone Infection. The
488   Enzymes 2018, 44, 35-59.
489 12. Mora-Raimundo, P; Lozano, D; Manzano, M; Vallet-Regí, M. Nanoparticles to Knockdown
490   Osteoporosis-Related Gene and Promote Osteogenic Marker Expression for Osteoporosis Treatment. ACS
491   Nano 2019, 13, 5451-5464.
492 13. Zhao, Y.; Zhang, Z.; Feng, W. Toxicology of Nanomaterials. Wiley-VCH Verlag GmbH & Co. KGaA 2016.
493 14. Vallet-Regí, M.; Rámila, A.; del Real RP, Pérez-Pariente, J. A new property of MCM-41 as drug delivery
494   system. Chem. Mater. 2001, 13, 308-311.
495 15. Vallet-Regí, M.; Colilla, M.; Izquierdo-Barba, I.; Manzano, M. Mesoporous silica nanoparticles for drug
496   delivery: current insights. Molecules 2018, 23, NA 47.

497 16. Villaverde, G.; Alfranca, A.; Gonzalez-Murillo, A.; Melen, G.J.; Castillo, R.R.; Ramirez, M.; Baeza, A.;
498   Vallet-Regí, M. Molecular Scaffolds as Double-Targeting Agents For the Diagnosis and Treatment of
499   Neuroblastoma. Angew. Chem. Int. Ed. 2019, 58, 3067-3072.
500 17. Campoccia, D.; Montanaro, L.; Arciola, C.R. The significance of infection related to orthopaedic devices
501   and issues of antibiotic resistance, Biomaterials 2006, 27, 2331-2339.
502 18. Dong, K.; Ju, E.; Gao, N.; Wang, Z.; Ren, J.; Qu, X. Synergistic eradication of antibiotic-resistant bacteria
503   based biofilms in vivo using a NIR-sensitive nanoplatforms. Chem. Commun. 2016, 52, 5312-5315.
504 19. Chen, Z.; Ji, H.; Liu, C.; Bing, W.; Wang, Z.; Qu, X. A multinuclear metal complex based DNAse-mimetic
505   artificial enzyme: matrix cleavage for combating bacterial biofilms. Angew. Chem. Int. Ed. 2016, 55,
506   10732-10736.
507 20. Huh, A.J.; Kwon, Y.J. ''Nanoantibiotics'': A new paradigm for treating infectious diseases using
508   nanomaterials in the antibiotics resistant era. J. Control. Release 2011, 156, 128-145.
509 21. Zhang, L.; Pornpattananangku, D.; Hu, C.M.J.; Huang, C.M. Development of nanoparticles for
510   antimicrobial drug delivery. Curr. Med. Chem. 2010, 17, 585-594.
511 22. Moritz, M.; Geszke-Moritz, M. The newest achievements in synthesis, immobilization and practical
512   applications of antibacterial nanoparticles. Chem. Eng. J. 2013, 228, 596-613.
513 23. Li, X.; Robinson, S.M.; Gupta, A.; Saha, K.; Jiang, Z.; Moyano, D.F.; Sahar, A; Riley, M.A.; Rotello, V.M.
514   Functional gold nanoparticles as potent antimicrobial agents against multi-drug-resistant bacteria. ACS
515   Nano 2014, 8, 10682-10686.
516 24. Lam, S.J.; O'Brien-Simpson, N.M.; Pantarat, N.; Sulistio, A.; Wong, E.H.H.; Chen, Y.-Y.; Lenzo, Holden,
517   J.A.; Blencowe, A.; Reynolds, E.C.; Qiao, G.G. Combating multidrug-resistant Gram-negative bacteria with
518   structurally nanoengineered antimicrobial peptide polymers. Nat. Microbiol. 2016, 1, NA 16162.
519 25. Mu, H.; Tang, J.; Liu, Q.; Sun, C.; Wang, T.; Duan, J. Potent antibacterial nanoparticles against biofilm and
520   intracellular bacteria. Sci. Rep. 2016, 6, NA 18877.
521 26. Bandyopadhyay, A.; McCarthy, K.A.; Kelly, M.A.; Gao, J. Targeting bacteria via iminoboronate chemistry
522   of amine-presenting lipids. Nat. Commun. 2015, 6, NA 6561.
523 27. Radovic-Moreno, A.F.; Lu, T.K.; Puscasu, V.A.; Yoon, C.J.; Langer, R.; Farokhzad, O.C. Surface
524   charge-switching polymeric nanoparticles for bacterial cell wall targeted delivery of antibiotics, ACS Nano
525   2012, 6, 4279-4287.
526 28. Andiappan, K.; Sanmugam, A.; Deivanayagam, E.; Karuppasamy, K.; Kim, H.S.; Vikraman, D. Schiff base
527   rare earth metal complexes: Studies on functional, optical and thermal properties and assessment of
528   antibacterial activity. Int J Biol Macromol. 2019, 124, 403-410.
529 29. Sanmugam, A.; Vikraman, D.; Park, H. J.; Kim, H.-S. One-Pot Facile Methodology to Synthesize
530   Chitosan-ZnO-Graphene Oxide Hybrid Composites for Better Dye Adsorption and Antibacterial Activity.
531   Nanomaterials 2017, 7, 363.
532 30. Packirisamy, R.G.; Govindasamy, C.; Sanmugam, A.; Venkatesan, S.; Kim, H.-S.; Vikraman, D. Synthesis
533   of novel Sn1-xZnxO-chitosan nanocomposites: Structural, morphological and luminescence properties
534   and investigation of antibacterial properties. International Journal of Biological Macromolecules 2019, 138,
535   546-555.
536 31. Sánchez-Salcedo, S.; Colilla, M.; Izquierdo-Barba I.; Vallet-Regí, M. Preventing bacterial adhesion on
537   scaffolds for bone tissue engineering. Int. J. Bioprinting 2016, 2, 20-34.
538 32. Anselme, K.; Davidson, P.; Popa, A.M.; Giazzon, M.; Liley, M.; Ploux, L. The interaction of cells and
539   bacteria with surfaces structured at the nanometre scale. Acta Biomater. 2010, 6, 3824-3846.
540 33. Izquierdo-Barba, I.; Colilla, M.; Vallet-Regí, M. Zwitterionic ceramics for biomedical applications. Acta
541   Biomater. 2016, 40, 201-211.
542 34. Goudie, M.J.; Singha, P.; Hopkins, S.P.; Brisbois, E.J.; Handa, H. Active release of an antimicrobial and
543   antiplatelet agent from a non-fouling surface modification. ACS Appl. Mater. Interfaces 2019, 11,
544   4523-4530.
545 35. Modaresifar, K.; Azizian, S.; Ganjian, M.; Fratila-Apachitei, L.E.; Zadpoor, A.A. Bactericidal effects of
546   nanopatterns: A systematic review. Acta Biomater. 2019, 83, 29-36.
547 36. Colilla, M.; Izquierdo-Barba, I.; Vallet-Regí, M. The Role of Zwitterionic Materials in the Fight against
548   Proteins and Bacteria. Medicines 2018, 5, NA 125.
549 37. Zazo, H.; Colino, C.I.; Lanao. J.M. Current applications of nanoparticles in infectious diseases. J. Control.
550   Release 2016, 224, 86-102.

551 38. Gao, W.; Chen, Y.; Zhang, Y.; Zhang, Q.; Zhang, L. Nanoparticle-based local antimicrobial drug delivery.
552 Adv. Drug Delivery Rev. 2018, 127, 46-57.

553 39. Campoccia, D.; Montanaro, L.; Arciola, C.R. A review of the biomaterials technologies for infection-
554 resistant surfaces. Biomaterials 2013, 34, 8533-5854.

555 40. Puckett, S.D.; Taylor, E.; Raimondo, T.; Webster, T.J. The relationship between the nanostructure of
556 titanium surfaces and bacterial attachment. Biomaterials 2010, 31, 706-713.

557 41. Encinas, N.; Angulo, M.; Astorga, C.; Colilla, M.; Izquierdo-Barba, I.; Vallet-Regí, M. Mixed-charge
558 pseudo-zwitterionic mesoporous silica nanoparticles with low-fouling and reduced cell uptake properties.
559 Acta Biomater. 2019, 84, 317-327.

560 42. Sun, J.T.; Yu, Z.Q.; Hong, C.Y.; Pan, C.Y. Biocompatible zwitterionic sulfobetaine copolymer-coated
561 mesoporous silica nanoparticles for temperature responsive drug release. Macromol. Rapid Commun.
562 2012, 33, 811-818.

563 43. Lalani, R.; Liu, L. Electrospun zwitterionic poly(sulfobetaine methacrylate) for nonadherent,
564 superabsorbent, and antimicrobial wound dressing applications. Biomacromolecules 2012, 13, 1853-1863.

565 44. Zhang, Z.; Chen, S.; Chang, Y.; Jiang, S. Surface grafted sulfobetaine polymers via atom transfer radical
566 polymerization as superlow fouling coatings. J. Phys. Chem. B 2006, 110, 10799-10804.

567 45. Zhang, Z.; Chao, T.; Chen, S.; Jiang, S. Superlow fouling sulfobetaine and carboxybetaine polymers on
568 glass slides. Langmuir 2006, 22, 10072-10077.

569 46. Rosen, J.E.; Gu, F.X. Surface functionalization of silica nanoparticles with cysteine: a low-fouling
570 zwitterionic surface. Langmuir 2011, 27, 10507-10513.

571 47. Villegas, F.M.; Garcia-Uriostegui, L.; Rodríguez, O.; Izquierdo-Barba, I.; Salinas, A.J.; Toriz, G.; Vallet-Regí,
572 M.; Delgado, E. Lysine-grafted MCM-41 silica as an antibacterial biomaterial. Bioengineering 2017, 4, NA
573 80.

574 48. Sánchez-Salcedo, S.; García, A.; Vallet-Regí, M. Prevention of bacterial adhesion to zwitterionic
575 biocompatible mesoporous glasses. Acta Biomaterialia 2017, 57, 472-486.

576 49. Colilla, M.; Izquierdo-Barba, I.; Sánchez-Salcedo, S.; Fierro, J.L.G.; Hueso, J.L.; Vallet-Regí, M. Synthesis
577 and characterization of zwitterionic SBA-15 nanostructured materials. Chem. Mater. 2010, 23, 6459-6466.

578 50. Izquierdo-Barba I.; Sánchez-Salcedo, S.; Colilla, M.; Feito, M.J.; Ramírez-Santillán, C.; Portolés, M.T.;
579 Vallet-Regí, M. Inhibition of bacterial adhesion on biocompatible zwitterionic SBA-15 mesoporous
580 materials. Acta Biomater. 2011, 7, 2977-2985.

581 51. Colilla, M.; Martínez-Carmona, M.; Sánchez-Salcedo, S.; Ruiz-González, M.L.; González-Calbet, J.M.;
582 Vallet-Regí, M. A novel zwitterionic bioceramic with dual antibacterial capability, J. Mater. Chem. B 2014,
583 2, 5639-5651.

584 52. Rodriguez-Palomo, A.; Monopoli, D.; Afonso, H.; Izquierdo-Barba I.; Vallet-Regí, M. Surface
585 zwitterionization of customized 3D Ti6Al4V scaffolds: a promising alternative to eradicate bone infection.
586 J. Mater. Chem. B 2016, 4, 4356-4365.

587 53. Sánchez-Salcedo, S.; Colilla, M.; Izquierdo-Barba, I.; Vallet-Regí, M. Design and preparation of
588 biocompatible zwitterionic hydroxyapatite. J. Mater. Chem. B 2013, 1, 1595-1606.

589 54. Díaz, C.; Schilardi, P.L.; Salvarezza, R.C.; Fernández-Lorenzo, M.; Mele, D. Nano/ microscale order affects
590 the early stages of biofilm formation on metal surfaces. Langmuir 2007, 23, 11206-11210.

591 55. Chouirfa H.; Bouloussa, H.; Migonney, V.; Falentin-Daudré, C. Review of titanium surface modification
592 techniques and coatings for antibacterial applications. Acta Biomater. 2019, 83, 37-54.

593 56. Narendrakumar, K.; Kulkarni, M.; Addison, O.; Mazare, A.; Junkar, I.; Schmuki, P.; Sammons, R.; Iglic, A.
594 Adherence of oral streptococci to nanostructured titanium surfaces. Dent. Mater. 2015, 31, 1460-1468.

595 57. Ercan, B.; Taylor, E.; Alpaslan, E.; Webster, T.J. Diameter of titanium nanotubes influences anti-bacterial
596 efficacy. Nanotechnology 2011, 22, NA 295102.

597 58. Barbour, E.; Gandhi, N.; El-Turki, A.; Oullivan, D.J., Jagger, C. Differential adhesion of Streptococcus
598 gordonii to anatase and rutile titanium dioxide surfaces with and without functionalization with
599 chlorhexidine. J. Biomed. Mater. Res., Part A 2009, 90A, 993-998.

600 59. Mei, S.; Wang, H.; Wang, W.; Tong, L.; Pan, H.; Ruan, C.; Ma, Q.; Liu, M.; Yang, H.; Zhang, L.; Cheng, Y.;
601 Zhang, Y.; Zhao, L.; Chu, P.K. Antibacterial effects and biocompatibility of titanium surfaces with graded
602 silver incorporation in titania nanotubes. Biomaterials 2014, 35, 4255-4265.

603 60. Alvarez, R.; García-Martín, J.M.; Macías-Montero, M.; González-García, L.; González, J.C.; Rico, V.;
604 Perlich, J.; Cotrino, J.; González-Elipe, A.R.; Palmero, A. Growth regimes of porous gold thin films

deposited by magnetron sputtering at oblique incidence: from compact to columnar microstructures. Nanotechnology 2013, 24, NA 045604.

61. Izquierdo-Barba, I.; García-Martín, J.M.; Álvarez, R.; Palmero, A.; Esteban, J.; Pérez-Jorge, C.; Arcos, D.; Vallet-Regí, M. Nanocolumnar coatings with selective behavior towards osteoblast and Staphylococcus aureus proliferation. Acta Biomater. 2015, 15, 20-28.

62. Lauzon, M.A.; Daviau, A.; Marcos, B.; Faucheux, N. Nanoparticle-mediated growth factor delivery systems: a new way to treat Alzheimer's disease. J. Control. Release 2015, 206, 187-205.

63. Slavin, Y.N.; Asnis, J.; Hafeli, U.O.; Bach, H. Metal nanoparticles: understanding the mechanisms behind antibacterial activity. J. Nanobiotechnology 2017, 15, NA 65

64. Baptista, P.V.; McCusker, M.P.; Carvalho, A.; Ferreira, D.A.; Mohan, N.M.; Martins, M.; Fernandes, A.R. Nano-Strategies to Fight Multidrug Resistant Bacteria—"A Battle of the Titans" Front. Microbiol. 2018, 9, NA 1441.

65. Wang, L.; Hu, C.; Shao, L. The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int. J. Nanomedicine 2017, 12, 1227-1249.

66. AlMatar, M.; Makky, E.A.; Var, I.; Koksal, F. The role of nanoparticles in the inhibition of multidrug-resistant bacteria and biofilms. Curr. Drug Deliv. 2017, 15, 470-484.

67. Bassegoda, A.; Ivanova, K.; Ramon, E.; Tzanov, T. Strategies to prevent the occurrence of resistance against antibiotics by using advanced materials. Appl. Microbiol. Biotechnol. 2018, 102, 2075-2089.

68. Hemeg, H.A. Nanomaterials for alternative antibacterial therapy. Int. J. Nanomed. 2017, 12, 8211-8225.

69. Natan, M.; Banin, E. From nano to micro: using nanotechnology to combat microorganisms and their multidrug resistance. FEMS Microbiol. Rev. 2017, 41, 302-322.

70. Dwivedi, S.; Wahab, R.; Khan, F.; Mishra, Y. K.; Musarrat, J.; Al-Khedhairy, A.A. Reactive oxygen species mediated bacterial biofilm inhibition via zinc oxide nanoparticles and their statistical determination. PLoS ONE 2014, 9, NA 111289.

71. Rudramurthy, G.R.; Swamy, M.K.; Sinniah, U.R.; Ghasemzadeh, A. Nanoparticles: alternatives against drug-resistant pathogenic microbes. Molecules 2016, 21, NA 836.

72. Glaeser, J.; Nuss, A.M.; Berghoff, B.A.; Klug, G. Singlet oxygen stress in microorganisms. Adv Microb Physiol. 2011, 58, 141-173.

73. Gurunathan, S.; Han, J.W.; Dayem, A.A.; Eppakayala, V.; Kim, J.H. Oxidative stress-mediated antibacterial activity of graphene oxide and reduced graphene oxide in Pseudomonas aeruginosa. Int. J. Nanomedicine 2012, 7, 5901-5914.

74. Su, Y.; Zheng, X.; Chen, Y.; Li, M.; Liu, K. Alteration of intracellular protein expressions as a key mechanism of the deterioration of bacterial denitrification caused by copper oxide nanoparticles. Sci. Rep. 2015, 5, NA 15824

75. Zakharova, O.V.; Godymchuk, A.Y.; Gusev, A.A.; Gulchenko, S.I.; Vasyukova, I.A.; Kuznetsov, D.V. Considerable variation of antibacterial activity of Cu nanoparticles suspensions depending on the storage time, dispersive medium, and particle sizes. Biomed. Res. Int. 2015, 2015, NA 412530.

76. Leung, Y.H.; Ng, A.M.; Xu, X.; Shen, Z.; Gethings, L.A.; Wong, M.T.; Chan, C.M.; Guo, M.Y.; Ng, Y.H.; Djurišić, A.B.; Lee, P.K.; Chan, W.K.; Yu, L.H.; Phillips, D.L.; Ma, A.P.; Leung, F.C. Mechanisms of antibacterial activity of MgO: non-ROS mediated toxicity of MgO nanoparticles towards Escherichia coli. Small 2014, 10, 1171-1183.

77. Navarre, W.W.; Schneewind, O. Surface Proteins of Gram-Positive Bacteria and Mechanisms of Their Targeting to the Cell Wall Envelope. Microbiol. Mol. Biol. Rev. 1999, 63, 174-229.

78. Beveridge, T.J. Structures of Gram-Negative Cell Walls and Their Derived Membrane Vesicles. J. Bacteriol. 1999, 181, 4725-4733.

79. Lesniak, A.; Salvati, A.; Santos-Martinez, M.J.; Radomski, M.W.; Dawson, K.A.; Åberg, C. Nanoparticle adhesion to the cell membrane and its effect on nanoparticle uptake efficiency. J. Am. Chem. Soc. 2013, 135, 1438-1444.

80. Lam, S.J.; O'Brien-Simpson, N.M.; Pantarat, N.; Sulistio, A.; Wong, E.H.H.; Chen, Y-Y.; Lenzo, J.C.; Holden, J.A.; Blencowe, A.; Reynolds, E.C.; Qiao, G.G. Combating multidrug-resistant Gram-negative bacteria with structurally nanoengineered antimicrobial peptide polymers. Nature Microbiology 2016, 1, NA 16162.

81. Kim, J.S.; Kuk, E.; Yu, K.N.; Kim, J.-H.; Park, S.J.; Lee, H.J.; Kim, S.H.; Park, Y.K.; Park, Y.H.; Hwang, C.-Y.; Kim, Y.-K.; Lee, Y.-S.; Jeong, D.H.; Cho, M.-H. Antimicrobial effects of silver nanoparticles. Nanomedicine 2007, 3, 95-101.

82. Payne, J.N.; Waghwani, H.K.; Connor, M.G.; Hamilton, W.; Tockstein, S.; Moolani, H.; Chavda, F.; Badwaik, V.; Lawrenz, M.B.; Dakshinamurthy, R. Novel synthesis of kanamycin conjugated gold nanoparticles with potent antibacterial activity. Front. Microbiol. 2016, 7, NA 607.

83. Shaker, M.A.; Shaaban, M.I. Formulation of carbapenems loaded gold nanoparticles to combat multi-antibiotic bacterial resistance: in vitro antibacterial study. Int. J. Pharm. 2017, 525, 71-84.

84. Huh, A.J.; Kwon, Y.J. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. J. Control. Release 2011, 156, 128-145.

85. Perelshtein, I.; Lipovsky, A.; Perkas, N.; Gedanken, A.; Moschini, E.; Mantecca, P. The influence of the crystalline nature of nano-metal oxides on their antibacterial and toxicity properties. Nano Res. 2015, 8, 695-707.

86. Gold, K.; Slay, B.; Knackstedt, M.; Gaharwar. A.K. Antimicrobial Activity of Metal and Metal-Oxide Based Nanoparticles. Adv. Therap. 2018, 1, NA 1700033.

87. Burdusel, A.-C.; Gherasim, O.; Grumezescu, A.M.; Mogoanta, L.; Ficai, A.; Andronescu E. Biomedical Applications of Silver Nanoparticles: An Up-to-Date Overview. Nanomaterials 2018, 8, NA 681.

88. Yan, X.; He, B.; Liu, L.; Qu, G.; Shi, J.; Hu, L.; Jiang, G. Antibacterial mechanism of silver nanoparticles in pseudomonas aeruginosa: Proteomics approach. Metallomics 2018, 10, 557-564.

89. Manikprabhu, D.; Lingappa, K. Anti-bacterial activity of silver nanoparticles against methicillin-resistant Staphylococcus aureus synthesized using model Streptomyces sp. pigment by photo-irradiation method. J. Pharm. Res. 2013, 6, 255-260.

90. Su, H.-L.; Lin, S.-H.; Wei, J.-C.; Pao, I.-C.; Chiao, S.-H.; Huang, C.-C.; Lin, S.-Z.; Lin J.-J. Novel nanohybrids of silver particles on clay platelets for inhibiting silver resistant bacteria. PLoS ONE 2011, 6, NA 21125.

91. Otari, S.V.; Patil, R.M.; Waghmare, S.R.; Ghosh, S.J.; Pawar, S.H. A novel microbial synthesis of catalytically active Ag–alginate biohydrogel and its antimicrobial activity. Dalton Trans. 2013, 42, 9966-9975.

92. Chernousova, S.; Epple, M. Silver as Antibacterial Agent: Ion, Nanoparticle, and Metal. Angew. Chem. Int. Ed. 2013, 52, 1636-1653.

93. Eckhardt, S.; Brunetto, P.S.; Gagnon, J.; Priebe, M.; Giese, B.; Fromm, K.M. Nanobio Silver: Its Interactions with Peptides and Bacteria, and Its Uses in Medicine. Chem. Rev. 2013, 113, 4708-4754.

94. Thapa, R.; Bhagat, C.; Shrestha, P.; Awal, S.; Dudhagara, P. Enzyme-mediated formulation of stable elliptical silver nanoparticles tested against clinical pathogens and MDR bacteria and development of antimicrobial surgical thread. Ann. Clin. Microbiol. Antimicrob. 2017, 16, 1-10.

95. Siddiqi, K.S.; Husen, A.; Rao, R.A.K. A review on biosynthesis of silver nanoparticles and their biocidal properties. J. Nanobiotechnology 2018, 16, NA 14.

96. Ramalingam, B.; Parandhaman, T.; Das, S.K. Antibacterial effects of biosynthesized silver nanoparticles on surface ultrastructure and nanomechanical properties of gram-negative bacteria viz. Escherichia coli and Pseudomonas aeruginosa. ACS Appl. Mater. Interfaces 2016, 8, 4963-4976.

97. Roy, A.; Bulut, O.; Some, S.; Mandal, A.K.; Yilmaz, M.D. Green synthesis of silver nanoparticles: biomolecule-nanoparticle organizations targeting antimicrobial activity. RSC Advances 2019, 9, 2673-2702.

98. Santos, L.M.; Stanisic, D.; Menezes, U.J.; Mendonça, M.A.; Barral, T.D.; Seyffert, N.; Azevedo, V.; Durán, N.; Meyer, R.; Tasic, L.; Portela, R.W. Biogenic Silver Nanoparticles as a Post-surgical Treatment for Corynebacterium pseudotuberculosis Infection in Small Ruminants. Front Microbiol. 2019, 10, NA 824.

99. Xie, K.; Zhou, Z.; Guo, Y.; Wang, L.; Li, G.; Zhao, S.; Liu, X.; Li, J.; Jiang, W.; Wu, S.; Hao, Y. Long-Term Prevention of Bacterial Infection and Enhanced Osteoinductivity of a Hybrid Coating with Selective Silver Toxicity. Adv. Healthc. Mater. 2019, 8, NA 1801465.

100. He, W.; Zhou, Y.T.; Wamer, W.G.; Hu, X.; Wu, X.; Zheng, Z.; Boudreau, M.D.; Yin, J.J. Intrinsic catalytic activity of Au nanoparticles with respect to hydrogen peroxide decomposition and superoxide scavenging. Biomaterials 2013, 34, 765-773.

101. Tao, Y.; Ju, E.; Ren, J.; Qu, X. Bifunctionalized mesoporous silica supported gold nanoparticles: intrinsic oxidase and peroxidase catalytic activities for antibacterial applications. Adv. Mater. 2015, 27, 1097-1104.

102. Zheng, K.; Setyawati, M.I.; Leong, D.T.; Xie, J. Antimicrobial Gold Nanoclusters. ACS Nano 2017, 11, 6904-6910.

103. Shamaila, S.; Zafar, N.; Riaz, S.; Sharif, R.; Nazir, J.; Naseem, S. Gold Nanoparticles: An Efficient Antimicrobial Agent against Enteric Bacterial Human Pathogen. Nanomaterials 2016, 6, NA 71.

104. Ibelli, T.; Templeton, S.; Levi-Polyachenko, N. Progress on utilizing hyperthermia for mitigating bacterial infections. Int. J. Hyperthermia 2018, 34, 144-156

105. Zhao, Y.; Guo, Q.; Dai, X.; Wei, X.; Yu, Y.; Chen, X.; Li, C.; Cao, Z.; Zhang, X. A biomimetic non-antibiotic approach to eradicate drug-resistant infections. Adv. Mater. 2019, 31, NA 1806024.

106. Raghunath, A.; Perumal, E. Metal oxide nanoparticles as antimicrobial agents: a promise for the future. Int. J. Antimicrob. Agents 2017, 49, 137-152.

107. Singh, R.; Smitha, M.S.; Singh, S.P. The role of nanotechnology in combating multi-drug resistant bacteria. J. Nanosci. Nanotechnol. 2014, 14, 4745-4756.

108. Kadiyala, U.; Kotov, N.A.; Van Epps, J.S. Antibacterial metal oxide nanoparticles: challenges in interpreting the literature. Curr. Pharm. Des. 2018, 24, 896-903.

109. Garino, N.; Sanvitale, P.; Dumontel, B.; Laurenti, M.; Colilla, M.; Izquierdo-Barba, I.; Cauda, V.; Vallet-Regí, M. Zinc oxide nanocrystals as a nanoantibiotic and osteoinductive agent. RSC Adv. 2019, 9, 11312-11321.

110. Arias, L.S.; Pessan, J.P.; Miranda Vieira, A.P.; Toito de Lima, T.M.; Botazzo Delbem, A.C.; Monteiro, D.R. Iron Oxide Nanoparticles for Biomedical Applications: A Perspective on Synthesis, Drugs, Antimicrobial Activity, and Toxicity. Antibiotics 2018, 7, NA 46.

111. Chaurasia, A.K.; Thorat, N.D.; Tandon, A.; Kim, J.H.; Park, S.H.; Kim, K.K. Coupling of radiofrequency with magnetic nanoparticles treatment as an alternative physical antibacterial strategy against multiple drug resistant bacteria. Sci Rep. 2016, 6, NA 33662.

112. Novickij, V.; Stanevičienė, R.; Vepštaitė-Monstavičė, I.; Gruškienė, R.; Krivorotova, T.; Sereikaitė, J.; Novickij, J.; Servienė, E. Overcoming Antimicrobial Resistance in Bacteria Using Bioactive Magnetic Nanoparticles and Pulsed Electromagnetic Fields. Front. Microbiol. 2017, 8, NA 2678.

113. Paris, J.L.; Manzano, M.; Cabañas, M.V.; Vallet-Regí, M. Mesoporous silica nanoparticles engineered for ultrasound-induced uptake by cancer cells. Nanoscale 2018, 10, 6402-6408.

114. Montalvo-Quiros, S.; Aragoneses-Cazorla, G.; Garcia-Alcalde, L.; Vallet-Regí, M.; González, B.; Luque-Garcia, J.L. Cancer cell targeting and therapeutic delivery of silver nanoparticles by mesoporous silica nanocarriers: insights into the action mechanisms using quantitative proteomics. Nanoscale 2019, 11, 4531-4545.

115. Villegas, M.R.; Baeza, A.; Noureddine, A.; Durfee, P.N.; Butler, K.S.; Agola, J.O.; Brinker, C.J.; Vallet-Regí, M. Multifunctional Protocells for Enhanced Penetration in 3D Extracellular Tumoral Matrices. Chem. Mater. 2018, 30, 112-120.

116. Pedraza, D.; Díez, J.; Izquierdo-Barba, I.; Colilla, M.; Vallet-Regí, M. Amine-Functionalized Mesoporous Silica Nanoparticles: A New Nanoantibiotic for Bone Infection Treatment. Biomedical Glasses 2018, 4, 1-12.

117. González, B.; Colilla, M.; Díez, J.; Pedraza, D.; Guembe, M.; Izquierdo-Barba, I.; Vallet-Regí, M. Mesoporous silica nanoparticles decorated with polycationic dendrimers for infection treatment. Acta Biomater. 2018, 68, 261-271.

118. Martínez-Carmona, M.; Izquierdo-Barba, I.; Colilla, M.; Vallet-Regí, M. Concanavalin A-targeted mesoporous silica nanoparticles for infection treatment. Acta Biomater. 2019, https://doi.org/10.1016/j.actbio.2019.07.001

Title of Site. Available online: URL (accessed on Day Month Year).