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Antimicrobial nanoparticle coatings for medical implants: design challenges and prospects

Running title: Antimicrobial nanoparticle coatings

Running Authors: Li et al.

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Microbial colonization, infection and biofilm-formation are major complications in the use of implants and are the predominant risk factor in implant failure. Although aseptic surgery and administration of antimicrobial drugs may reduce the risk of infection, systemic use of antibiotics can lack efficacy, increase the risk of tissue toxicity and development of drug resistant infections. To reduce implant related infections, antimicrobial materials are increasingly being investigated and applied to implant surfaces using various methods depending on the agents and their microbicidal mechanisms. Through the development of biomaterials and nanotechnology, antimicrobial nanoparticles are becoming promising candidates for implant coatings, as their multifactorial antimicrobial mechanisms combat microbial adherence, viability and biofilm formation. Despite their antimicrobial promise, the application of nanoparticles onto implant surfaces while retaining their antimicrobial potency faces many challenges. Herein, we review the potential and challenges associated with the design and implementation of antimicrobial nanoparticle coatings for the medical implant industry, particularly focusing on manufacturing considerations, sterilization, long-term stability, protein fouling, regulation and safety, with a view to provide researchers the necessary tools to aid translation of materials from the bench to the clinic.

I. INTRODUCTION

Medical implants have been widely applied clinically to overcome the disfunction or loss of specific tissues. Generally, integration of an implant with its surrounding tissue is desired in order to achieve optimal functional repair. However, implant surfaces that encourage mammalian cell attachment also tend to provide an ideal environment for

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bacterial growth[1]. In this case, host cells need to compete for adhesion to the surface with microorganisms that may be present, commonly termed "the race for the surface"[2]. If pathogens adhere first, there will be a high risk of microbial colonization on the biomaterial surface to form biofilms and cause infection. Although the prophylactic measures and aseptic surgery techniques have effectively reduced the incidence rate of implant-related infections, the infection rate remains significant[3]. For example, the incidence of implant-related infections was reported to be 5%-10% in orthopedic trauma patients[4]. Implant-related infections can lead to serious consequences, such as implant failure, requirements for revision surgery, possible systemic infections and sepsis, which can cause severe health and financial impacts on patients and the healthcare system[5, 6]. Current treatments rely on local or systemic administration of antibiotics, but microorganisms within biofilms can be recalcitrant to antibiotics, leading to persistence or recurrence of infections. Furthermore, antimicrobial resistance is an increasing global challenge, reducing the efficacy and range of antibiotic and antifungal drugs available, and high doses of these drugs can be toxic to human tissues[7].

Therefore, in order to prevent and combat biomaterial-associated infections, different designs of medical implants have been explored to inhibit biofilm formation on implants, such as changing the surface topology or chemistry to inhibit bacterial attachment and local administration of antimicrobials through targeted delivery or surface coatings. An attractive alternative is to deliver antimicrobial drugs adjacent to an implant by loading them in an injectable hydrogel. This approach was demonstrated by loading gentamicin in a thermo-responsive hyaluronic acid-based hydrogel, which showed good antibacterial efficacy when injected over an implant in a rabbit model[8]. Another recent report described

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a novel bio-orthogonal strategy using systemic injection of a prodrug that was activated at a local site to treat infections, which could reduce the side effects of systemic administration of antibiotics; but this requires further evaluation *in vivo* and is restricted by the need to identify suitable prodrugs[9].

A variety of promising designs for local administration of antimicrobial agents via implanted biomaterials has been explored. Local delivery can be achieved by coating the agents onto biomaterial surfaces or loading them into the bulk of medical implants. Some agents may effectively inhibit microbial adhesion and/or kill microbes in the vicinity of the surface whilst attached to the surface, but many agents need to be released in order to exert their antimicrobial effects. Comparing with loading agents in the bulk material of an implant, surface coating is often preferable due to provision at the site of need and minimal impact on the bulk material properties, as well as being applicable to established devices post-manufacture, reducing development costs. Antimicrobial coatings could be applied on a wide range of medical devices, such as catheters, implants, guide wires, and surgical tools, which points to the significant potential for applications of antimicrobial coatings Hence, there is great market potential for antimicrobial coatings for medical devices, with an estimated global market by 2026 of 2.88 billion USD[10].

As shown in Fig.1, there are several different potential strategies to create antimicrobial coatings on surfaces[11–13]: 1) immobilizing functional groups on the surfaces or changing the surface topology to prevent bacterial adhesion and biofilm formation; 2) coating antimicrobial agents on the surfaces of an implant to damage pathogens after being released from the surface; 3) immobilizing antimicrobial agents on implant surfaces to act only on pathogens that contact the surface; 4) combining anti-adhesive

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moieties with antimicrobial properties in a surface coating to prevent microorganism attachment while also damaging pathogens that approach the surface; 5) coating a bioactive layer to encourage host tissue integration to inhibit biofilm formation.

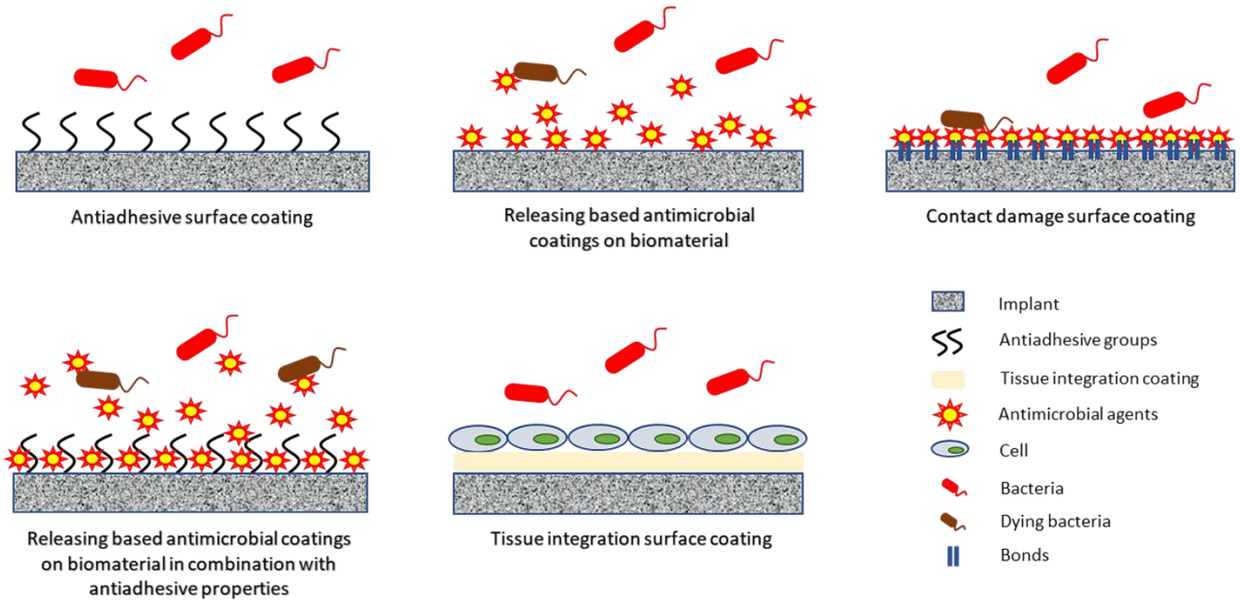


Fig. 1. Different potential design strategies for antimicrobial surface coatings.

Infections and biofilms on implant surfaces can contain polymicrobial populations of one or more strains of Gram-positive or Gram-negative bacteria or fungi. However, the majority of research on antimicrobial biomaterial coatings to date has focussed on bacterial infections, often studying the effect of coatings on only one type of bacteria at a time. There are numerous different antimicrobial agents that could be used for biomaterial surface coating applications, including antibiotics, antimicrobial nanoparticles (AMNPs), antimicrobial peptides (AMPs), antibacterial enzymes, antimicrobial polymers, and antifungal drugs. Several antimicrobial surface coating products have been made available commercially. For example, RepelaCOAT® is a

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coating product for medical devices based on sustained release of silver salts and antibiotics from a supporting polymer[14]. Another example is Kastus®, which is a transparent film based on nanotechnology that could be coated on glass and ceramics, damaging bacteria through reactive oxygen species[15].

A. Antibiotics and antifungal drugs

With known antibacterial spectra, antibiotics have been used as eluting drugs in a range of medical devices, such as gentamicin with its broad spectrum against most Gram-positive and Gram-negative bacteria[16]. Considering the targeted pathogens and the risk of developing drug resistance, they can be applied individually or in combination with other antimicrobial agents. For example, rifampin combined with minocycline or novobiocin was coated on indwelling catheters and showed bactericidal effectiveness against biofilm-associated staphylococci[17]. *In vivo* tests showed that a gentamicin-loaded poly(D,L-Lactide)(PDLLA) coating on titanium implants prevented local infections[18]. Gentamicin loaded in polymethyl methacrylate (PMMA) was also reported to be an effective drug releasing system to prevent or treat orthopaedic implant-related infections; however, such systems may induce a high risk of antibiotic resistance if the released dose falls below the minimum bactericidal level[19]. In certain cases, antimicrobial molecules can retain their bioactivity when immobilized on biomaterial surfaces. Griesser and coworkers have reported antifungal and anti-biofilm activity by covalently binding antifungal agents to surfaces[20].

A further challenge arises as many bacterial species tend to resist to more than one type of antibiotic due to the overuse of antibiotics and the evolution of bacteria, which leads to complexity in the treatment of infections. Antifungal drugs face similar

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challenges as fungal infections cause significant morbidity and mortality, and resistance to antifungal drugs is also emerging[7, 21, 22]. Therefore, simply applying antimicrobial drugs to be eluted from a surface coating is not an ideal option to prevent infections and biofilms associated with medical implants.

B. Antimicrobial peptides as coatings on biomaterials

Antimicrobial peptides (AMPs) are a group of peptides that contain different numbers of amino acids, with molecular weights usually less than 10 kDa[23]. One of the first reports of AMPs was published in 1939[24–26], and they were later reported to be widely existing in nature, being produced in human cells, plant cells, bacteria and fungi[23, 27]. Nowadays, both natural and synthetic AMPs are applied in antimicrobial research.

Due to AMPs' antimicrobial activity, bioactive functions and potential for biocompatibility, they have been applied as coatings on medical implants in biomedical research. For example, Kazemzadeh-Narbat *et al.*[28] reported that the AMP Tet213 loaded in a calcium phosphate layer on titanium could inhibit both *Staphylococcus aureus* and *Pseudomonas aeruginosa*, but it was nontoxic to osteoblast-like cells. A surface structured with the AMP melittin was reported to inhibit both Gram-positive and Gram-negative bacteria[29]. In addition, AMPs can be synthesised in the form of nanoparticles to act as antibacterial agents. Lam *et al.*[30] synthesized star-shaped peptide polymer nanoparticles, that were able to combat multi-drug resistant Gram-negative bacteria in an animal model. These findings indicate a remarkable potential to apply AMPs to inhibit implant-associated infections.

However, AMPs can be degraded by proteases in the body, and they are often cytotoxic towards human cells. Furthermore, there are challenges in their application as

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antimicrobial coatings on solid substrates. Their antimicrobial activity may be adversely altered by the binding mechanism used to attach them and could be reduced or even inactivated when they are attached to a surface[31]. For instance, in a layer-by-layer assembly process, AMPs may not be able to directly interact with pathogens, and their diffusion and release may be hindered[32]. AMPs, like other surface coatings, may also be subject to fouling in physiological fluids, particularly by serum proteins, which may block their antimicrobial activity. In addition, other factors, such as concentration and arrangement of AMPs on a surface, can affect their antimicrobial activity[32]. These factors make it challenging to develop sufficiently robust coatings for use on medical devices using AMPs.

C. Nanoparticles as antimicrobial coatings on biomaterials

Antimicrobial nanoparticles (AMNPs) have been studied for their potential use and mechanisms of action against different microbial pathogens. They can be divided into different categories based on their material composition. This includes metallic, oxide, ceramic, organic, polymeric, and composite particless. Examples of particles in each of these classes are described below. Commonly studied metallic NPs include silver[33-36] and gold[37,38], while silver is the most common one (Table 1). Examples of oxide NPs include zinc oxide[39], titanium dioxide[40], aluminium oxide[41] and copper oxide[42]. Commonly studied inorganic non-metallic NPs include selenium[43, 44], carbon[45] and tellurium[46] NPs. An example of polymeric NPs is the structurally nanoengineered antimicrobial peptide polymers (SNAPPs)[30]. Comparing to a solid layer coating, a nanoparticle coating can have many advantages, such as reduced material usage, higher

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antimicrobial efficacy and lower toxicity. For example, the use of Ag NPs as antibacterial coatings on medical implants was investigated due to the toxic effects that bulk silver brings to the blood and tissues[47]. Besides, bulk silver showed insufficient antimicrobial activity as its antibacterial activity only relies on the release of silver ions[35, 48]. In contrast, both Ag NPs themselves and the silver ions released from these Ag NPs have antibacterial activity, and the Ag NPs could show higher release rate of silver ions because of their high surface area to volume ratio. On the other hand, some elements like selenium have low solubility under physiological conditions, and their elemental form is unlikely to release ions meaning they cannot perform their antibacterial activity through the release of ions, while their nanoparticle form has favourable antibacterial properties[44].

AMNPs could be delivered as coatings decorated on medical implants, as depicted in Fig.1. Based on previous research, AMNPs can disturb bacterial functions through a complicated combination of mechanisms[49]. Thus, it may be difficult for pathogens to develop antimicrobial resistance (AMR) to AMNPs, which provides them with a significant potential advantage in combating infections. For example, silver NPs (Ag NPs), the most commonly used elemental nanoparticles for antimicrobial applications, have been shown to have broad-spectrum antibacterial activity through multiple mechanisms, and ionic silver can also be released from the NPs to enhance their bactericidal effects[33–35]. Ag NPs coated on plasma polymerized surfaces on a variety of types of substrates through electrostatic attraction have been shown to exhibit good antibacterial ability[50]. Ag NPs can also be covalently bound on plasma-polymers, showing both antimicrobial and cyto-compatible properties[51]. However, there is a lack of data on the release of Ag NPs and *in vivo* analysis for such surfaces, and their

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mechanisms of action need to be further explored. In addition, local argyria leading to skin discoloration and cytotoxicity can be caused by coatings based on silver[19].

Selenium, as an essential trace element, can also be formed into nanoparticles with potential in antimicrobial coatings. Direct surface coating with silver or selenium nanoparticles (Se NPs) can be achieved by *in situ* chemical synthesis in the presence of biomaterial samples[52]. For example, coating Se NPs (at a concentration lower than 31μg/mL) on polyvinyl chloride did not induce cytotoxicity on fibroblasts *in vitro*, indicating potential for application in endotracheal tube coatings to prevent ventilator-associated pneumonia[53]. Additionally, Se NPs with a diameter of 30-70 nm were demonstrated as an anti-infective coating on titanium to fight methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus epidermidis in vivo*[43]. This confirmed that non-metallic inorganic nanoparticles could also be applied on medical implants to prevent local infections, although their mechanisms of action were not fully elucidated. Besides biocompatibility, other factors including stability, degradation, metabolism, biodistribution, and *in vivo* antimicrobial ability, all needed to be considered when applying nanoparticles as antimicrobial coatings on biomaterials.

In this review, we highlight the potential and challenges of antimicrobial nanoparticle coatings to prevent infection and biofilm formation on medical implants. All types of implant surfaces are considered, noting that the majority of references published to date have focused on metal substrates. Issues including manufacturing requirements, sterilization, long-term stability, protein fouling, regulation and safety are addressed. Table 1 summarizes published studies on nanoparticles as surface coatings on biomaterials with potential in antimicrobial applications in the last five years (Web of

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Science search: 'nanoparticle' and 'implant' and 'surface coating'), where silver is the most popular NP element and titanium is the major biomaterial.

Table 1 Selected studies reported 2016-2020 on applications of nanoparticles as surface coatings on biomaterials, highlighting NP compositions and sizes, additional coating components and methods, and their reported functions.

NPs	Combinations	Coating methods	Coating materials	NPs sizes	Functions	Ref.
Ag NPs	TiO_2 nanotubes, then multilayer films of chitosan and dialdehyde alginate	dip-coating and UV exposure	titanium	N/A	antibacterial, osteogenic	54
Ag NPs	HAp	Ag mirror reaction	titanium	ca. 20 nm, 25 nm	antibacterial, osteogenic	55
Ag NPs		*in situ* reduction by PDA	porous titanium	30-50 nm	promoting mineralization	56
Ag NPs	chitosan/hyaluronic acid multilayer	layer-by-layer	titanium	30 nm	antimicrobial	57
Ag NPs	polypropylene, polyethylene glycol	dip-coating	titanium pedicle screw	N/A	antimicrobial	58
Ag NPs	diamond-like carbon	dip-coating with PVP/Ag NPs, then plasma immersion ion implantation-induced densification	titanium	avg. 9 nm, max. 30 nm	antimicrobial	59
Ag NPs	HAp coatings with oriented block arrays	facile Ag mirror reaction	Ti6Al4V alloy	10-30 nm	bactericidal, osteoinductive	60
Ag NPs		direct liquid injection atomic layer deposition	open-porous titanium	avg. 49 nm	osseointegration	61
Ag NPs	graphene oxide and type I collagen, hybrid coating	UV exposure to form graphene oxide/Ag NPs, then dip-coating PDA/Ti	titanium	ca. 6 nm	bactericidal	62
Ag NPs	loaded in TiO_2 nanorods with PDA	soak in $AgNO_3$ solution then UV exposure	titanium alloy	ca. 30 nm, 25 nm	long-lasting bactericidal	63, 64

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Ag NPs		ion implantation + physical vapor deposition by magnetron sputtering + annealing	titanium	avg. 58 nm	antibacterial	[65]
Ag NPs		electrochemical deposition	Ti6Al4V alloy	5 nm, 30 nm	cytocompatible, antibacterial	[66]
Ag NPs		plasma electrolytic oxidation	ZrNb alloy	ca. 27 nm	antibacterial	[67]
Ag NPs	in Ca-P coating	electrochemical deposition	titanium	N/A	antimicrobial	[68]
Ag NPs		aerosol-assisted chemical vapor deposition	316L stainless steel	varied, 100-300 nm had best performance	anticorrosion	[69]
Ag NPs	within a titanate nanowire film, capped with chitosan	UV reduction	titanium	3-5 nm	antibacterial	[70]
Ag NP-loaded oxidized carbon nanotube	coated by dopamine-grafted chitosan and sulfonated heparin-like polymer	spray-coating assisted layer-by-layer assembly	PVDF, silicon, glass, PVC	ca. 10-30 nm	antimicrobial	[71]
Ag or Au NPs		*in situ* reduction by PDA	magnesium alloy (AZ31)	Ag: ca. 168 nm; Au: ca. 151 nm	cytocompatible, antibacterial, anticorrosion	[37]
Ag/Pt nanopatches		sputtering	titanium	Nanopatches: 1.3–3.9 nm in thickness, 3–60 nm in extension	antibacterial	[72]
Ag NPs	Ag NPs on TiO_2 to form photocatalyst, then dodecyl-sulfate to obtain hydrophobicity	coating Ag NPs on TiO_2 by photodeposition then spraying on substrate	titanium	N/A	photocatalytic antibacterial activity	[73]
Ag NP and Au NPs		covalent binding through silanization on titanium surface	titanium	30 nm	cytocompatible, antibacterial	[74]
silica-gentamicin NPs	with a chitosan and gelatin coating	electrophoretic deposition	titanium	avg. 200 nm	bioactive, antibacterial	[75]

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chitosan-58S bioactive glass nanocomposite	TiO_2 nanotube layer	dip-coating	titanium	743 nm - 392 μm	bioactive	[76]
gentamicin-loaded mesoporous silica NPs		drop-by-drop	6 precursor layers of polyelectrolytes	N/A	prolonged and continuous antibacterial and anti-biofilm	[77]
Au NPs		sedimentation	gold-coated silicon wafer	100-150 nm	bioactive, antibacterial	[78]
Au NPs		*in situ* growth on surface through reduction by PDA coated on PCL	PCL	N/A	osteogenic	[79]
HAp/TiO2 nanocomposite		pulse electrodeposition	Ti6Al4V alloy	80-120 nm	anticorrosion	[80]
Mytilus edulis foot protein-1/Ag NPs	constructed on titania nanotubes	dip-coating	titanium	ca. 10 nm	cytocompatible, antibacterial	[81]
tetracycline-loaded 57S mesoporous bioactive glass	polyelectrolyte multilayer (collagen, chitosan, γ-poly-glutamic acid)	spin-coating	316L stainless steel	500 nm	antibacterial, osteoconductive	[82]
PLGA(Ag-Fe_3O_4) composites		coating under an extracorporeal magnetic field	implant tooth	N/A	antibacterial, osteogenic	[83]
norfloxacin-loaded PLGA NPs		layer-by-layer (alternating with chitosan)	titanium	140 nm	bacteriostatic	[84]
CuO NPs	with polydimethylsiloxane-SiO_2 as nanocomposite coating	dip-coating	316L stainless steel	rod-like shape with size ca. 200 nm	biocompatible, antibacterial, anticorrosion	[42]
Se NPs		*in situ* growth on surface through reduction reaction	titanium	near-spherical 30-70 nm	anti-infective	[43]
Strontium titanate NPs	with TiO_2 nanotube to form a heterostructure	hydrothermal method	microporous titanium	N/A	osteogenic	[85]
ZnO NPs		layer-by-layer	polystyrene pegs (Calgary Biofilm Device)	pyramids, spheres and plates: 3.5 - 20 nm	antibacterial	[39]

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Squaraine-functionalized ZnO NPs		N/A	titanium	ca. 24 nm	antibacterial and antibiofilm	[86]
MoS_2 nanosheets	on TiO_2 nanotube with PDA-RGD	hydrothermal method	titanium foils	N/A	antimicrobial, biocompatible, osteointegration	[87]
nanodiamond		dip-coating	3D-printed titanium	120 nm	antifouling	[45]
nanodiamond		ultrasonicating substrates in the nanodiamond solution	glass	5-10 nm	cellular interaction	[88]

HAp: hydroxyapatite, PCL: polycaprolactone, PDA: polydopamine, PLGA: poly(lactic-co-glycolic acid), PVC: polyvinyl chloride, PVDF: polyvinylidene fluoride, PVP: polyvinylpyrrolidone.

II. MANUFACTURING OF ANTIMICROBIAL NANOPARTICLE COATINGS

The promise of AMNPs as coatings on biomaterials arises particularly due to their multimodal antimicrobial mechanisms. The antimicrobial actions of AMNPs can occur at the NP-microbe interface or intracellularly once the NPs are taken up by the cells. Their mechanisms can include generating reactive oxygen species, disturbing membrane permeability, interrupting electron transport across the cell membranes, damaging DNA and proteins, affecting organelle function or ATP production, and inhibiting quorum sensing[49, 89]. At least some of these mechanisms require the NPs to be released from a surface in order to interact closely with the microbial cell membranes or be taken up by the cells[33]. A NP releasing coating can thus exploit the multiple antimicrobial mechanisms of AMNPs whilst also localizing the administration of the NPs to the implant site, thereby minimizing losses and side effects that may occur with systemic delivery.

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For AMNPs to be used in surface coatings, the synthesis and properties of the NPs should first be well controlled and characterized. Factors including the NP size, surface properties, required dose and release rate, biodistribution and metabolism *in vivo*, stability, and safety need to be thoroughly evaluated to inform the antimicrobial coating design. To date, chemical synthesis is the most common choice to produce NPs with desired properties, although biological synthesis is also possible for some types of AMNPs[90]. Taking Ag NPs as an example, the NP synthesis can be achieved simply through a reduction reaction between silver nitrate and sodium borohydride under certain mixing conditions[91], or through so called green synthesis based on the reaction between silver compounds and plant extracts or microorganisms[92]. However, the NPs obtained may be different in size, surface charge, or even morphology, all of which can impact their performance. Many newly developed synthesis methods remain at relatively early research stages and may not be suitable for large-scale production. In order to scale-up successfully, factors such as mixing hydrodynamics, temperature and pH control, batch-to-batch variability and quality control of critical NP properties will need to be thoroughly managed before being ready for use in the medical implant market.

On the other hand, the selection of coating mechanism is also significant for decorating NPs on the surface of an implant. Current surface coating methods include dip-coating based on either electrostatic force[93] or self-assembly[94], spin-coating[82], freeze-thaw processes[95], layer-by-layer assembly[96], plasma spraying[97], electrospinning[98], magnetron sputtering[99], electrochemical deposition[66] and pulsed electro-deposition[100], and covalent binding[74]. Although they are based on different techniques, commonly desired properties of the resulting antimicrobial surfaces are the same, which include

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controlled structure of the coated layer (surface roughness, porosity, etc.), coating homogeneity, practicability, functionality, biocompatibility, antimicrobial function and stability. The choice of the most appropriate coating scheme depends on the properties of the biomaterial surfaces and the nanoparticles, their antimicrobial mechanisms, the complexity of the process, and resulting manufacturing time and cost. For example, Ag NPs stabilized by 2-mercaptosuccinic acid were shown to be substrate-independent by modifying different substrates through allylamine plasma polymerization and to have similar silver content on different substrates with antimicrobial efficacy, which showed good potential for this method of applying Ag NPs as antimicrobial coatings[101]. However, it should be noted that another step was required to modify the surface of the substrates and only 2D surfaces were analysed in the research. It can be much harder to achieve coating homogeneity for complicated 3D structures such as a porous orthopaedic implant. Tran *et al.*[43] used *in situ* chemical formation of selenium nanoparticles on titanium plates and screws (Fig.2), which indicated the potential to apply Se NPs on 3D medical implants through a simple coating process. Similar coatings were also previously successfully applied on polymeric biomaterials[102]. In addition, it should also be noted that the weak bonding between the antimicrobial coating and the implant surface can lead to rapid release[103]. For many nanoparticle coatings, the nanoparticles are embedded in a layer of polymers to improve bonding strength to the surface[104], and the presence of this polymer layer can also provide controlled release capability to the coating[105]. The choice of the polymeric coating material depends on its bonding strength to the substrate, its chemical compatibility with antibacterial nanoparticles, the desired release mode, stability and biocompatibility.

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Although a range of methods has been demonstrated to create AMNP coatings on biomaterials, there is limited data on the release of the AMNPs from such surface coatings and their mechanisms of action, particularly *in vivo*. Thus, further analysis of their release from different coatings and bioactivity *in vitro* and *in vivo* is required to inform the design and robust manufacturing process selection for development of AMNP-coated medical implants. Moreover, from an industrial perspective, whether the coating process will increase the complexity of the manufacture and impact the quality control of the final products may be a bottleneck for NP-coated medical implants to move into the market. Therefore, it is vital to comprehensively analyse the above-mentioned factors during the early stages of the development of AMNPs coatings on medical devices, which may later affect the manufacturing process.

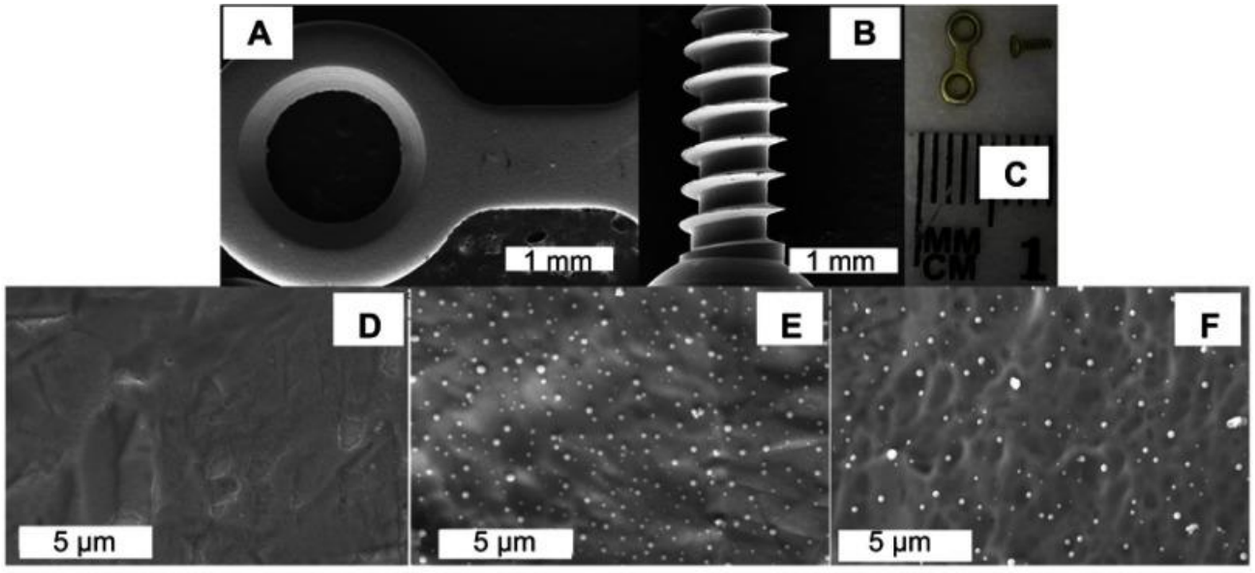


Fig. 2. Titanium implants used in *in vivo* experiments. A) and B) are scanning electron microscopy (SEM) images of titanium plate and screw (scale bar: 1 mm), C) is a photo of uncoated implants, D) is a SEM surface image of an uncoated titanium implant (scale bar: 5 µm), E) and F) are SEM images of Se NP coated titanium plate and screw surfaces

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(scale bar: 5 μm), where the white dots are Se NPs[43]. (From International Journal of Nanomedicine 2019, reproduced with permission from Dove Medical Press limited.)

In addition, there is a gap among researchers, industry and regulators in the development and application of nanomaterial-containing medical implants, which may potentially slow down the approval and use of medical devices associated with nanomaterials[106]. Jones, Mi and Webster[106] also pointed out that there is a lack of research evidence, funding, and long-term studies of nanomaterial-associated medical devices, compared to those for nanomedicine drugs or drug delivery systems (Fig.3). This could consequently affect the production of NPs-associated medical devices.

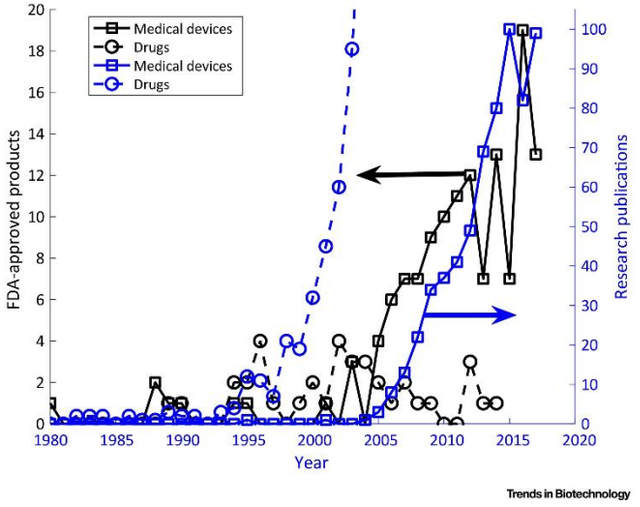


Fig. 3. Both research and regulatory product approvals for medical devices containing nanostructured materials have increased rapidly in the last two decades[106]. (Reprinted from Trends in Biotechnology, 37, Jones *et al.*, A Status Report on FDA Approval of Medical Devices Containing Nanostructured Materials, 117-120, Copyright (2019), with permission from Elsevier.)

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III. STERILIZATION OF NANOPARTICLE-COATED MEDICAL IMPLANTS

Before implantation, all implanted medical devices need to meet an acceptable sterility assurance level to reduce the risk of infection. Currently terminal sterilization methods that are commonly used in manufacturing of devices for clinical use include ethylene oxide (EtO), humid heat (autoclaving) and gamma radiation, which are recognized as established sterilization methods by regulatory authorities such as the US Food and Drug Administration (FDA)[107]. There are also other methods, such as ultraviolet (UV) exposure and ethanol disinfection, that may be useful, but they are not recognized as able to reach the sterility level required for clinical grade applications. Aseptic processing can also potentially be used for devices that cannot withstand terminal sterilization. All these methods aim to avoid viable pathogens being on or within medical implants to ensure patients' safety.

For AMNPs, the most common sterilization method is filtration, which is a commonly used method in pharmaceutical industry[108]. This has the advantage that it helps to retain the properties of nanoparticles which could be damaged during conventional terminal sterilization processes, depending on the AMNP composition. However, for use of AMNPs in surface coatings on medical implants, this would necessitate aseptic processes to apply the particles to the surfaces after filtration, which adds complexity and cost to the manufacture. Thus, taking account of the requirements for clinical use and market supply chains, it is preferable to sterilize the final product as a whole after packaging if possible.

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Autoclaving is one of the most widely used and simple sterilization methods. While not all biomaterials can withstand this treatment, it has been reported that autoclaving and ethanol treatment had no obvious influence on the size and morphology of peptide nanospheres in aqueous solution[109]. Tran *et al.*[43] used autoclaving to sterilize Se NP-coated titanium in their *in vivo* study and demonstrated that the implants retained their Se NP antibacterial effects. However, steam is corrosive to some metal implants, in which case their mechanical properties and biocompatibility can be diminished by the treatment. So, it may not applicable for all metal implants with AMNP coatings. Additionally, polymeric and biologically derived biomaterials often suffer structural and property changes when exposed to high temperatures, so may not be suitable to be autoclaved.

Property changes in both implant materials and surface coatings are observed not only in autoclaving, but also in other sterilization processes. For example, gamma irradiation can cause changes in the colour, molecular weight and mechanical strength of polymeric materials[110] as well as a crystalline phase change in Ag NPs[111]. Although ethylene oxide (EtO) has been widely applied as a standard sterilization method for clinical and research applications, it also changes the properties of some materials irreversibly. It has been reported that EtO changed mechanical strength of magnesium alloy samples even though it showed the best sterilization performance among EtO, steam, dry heat, and gamma radiation[112]. EtO may also be applied to certain types of nanoparticles, such as PEG-Au NPs[113], but it should be noted that particle aggregation[113] (Fig.4) and chemical changes of loaded drugs may occur due to EtO treatment[114]. In addition, residual EtO in the materials may lead to severe health issues if it is not fully

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evaporated after treatment. A challenge for design of AMNP coatings is that the most appropriate sterilization method depends on the specific types of nanoparticles and biomaterials to be used in a specific application scenario, so there is no clear theoretical basis to guide decisions. Comprehensive experimental evaluations are required to screen the optimal sterilization method for specific materials. Moreover, the potential changes of the properties of both the surface coating and bulk implant material need to be thoroughly considered, including ensuring that the antimicrobial properties of the AMNPs are retained after treatment.

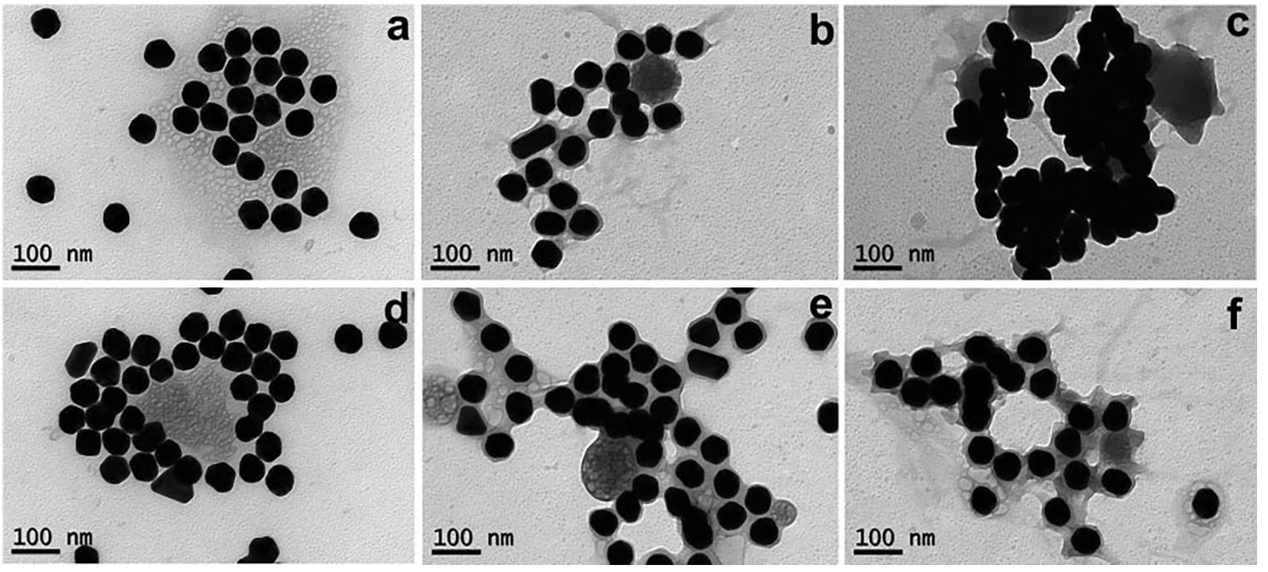


Fig. 4. Transmission electron microscopy characterization of the effect of sterilization methods on the size and morphology of PEG-Au nanoparticles: a) control, b) UV irradiation, c) gas-plasma treatment, d) ethylene oxide treatment, e) formaldehyde treatment, and f) autoclaving. (Reprinted with permission[113], Copyright (2010), Wiley.)

IV. LONG-TERM STABILITY

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The long-term stability considerations for AMNP coatings include the stability both before and after implantation. Once an antimicrobial medical implant is manufactured, it will be packed as sterile and stored for a certain time. From an industry and hospital perspective, the shelf life of the product is important to managing the manufacturing timing and supply chain. Coatings of antimicrobial agents should not only have similar stability from batch to batch, but also withstand the storage conditions and ideally retain their antimicrobial activity for a relatively long time until use (months to years). Well-controlled storage conditions (temperature, humidity, light exposure, etc.) may help preserve coatings. However, for coatings designed to release antimicrobial agents, degradation of the coating layer, leaking of active agents, cleavage of chemical bonds, and diffusion of active agents over time could create challenges for long-term storage of antimicrobial medical implants. This requires product design and highly controlled manufacturing conditions to ensure sufficient product stability during sterilization and storage.

A further challenge in the design of the stability coatings is optimizing their releasing regimen and the local doses of AMNPs that will be delivered after implantation. Non-covalently bound antimicrobial agents on surfaces often experience a high release rate or burst release at the early stages after implantation, which may be valuable to help reduce the postoperative infection risk. However, there is a higher risk of cytotoxicity to mammalian cells with a higher released dose, so this needs to be controlled. Makvandi *et al.*[89] reviewed some metal-based nanoparticles and their cytotoxicity in biomedical applications, indicating that cytotoxicity depends on various factors such as NP size, NP dose, and the NP location in upon uptake cells. The antimicrobial ability and cytotoxicity

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of the inorganic non-metallic NPs was also shown to depend on dose, size and surface chemistry[115, 44]. So, balancing the antimicrobial ability and cytocompatibility by controlling the coating composition and design, release regimen and dose remains challenging.

Meanwhile, although a high concentration of AMNPs at the early post-operative stage is expected to be helpful in preventing early infection post-implantation, longer-term release could also be beneficial to protect the implant surface from later microbial attachment and biofilm formation. Cloutier *et al.*[33] summarized the importance of long-term release of AM agents for tissue integration and revision surgery. However, it must be noted that the continuous release of antimicrobial agents at low doses could contribute to the development of antimicrobial resistance, leading to potentially worse outcomes for patients. Additionally, a recent report incorporated zinc with nano-crystalline hydroxyapatite, providing a biocompatible surface that encouraged bone tissue integration while potentially being antimicrobial[116]. This highlights the potential of bioactive-antimicrobial surface design. Enhanced tissue integration may not only help with 'the race for the surface'[2] therefore providing steric repulsion to the bacteria, but also enhance long-term stability of the implant thus to improve the rate of successful implantation. Further research on the combined biological and antimicrobial effects of such coatings would be valuable to verify this point of view. Thus, long-term stability needs to be well controlled with consideration of the shelf-life, release rates and biocompatibility of antimicrobial nanoparticles.

V. PROTEIN ADSORPTION

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After implantation, proteins will adsorb rapidly to the surfaces of most medical devices. This can result in immune activation due to protein denaturation, whilst also supporting host cell attachment and integration. A biomaterial that is implanted into the human body may be recognized as a foreign body and therefore induce cell-mediated immune responses. To reduce the risk of adverse host responses, it is essential for implanted biomaterials to be biocompatible in their target applications[117]. In addition, a protein-coated surface that supports human cells is also likely to be attractive to microbial pathogens, in which case these pathogens may transition from a planktonic to an adhered state, and lead to formation of a biofilm[118, 119]. Biofilms help pathogens not only to evade the immune system, but also to survive the presence of antimicrobial agents due to the low penetration ability of antimicrobials into the biofilm. Such biofilm-induced infections account for most biomaterial-associated infections, and can be caused by both non-specific pathogens within the surrounding environment or hospital-acquired pathogens existing in perioperative environments[11, 120]. However, it should be noted that protein adsorption on biomaterials can be guided through surface modification with antiadhesive moieties or specific ligands to encourage target protein adsorption and cell integration[119, 121].

Another important aspect of protein adsorption is the biocorona formed on released AMNPs. Once the NPs interact with body fluids, biomolecules, particularly serum proteins, will rapidly adsorb on them. These molecules form a layer on the surface of NPs, which may consequently alter or cause disfunction of the NPs. This layer is known as a biocorona. It includes both a 'soft' corona that contains proteins loosely attached and a 'hard' corona in which proteins are tightly adhered. Whether this protein

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corona will adversely affect the properties of AMNPs remains difficult to predict. Factors such as the charge and size of the NPs will impact how proteins adsorb, resulting in variations in the amount and composition of the biocorona formed on NPs[122,123]. This adsorbed material may lead to altered interaction of AMNPs with microbes and human cells and may also induce their phagocytosis by macrophages or reduce their antimicrobial efficacy[124]. For example, it has been shown that the uptake of Ag NPs and Se NPs by human cell lines changed after adsorption of blood proteins[125, 126], and this can also affect their antimicrobial ability[127]. This altered cellular uptake may also influence the cytotoxicity of AMNPs, which links to the safety of the application of AMNPs as surface coatings. Biocorona was also supposed to modulate the biotransformation of Ag NPs to Ag_2S[128], which was later observed in an *in vivo* study[61], indicating the important role of protein corona in cellular interaction with NPs. However, AMNPs can be designed to resist protein corona formation through controlled surface chemistry, which may help protect the AMNPs from immune responses *in vivo*[129] and restore their antimicrobial ability[127]. Ag NPs capped with PEG were reported to retain substantial antimicrobial capacity compared to starch-capped AgNPs after being conjugated with proteins (Fig.5), probably due to the reduced the interaction with and conformational changes of proteins[130]. Similar mitigated protein influence and enhanced bacterial targeting ability were also found in copper-based NPs with surface functionalization[131]. Therefore, it is important to thoroughly evaluate the influence of protein corona formation on AMNPs in designing systems for biomaterial surface coatings.

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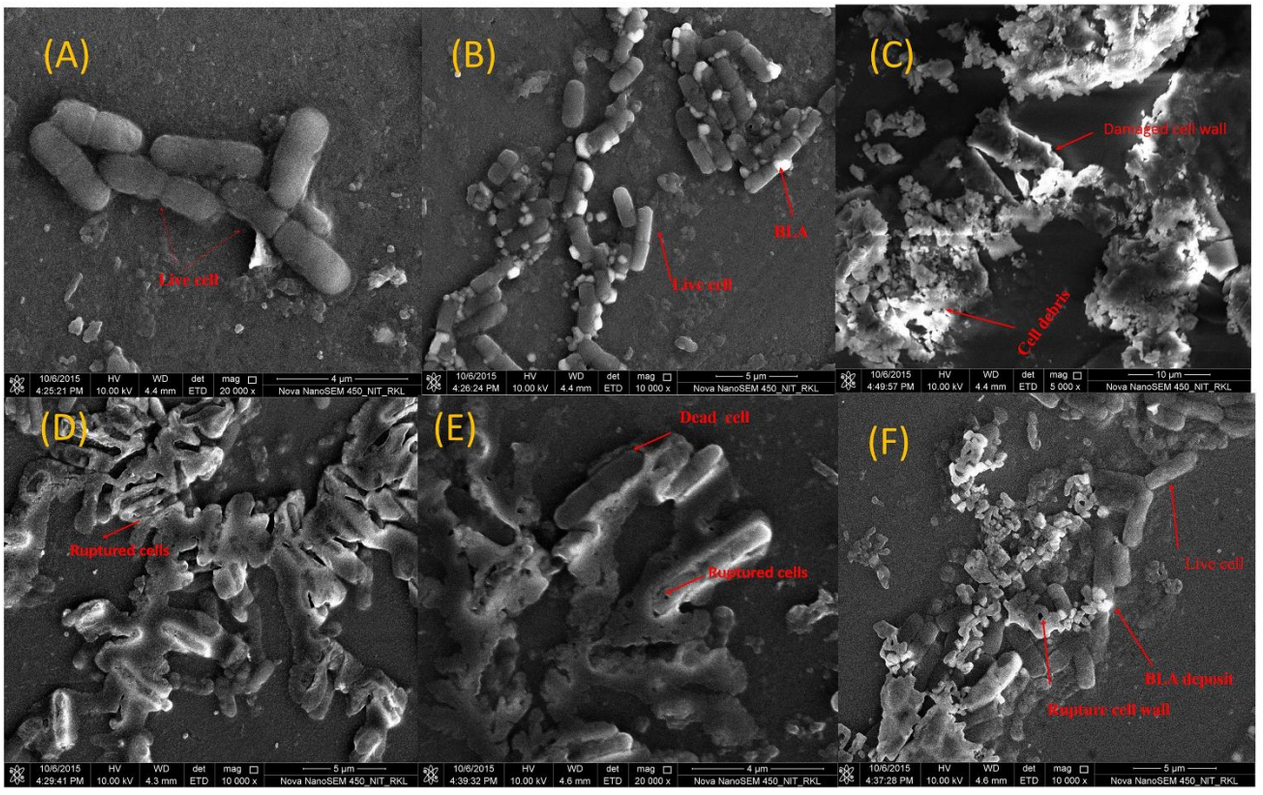


Fig. 5. Field emission scanning electron microscopy images of *E. coli* after treatment with: A) bacterial culture media alone; B) bovine α-lactalbumin (BLA); C) starch-capped Ag NPs; D) PEG-capped Ag NPs; E) PEG-capped Ag NPs conjugated with BLA; E) starch-capped Ag NPs conjugated with BLA[130]. (Reprinted from Colloids and Surfaces B: Biointerfaces, 146, D. K. Ban and B. S. Paul, Protein corona over silver nanoparticles triggers conformational change of proteins and drop in bactericidal potential of nanoparticles: Polyethylene glycol capping as preventive strategy, 577-584, Copyright (2016), with permission from Elsevier.)

VI. SAFETY OF NANOPARTICLES AS ANTIMICROBIAL COATINGS

Another important consideration associated with the application of nanoparticles is safety. Safety and efficacy are the two key requirements for a medical device.

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Nanoparticles have been approved to enter the market or be used in clinical trials for drug delivery for several years. Anselmo and Mitragotri[132, 133] comprehensively reviewed the nanomedicines that were approved by FDA and/or European Medicines Agency (EMA), most of which are related to cancer treatments. For medical devices, the FDA "does not categorically judge all products containing nanomaterials … as intrinsically benign or harmful" and numerous products containing nanomaterials have received approval to date[134]. In Australia, five nanoparticle-containing products are registered as therapeutic goods on the Australian Register of Therapeutic Goods, such as ABRAXANE (nanoparticle albumin-bound paclitaxel) for cancer treatment[135]. In addition, even though ZnO and TiO_2 NPs are often used in sunscreens as a skin contacting ingredient[135], the Australian Therapeutic Goods Administration regularly reviews the safety of ZnO and TiO_2 NPs in sunscreens[136, 137]. Another consideration is the future safety of the use of nanoparticles. Not only health issues but also environmental risks that may cause health problems should be included in the development and use of nanoparticles, although this may not be well-controlled under current government policies[106].

Controlled release of AMNPs with specific doses can be difficult to achieve predictably under an *in vivo* environment, due to variable conditions such as inflammation and microbial activity compared to simulated *in vitro* conditions. Low dose release over extended time periods could also raise the risk of microbes in the local tissue environment developing resistance to the AMNPs. Thus, how to optimize the NP coating design to satisfy safety requirements becomes challenging. Tran *et al.*[43] demonstrated the potential antibacterial coating of Se NPs on titanium fixation implants *in vivo* in a small animal model, indicating their potential for safe use but also highlighted the need for

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release studies to further their evaluation. In addition, AMNPs released at a bactericidal concentration may be harmful to tissue cells[138], so a dose range that balances biocompatibility and antimicrobial ability must be achieved to ensure safety. Ag NPs, which are the most common selection as an antibacterial NP agent due to their broad-spectrum capacity to kill microbes, are known to exhibit toxicity that can be at an organ or a cellular level through various mechanisms[139]. So, their use must be limited in maximum dose to avoid compromising patient safety. Moreover, surface functionalization of nanoparticles may also affect their biocompatibility. A recent study reported that altered surface chemistry may affect the cytotoxicity of polymer stabilized Se NPs, due to differences in the surface charge of the polymers[140], reinforcing the importance of detailed safety assessments when designing AMNPs.

Evidence of *in vivo* biodistribution and metabolism or excretion are also necessary for assessing the safety of NPs in humans. Notably, the *in vivo* release rates of antimicrobial agents from coatings are likely to be different from their *in vitro* rates. This is due to the complexity of *in vivo* microenvironments in which processes including enzymatic and hydrolytic degradation may accelerate the breakdown of the coating layer and the release of antimicrobial agents. Besides, the cytotoxicity and antibacterial ability of AMNPs are mediated by various biological mechanisms, and these properties may be altered under *in vivo* circumstances. Such effects may reduce or potentially enhance the safety and efficacy of AMNPs *in vivo* compared to their *in vitro* performance. For example, Ag NPs can be cytotoxic and genotoxic, depending on the properties of the NPs, such as size and dose[139], but it was hypothesized that the biotransformation of Ag NPs to silver sulfide may reduce the toxic effects of silver ions, which may be attributed

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to the protein corona formed on Ag NPs[128]. This silver sulfide transformation was also observed in the surrounding osseous tissue where Ag NPs-coated porous titanium was implanted in a rabbit model[61]. Another study of the oral intake of Ag NPs in rats showed no cytotoxicity in bone marrow whereas the accumulation of Ag NPs in tissues could be observed[141], highlighting the complexity of the *in vivo* fate of AMNPs. Unfortunately, these studies lacked evaluations of antimicrobial properties, which may change due to the transformation of Ag NPs. Most reports on AMNPs have been restricted to *in vitro* studies to date, so further research is needed to improve understanding of their *in vivo* safety and performance and thereby enhance their design.

VII. CHALLENGES AND OPPORTUNITIES

To sum up, it cannot be denied that quite a few challenges exist in the application of AMNPs-coated medical implants. Additionally, NP-specific challenges may exist depending on the type of material from which the particles are formed, such as processing stability for polymeric NPs comparing to metallic NPs. These challenges are not separated but correlated to one another. Protein fouling on either NPs or implant surfaces may lead to changes in antimicrobial properties, and sterilization processes may further contribute to property alterations. Manufacture of both AMNPs and/or implants with AMNPs coatings depends on a series of factors, including properties of the NPs and bulk materials, antimicrobial efficacy, complexity of the process, and cost-effectiveness, while properties of biomaterials such as antibacterial ability and long-term stability can be affected by fouling and sterilization. In addition, all these factors are related to safety, which is the first priority for use of biomaterials in humans.

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The previous focus of nanomedicine on drug delivery in cancer therapy may overshadow the importance of nanomaterials in medical implants, but it also supports the potential opportunities to develop NPs associated with medical devices. NPs have promising potential in antimicrobial coatings on medical implants individually or in combination with other agents due to their ability to damage microbes through multiple mechanisms. AMR has drawn much attention due to the increasing strains of resistant microorganisms, the severe consequences and the complexity of treatments. Because of the complexity of antimicrobial mechanisms, AMNPs may offer new opportunities to help overcome AMR. However, as discussed above, there is a shortage of *in vivo* assessments of AMNPs to date. Polymicrobial infections caused by multiple types of bacteria and/or fungi make the situation even more challenging. Therefore, there is a need to further study the polymicrobial populations causing implant-related infections during the evaluation of AMNPs as surface coatings to face AMR. Even though there are predictable obstacles to apply AMNPs as releasing surface coatings, enhanced collaboration among researchers, clinicians, industry and regulatory parties could contribute to the development of nanoparticles for medical implants, helping to overcome the challenges that are addressed in this article.

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List of figure captions

Fig. 1. Different potential design strategies for antimicrobial surface coatings.

Fig. 2. Titanium implants used in *in vivo* experiments. A) and B) are scanning electron microscopy (SEM) images of titanium plate and screw (scale bar: 1 mm), C) is a photo of uncoated implants, D) is a SEM surface image of an uncoated titanium implant (scale bar: 5 µm), E) and F) are SEM images of Se NP coated titanium plate and screw surfaces (scale bar: 5 µm), where the white dots are Se NPs[43]. (From International Journal of Nanomedicine 2019, reproduced with permission from Dove Medical Press limited.)

Fig. 3. Both research and regulatory product approvals for medical devices containing nanostructured materials have increased rapidly in the last two decades[106]. (Reprinted from Trends in Biotechnology, 37, Jones *et al.*, A Status Report on FDA Approval of Medical Devices Containing Nanostructured Materials, 117-120, Copyright (2019), with permission from Elsevier.)

Fig. 4. Transmission electron microscopy characterization of the effect of sterilization methods on the size and morphology of PEG-Au nanoparticles: a) control, b) UV irradiation, c) gas-plasma treatment, d) ethylene oxide treatment, e) formaldehyde treatment, and f) autoclaving. (Reprinted with permission[113], Copyright (2010), Wiley.)

Fig. 5. Field emission scanning electron microscopy images of *E. coli* after treatment with: A) bacterial culture media alone; B) bovine α-lactalbumin (BLA); C) starch-capped Ag NPs; D) PEG-capped Ag NPs; E) PEG-capped Ag NPs conjugated with BLA; E) starch-capped Ag NPs conjugated with BLA [130]. (Reprinted from Colloids and Surfaces B: Biointerfaces, 146, D. K. Ban and B. S. Paul, Protein corona over silver nanoparticles triggers conformational change of proteins and drop in bactericidal potential of nanoparticles: Polyethylene glycol capping as preventive strategy, 577-584, Copyright (2016), with permission from Elsevier.)

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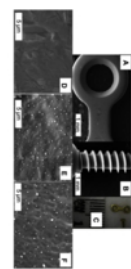
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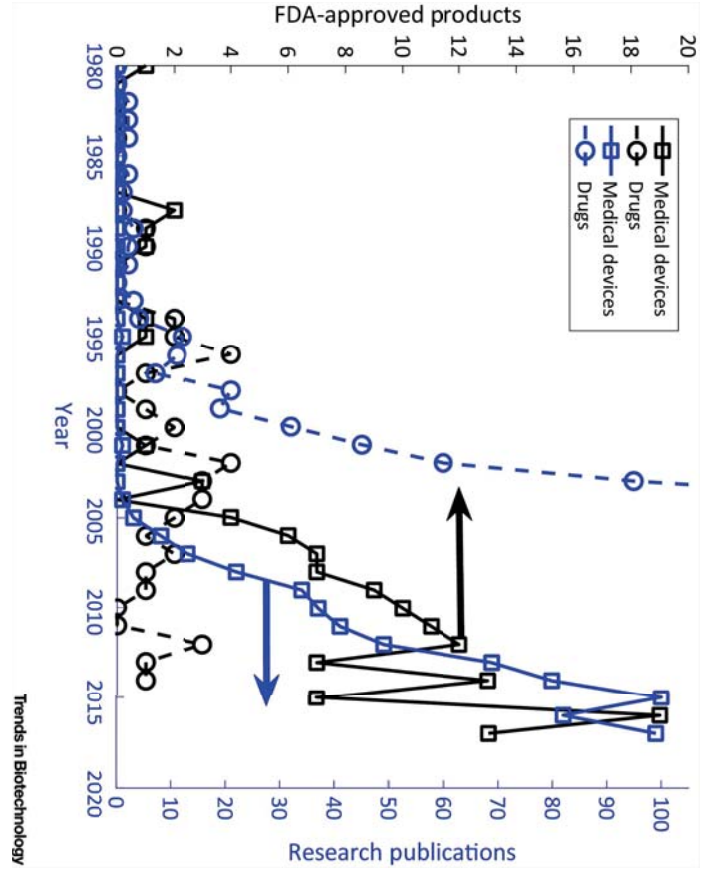
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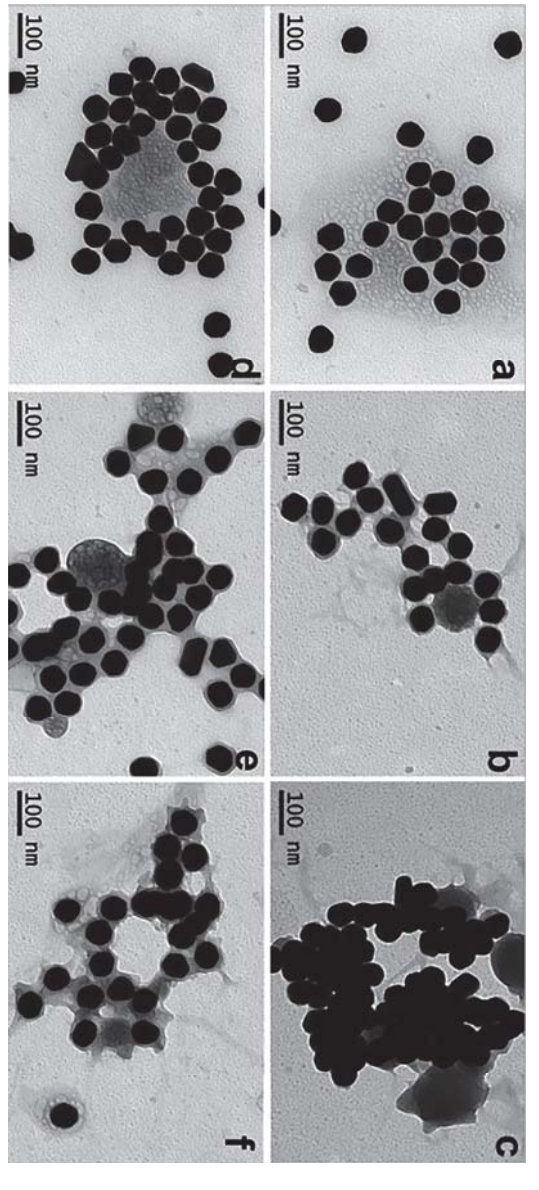
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