Review Paper

Strategies to Prevent Bacterial Infections on Titanium-BASED Orthopedic and Dental Implants.

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Titanium and titanium alloys remain the gold standard for dental and orthopedic implants. These materials are heavily used because they are bioinert, have strong mechanical properties, and promote integration with bone. However, implant-associated infections (IAIs) remain one of the leading causes of implant failure. Eradicating an IAI can be difficult since bacteria can form biofilms on the medical implant, protecting the bacterial cells against systemic antibiotics and the host's immune system. If the infection is not treated promptly and aggressively, device failure is inevitable, leading to costly multi-step revision surgeries. To circumvent this dire situation, scientists and engineers continue to fabricate novel strategies to protect the surface of medical implants from bacteria. In this review, we report on emerging strategies to prevent infection in titanium implants. These strategies include anti-adhesion properties provided by polymers, superhydrophobic, superhydrophilic, and liquid-infused surface coatings, as well as strategies and coatings employed to lyse the bacteria. We also explore commercially available technologies or under clinical trials and discuss future trends.

1. Introduction

Titanium and titanium alloys remain one of the most widely used materials for medical implants. Titanium alloys, especially medical grade titanium (Ti6Al4V), are commonly used in orthopedic and dental applications due to their high moduli, strength, and bioinert properties. The titanium oxide passivation layer protects the implant from corrosion and allows for direct fusion with bone to provide mechanical stability.[1] The process of osseointegration, or the direct structural and functional connection between implant and bone, is paramount for the long-term stability of a load-bearing implant.[1] Failure of orthopedic and dental implant can occur shortly after implantation (weeks post-surgery) or even years after implantation.[2] Among the causes of device failure, implant-associated infections (IAI) remain the top cause for revision surgeries in orthopedic and dental implants in North America, primarily because bacterial infections cause bone resorption and device loosening.[2–5] Eradication of IAI is particularly challenging because surface-dwelling bacteria are protected by a self-secreted biofilm, making them 1000x less susceptible to antibiotics than planktonic bacteria and also reduces the effectivity of the host's immune cells.[6] A bacterial biofilm can be defined as a surface-associated bacterial community embedded in an extracellular matrix (ECM).[7] The ECM is composed of polysaccharides, extracellular DNA, and glycoproteins, which create a polymeric habitat for the bacteria. Furthermore, the ECM enhances cell-to-cell communication among bacteria (quorum sensing) and provides an optimal surface for bacteria recolonization if needed.[6,8–11] In Canada alone, the cost of orthopedic revision surgeries

involving an IAI costs 2.3 times that of the initial implantation surgery at a cost of $25,000, while in the US, the cost for a revision arthroplasty surgery costs around $49,000.[3,12] The higher costs come from the surgical treatments, requiring single- or multi-stage procedures.[13] In the revision arthroplasty, the infected implant is removed, the necrotic tissue is debrided, and the patient is treated with systemic antibiotics for an extended period of time to eradicate the infection.[13] Once the bone has healed and there are no signs of infection, a secondary surgery is required to introduce new long-term prosthetic.[13]

In the past, the main strategy to prevent IAI revolved around rapidly integrating the biomaterial with the host body and "win the race to the surface".[14] This is because any surface that has already been occupied by a connective (bone) or soft tissue, would not be available for bacteria colonization. This strategy focuses on coating the biomaterial with functional biomolecules, which enhance cell adhesion,[15] promote bone cells chemotaxis,[16–19] induce differentiation of immature cells into bone cells,[18–21] increase vascularization and healing of the peri-implant space,[22] or use immunomodulator molecules that promotes the hosts' anti-inflammatory response and tissue healing.[23,24] Review papers on osseointegration through these strategies can be found elsewhere.[20,23,25–28]

It is important to understand how bacteria can infect a medical device and how a biofilm can form. **Figure 1** illustrates the process of biofilm formation and bacteria colonization. First, planktonic bacteria attach to the surface and proliferate into a microcolony. Then, the colony grows in mass and secretes the ECM. Different bacteria stains may attach and form a symbiotic multi-strain colony at this stage. Finally, some bacteria are expelled into the planktonic phase to colonize new surfaces. It is noteworthy that an infected medical implant can occur prior to insertion of the device, as well as post-operation. In some cases, device infection can occur years after implantation, which are considered a ‘late infection’.[29] In this review, we will highlight the current state of surface coating technologies to prevent bacterial biofilms. These coatings, also highlighted in Table 1, can be divided into bacteria repulsive coatings, which prevent bacterial colonization, or bactericidal coatings, which lyses bacteria that come in contact with the coating or in the peri-implant space as shown in Figure 1b.

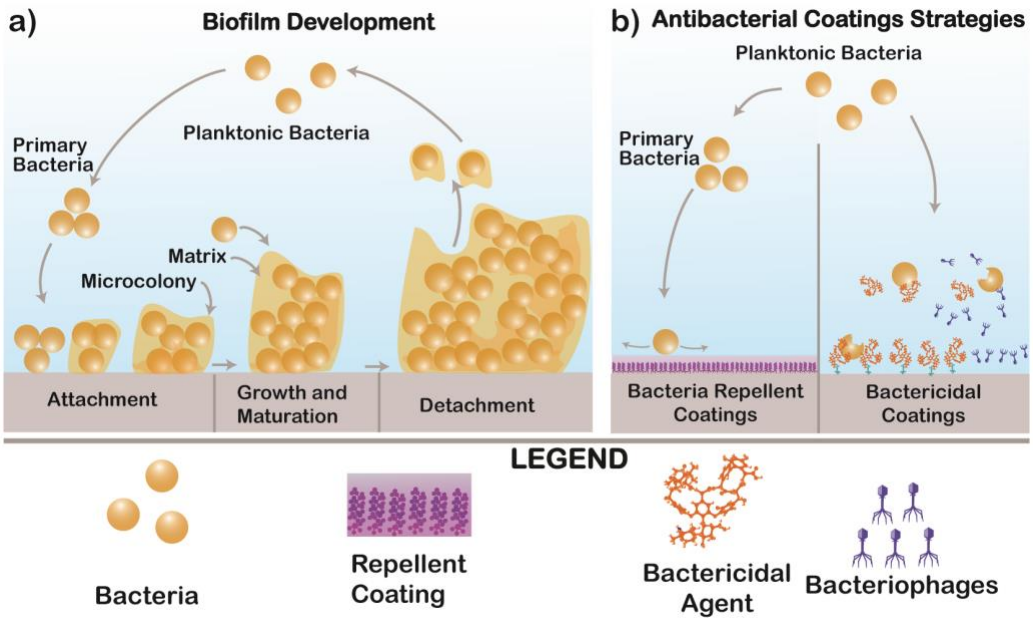

Figure 1. Schematic representation of antibacterial coatings. a) Schematic representation of biofilm formation and development. b) Schematic representation of different antibacterial coating strategies. Figure adapted with permission.[30] Copyright 2019 American Chemical Society.

Table 1. Table of antibacterial coatings.

Category	Components	Special Notes	Reference
Super Hydrophobic			
	Nanostructure via anodic oxidation Coated with fluorosilane	Surfaces showed low bacteria after 4-hour incubation	31
	Plasma etched with Ar, O_2, hexamethyldisiloxane	Used a one-step fabrication process. Low bacteria adhesion after 24-hour incubation	32
	Hydrothermal synthesis of nanoflower topographies	Superior hemocompatibility, low bacterial adhesion after 24-hours	33
	Titania nanotubes followed by silanization	Low biofilm formation after 24-hour bacterial incubation on superhydrophobic, compared to superhydrophilic coatings	34
	Femtosecond laser ablation	*S. aureus* colonized the surface to a greater extent than *P. aeruginosa* showing a geometry dependency	35
Liquid Infused Surfaces			
	Surface roughened with ultrashort laser ablation coated with a fluorinated polymer	Tested different combinations of surface roughness and different lubricants. Spike like nano/micro textures showed the highest bacterial reduction	36
	Spiked roughness through laser ablation and GPL 104 lubricant	Tested against different bacteria found in the oral cavity and displayed a 60% reduction in bacteria compared to Ti.	37
	Ti coated with Chitosan and fluorosilane Lubricated with perfluoroperhydro phenanthrene	Chitosan – LIS showed good biofilm reduction and osteoblast-like cell adhesion while traditional LIS did not support cell adhesion.	7
Polymeric Coatings			
	Silk-sericin and PMAA	Promoted osseointegration and reduced bacterial adhesion	38
	Silk-sericin and tannic acid	Reduction in *E. coli* and *S. aureus* adhesion	39
	PLL-*g*-PEG	Up to 93% reduction in *S. aureus after 24-hours*	40
	Multivalent PEGylated-peptides	Tetravalent titanium-binding peptides (TBP) reduced *S. aureus* biofilm formation after 5-hours.	41
	Citral and thymol and PEG	No biofilm was formed on the coating and bacteria was sparse compared to uncoated Ti	42
	Tannic acid and PEG	Compared one-step vs. two-step deposition processes. One-step procedure was 12-14% more efficient at repelling bacteria.	43

	Electrospinning of polyethylene oxide (PEO)	Hydrophilic nano fibers reduced *S. epidermidis* biofilm formation after a 24-hours incubation	44
	Electrospun chitosan mixed with PEO and bioactive glass particles	Coating showed reduced *S. epidermidis* attachment after a 48-hour incubation period.	45
	Hyperbranched poly-L-lysiene polymer	Antibacterial osteoconductive properties in an in vivo model	46
Metallic Coatings			
	Tantalum-nitride (TaN) vs. titanium nitride (TiN) coating	TaN showed lower biofilm formation and thickness after 14 days compared to the TiN coating	47
	Microporous coating composed of cobalt, fluorine, calcium, oxygen, phosphorus and different concentrations of strontium (0%, 6%, 11%,18%) wt.	90% decrease in bacteria compared with Ti after 28-days. Coatings that had 11% strontium content provided the best osteogenic properties.	48
	Calcium phosphate and zinc	89% reduction in *P. gingivalis* bacteria compared to CaP coated titanium.	49
	Strontium, calcium phosphate, and zinc	Increasing the concentration of zinc in the coating increased the bactericidal effects of the coating. Coatings showed no cytotoxic effects against MC3T3-E1 cells.	50
	Silver strontium	*S. aureus* reduction was proportional to silver concentration. Large silver concentrations had cytotoxic effects toward osteoblast.	51
	Silver and hydroxy apatite	Inhibited *S. aureus, E. coli,* and MRSA	52
	Silver nanocomposite on an amorphous hydrocarbon layer.	Reduced *E. coli* and *S. aureus* dependent on silver concentration. Coating was cytotoxic at high silver concentrations	53
Antibiotic Coatings			
	Vancomycin via AEEA linker	Covalent attachment of AEEA linker provided higher interactions and effectivity of vancomycin. Reducing bacteria by 88% after 2-hour incubation.	54
	Cefotaxime sodium antibiotic via Polydopamine	Surface was hemocompatibility, biocompatible and broad spectrum against gram-negative and gram-positive bacteria	55
	Vancomycin and SRP-1 peptide linker	Coating would degrade and antibiotic was released in the presence of enzyme produced by *S. aureus.*	56
	Gentamycin and bone morphogenetic protein in a biodegradable polymer	Coating prevented infection and had osteoinductive properties.	57
	Rifampicin was embedded into hydroxyapatite and poly-caprolactone polymer	Polymer provided osteoblast cell attachment and proliferation. 3-log reduction in bacteria after 24-hour incubation	58
	Chitosan-bioglass with tetracycline and melittin	3-log reduction of MRSA after 6-hours	59
	Levofloxacin into PDEGMA polymer brushes	Drug release was temperature dependent. Brushes provided an anti-adhesion surface which reduced biofilm formation after 7-days	60
	Vancomycin on ethylene glycol PEG7 brushes	Reduced *S. aureus* 20-fold after 21-days in an animal model.	61

	Doxycycline on TiZr	Coatings were tested *in vivo*. The coated samples showed less cytotoxicity, and upregulated bone healing	62
	Bacitracin bonded via dopamine	Coating was tested in a rat model and prevented osteolysis caused be *S. aureus* (2.2-log reduction in CFU)	63
	Polyetheretherketone coating with BMP-2 and gentamicin	The coating had different release profiles for gentamicin and BMP-2. The coating was tested *in vivo* and showed increased bone deposition.	57
Bacteriophage			
	HPMC gel with linezolid and *S. aureus* specific bacteriophage	Combined coating showed the highest bacterial reduction in an *in vivo* model.	64,65
Misc.			
	Photosensitizer Indocyanine Green and RGD peptide on mesoporous polydopanine nanorparticle polymer	Coating provided photothermal and photodynamic therapy upon laser irradiation, decreasing bacteria by 99.7% in an animal model. Some cytotoxicity was seen.	66

2. Anti-adhesion Coatings

Anti-adhesion surfaces mitigate bacterial infections by preventing the unwanted accumulation of microbes on the implant's surface, which typically leads to bacterial build-up and the formation of biofilms. In order to give a surface anti-adhesive property, a specialized coating must be added, or the surface must be physically or chemically altered. Many strategies have been used throughout the literature to create the desired anti-adhesion property of the surface. For one, superhydrophobic surfaces have proved to be short-term inhibitors of bacterial adhesion due to their low surface free energy.[67] Next, liquid-infused surfaces, which involve creating a smooth interface on a surface through infusion with a layer of viscous liquid, have been shown to prevent bacterial adhesion and biofouling.[30] Polymeric coatings, including PEG and silk, have also shown a promising solution to prevent bacterial adhesion.[38,41] Finally, UV treatment prior to implantation has been shown to impair the accumulation of bacteria without compromising the biocompatibility of the surface [68] These methods and corresponding experimental results will be discussed in more detail.

2.1. Superhydrophobic Coatings

Surface wettability is a simple method to measure the free energy of a surface and has become an increasingly popular research topic. Two primary states of wetting on a rough solid surface have been identified based on Young's model. Wenzel's theory describes that liquid will follow the surface roughness and fully penetrate the surface. In contrast, Cassie-Baxter's theory says liquid will be suspended due to trapped air in crevices on the rough surface.[69,70] With recent advances in micro/nanotechnology, scientists and engineers have produced superhydrophobic surfaces by increasing the surface roughness and changing the surface chemistry. Superhydrophobic surfaces have low surface energy and are defined by a water contact angle greater than 150°.[67,71] This large contact angle is explained by the Cassie-Baxter theory, stating that the contact angle increases when microstructures are present on the surface.[70] Superhydrophobic surfaces have enhanced corrosion resistance, improved hemocompatibility, and the ability to self-clean.[32,33,71] However, these surfaces have been distinguished as a viable inhibitor of bacterial adhesion and biofilm formation.

In recent years, various methods have been used to fabricate superhydrophobic surfaces on titanium. Furthermore, the produced superhydrophobic coatings have been effective at preventing bacterial adhesion. Generally, superhydrophobic surfaces are known to be created in a complex two-step fashion. The first step is to modify the surface roughness, and the second is to lower the surface free energy by adding a surface coating, increasing the

surface's hydrophobicity.[32] Although many methods exist to create a roughened surface topography in the first step; the second step normally involves adding hydrocarbon or fluorocarbon groups to render the surface superhydrophobic. While the two-step process is facile, there have also been attempts to simplify the process of making the superhydrophobic surface by employing a one-step technique. In a study performed by Lin *et al.*, the authors aimed to determine how the adhesion of *S. aureus* would vary on hydrophilic, hydrophobic, and superhydrophobic titanium surfaces.[31] Lin *et al.* created a superhydrophobic nanotube structure via anodic oxidation followed by PTES (1H, 1H, 2H, 2H-perfluorooctyltriethoxysilane) in a self-assembled technique [31] SEM images showed that fewer bacteria had adhered to the superhydrophobic surface compared to the other surfaces at the 4-hour mark.[31] The bacterial cells on the superhydrophobic surface were also scattered and did not tend to gather, making them easier to be removed.[31] In a different study, Souza *et al.* created a unique one-step superhydrophobic coating on titanium by glow discharge plasma. The process used Ar, O2, and hexamethyldisiloxane gases, which etched the surface of titanium and made it superhydrophobic.[32] The authors compared the bacterial adhesion on a superhydrophobic coating to a non-coated titanium surface that served as the control.[32] An *in vitro* assay was performed using saliva as the microbial inoculum to evaluate the anti-biofilm property of the surfaces against bacterial and fungal adhesion.[32] The results for the superhydrophobic surface at the 2-hour mark showed an 8-fold reduction in total microbial adhesion compared to the control.[32] After 24 hours, scanning electron microscopy (SEM) images showed a robust biofilm developed on control surfaces, while the superhydrophobic surface had small and sparsely distributed colonies. Although a bacterial infection was mitigated in the short-term, longer periods need to be tested to see if the surface colony does not develop into a mature biofilm.[32] Montgomerie *et al.* created a superhydrophobic Titania coating through hydrothermal synthesis and vapor-phase silanization.[33] The produced coating contained a fractal geometry that resembled a nanoflower (**Figure 2ai**). The nanoflower coating had superior hemocompatibility compared to flat Ti, showing fewer platelets and leukocyte adhesion.[33] Additionally, the surfaces were tested against the adhesion of *S. aureus* and *E.coli.* Images of the surface topography before bacterial incubation and after a 24-hs bacterial incubation show that a biofilm layer was forming on the control Titania for both *S. aureus* and *E. coli*. At the same time, there was no biofilm formation on the superhydrophobic surface, and very few bacteria were visible on the surface (Figure 2ai).[33] The biofilm formation on the surfaces was also quantified via fluorescent staining after 6 and 24-hour incubation periods. The results show that the superhydrophobic surface consistently had the lowest amount of live bacterial adhesion for both *S. aureus* and *E. coli* in comparison to all other surfaces ($p \leq 0.05$) (Figure 2a,ii-iii).[33] Barlet *et al.* conducted a

comparable study, testing the adhesion of gram-positive and gram-negative bacteria on superhydrophobic and superhydrophilic titania nanotubes.[34] The superhydrophobic surface in this study was created by anodizing and chemically etching titanium to form titania nanotube arrays. Then the titania nanotube arrays were silanized to modify the surface chemistry and introduce superhydrophobicity.[34] Similar results were yielded from this study, as the number of adhered bacteria (for both *S. aureus* and *P. aeruginosa*) on the superhydrophobic surface was significantly lower than on all other surfaces ($p < 0.05$), and no biofilm formation was observed within 24 hours.[34] In contrast, Fadeeva *et al.* used a femtosecond laser ablation technique to fabricate superhydrophobic structures on titanium. In this study, *S. aureus* cells could colonize the superhydrophobic titanium surface after 18 hrs; however, the *P. aeruginosa* cells did not adhere.[35] The authors hypothesized that the *S. aureus* cells adhered more easily to the surface because of their spherical shape, allowing them to stick to the surface without requiring a large surface area. In contrast, *P. aeruginosa* cells containing an elongated rod shape may require more surface contact to adhere.[35] Overall, it is evident from all these studies that the superhydrophobic surfaces mitigated bacterial adhesion, showing fewer and more dispersed bacteria compared to the controls.

A negative aspect of superhydrophobic coatings is that the effectiveness of the surface to prevent bacterial adhesion seems to diminish as time passes. A study by Hwang *et al*. identified that the superhydrophobic surface might even encourage bacterial adhesion during long-term exposure. This increase in bacteria can be due to the high surface area typically created on the superhydrophobic surface and the addition of proteins from the complex solution.[72,73] Although there is encouraging evidence that superhydrophobic surfaces mitigate bacterial adhesion in the short-term, further research should focus on long-term studies to investigate their effectiveness for medical implants.

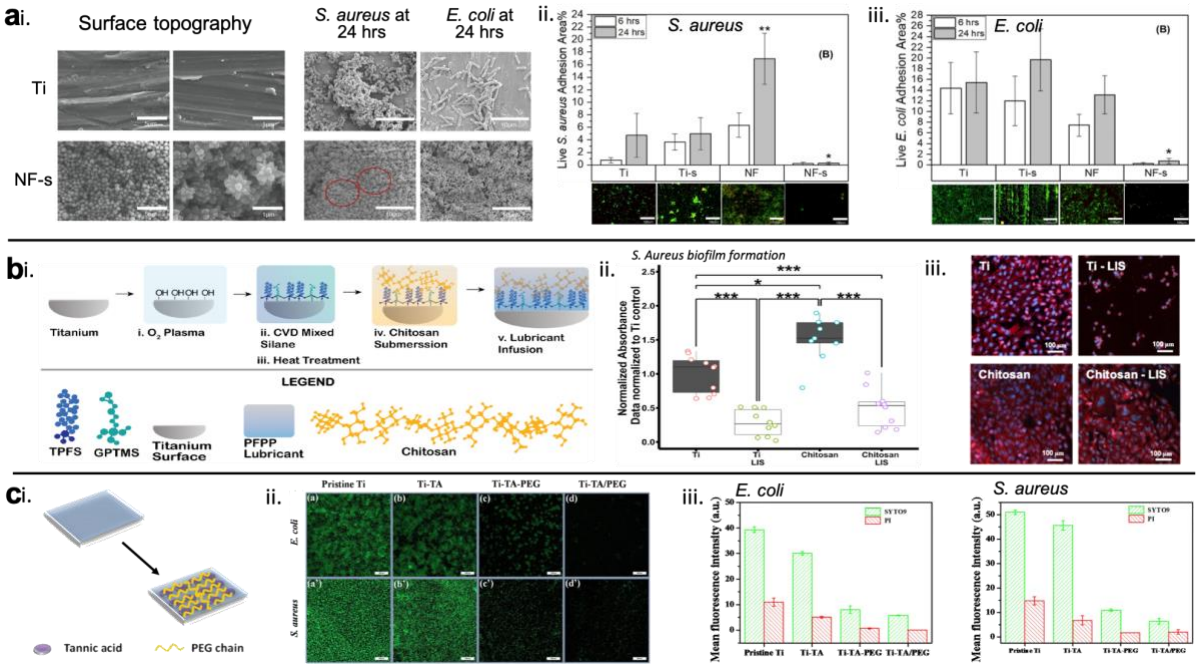

Figure 2 anti-adhesion coatings. **ai**) SEM images of control (Ti) and superhydrophobic titania nanoflower (NF-s) surfaces before bacterial incubation and 24 hours after bacterial incubation. Bacterial cell adhesion area percentage for live **aii)** S. aureus and **aiii)** E. coli. Figure 2a was adapted with permission.[33] **bi)** Schematic representation of chitosan-conjugated liquid-infused coatings on titanium. **bii**) Crystal violet evaluation of *S. aureus* biofilm formation. **biii)** Fluorescent microscopy images of SaOS-2 cell proliferation after seven-day cell cultures (nuclei: blue; microfilaments: red). Figure 2b was adapted with permission.[7] **ci)** Schematic representation of substrate coated with tannic acid and PEG. **cii)** Fluorescent images of bacteria adhesion for *E. coli* and *S. aureus*. **ciii)** Mean fluorescent intensity values of adherent bacteria for *E. coli* and *S. aureus.* Figure 2c was adapted with permissions.[43]

2.2. Liquid Infused Surfaces

Inspired by the Nepenthes pitcher plant, liquid-infused surfaces (LISs) are a class of functional materials with a tethered layer of liquid, creating a smooth interface.[74–82] LISs are recognized for their stable liquid-repelling behavior under low sliding angles.[83] For the LIS to be stable and repellant, the substrate must have a high affinity to the lubricant, and the lubricant must be immiscible with the liquid that needs repelling.[74,83,84] The lubricant should be selected for the specific application. For example, for medical implants, the lubricant must be biocompatible and immiscible with complex liquids, such as plasma or blood. For a LIS to have clinical applications, many factors must be considered, including cytotoxicity toward human cells, environmental toxicity, the effect of leached products or byproducts, and the stability and longevity of the liquid inside the body.[85] Throughout the literature, LISs have been documented to effectively reduce bacterial adhesion and biofilm formation.[78–81,86,87]

LISs exist in one- (1D), two- (2D), and three-dimensional (3D) forms, with 2D being the most popular.[74] In a 1D LIS, the surface structure retaining the lubricant exists in a single plane on the order of one to multiple monolayers.[74] The thin lubricant layer is adhered to the substrate through intermolecular interactions or is directly grafted to the substrate.[74] In a 2D LIS, the surface structure is roughened, and the lubricant is encased by capillary action through nano-topographical features.[74] Finally, 3D LISs trap lubricant through a 3D pore network that also stores lubricant.[74] This review will primarily discuss 2D LISs, as they are the most prevalent type of LIS reported in the literature. Two primary methods exist to prepare 2D LISs: (i) modify an existing rough surface with an adequate chemical coating to match the lubricant chemistry, or (ii) roughen the surface of a substrate with low-surface-energy.[83] Many methods exist to create nanostructures on the substrate surface, including emulsion and phase separation, chemical and physical etching, mold transcribing, spin-coating, spraying, electrochemical decomposition, and more.[83] It should be noted that although the textured surface improves the LIS interface and promotes lubricant retention, the most critical step in creating a LIS is obtaining compatible surface chemistry between the substrate and the lubricant.[83] Scientific literature has proven that many efficient LIS substrates exist, including metals, non-metals, and polymers. However, titanium (Ti) is this review's primary substrate of interest.

Titanium LIS have been created through various methods by scientists and engineers to repel liquid and solid materials. A recent study by Doll et al. created LIS and performed surface structuring using ultrashort laser ablation.[36] Doll et al. tested titanium SLIPS with four structures (hierarchical, micro-, and nanosized spikes, micro-sized grooves, nanosized ripples, and unstructured surfaces) and five infusing perfluoropolyether lubricants of different viscosities.[36] The SLIPS were fabricated by initially creating a rough surface, followed by dip coating the titanium with a fluorinated polymer.[36] Each lubricant was spin-coated onto each surface, creating a thin, homogenous liquid film.[36] Before the experiments, the LIS and uncoated Ti, were sterilized using UV irradiation.[36] Samples were tested against gram-positive *Streptococcus oralis* (*S. oralis*) bacterium. This study aimed to investigate biofilm formation and initial bacterial adhesion. For biofilm formation, samples were incubated in an inoculum of 4×10^{10} CFU/mL *S. oralis* for 18 hours under static conditions, and a maximum concentration of 0.3% lubricant in solution was used.[36] All SLIPS surface structures experienced reduced biofilm formation compared to their corresponding uncoated surfaces.[36] Spike SLIPS exhibited the highest reduction in biofilm formation of all tested surface structures. Each spike/lubricant combination was further investigated for biofilm volume and live/dead distribution of cells.[36] The biofilm volume on all spike SLIPS was 100-fold decreased compared to unstructured, uncoated Ti control.[36] For evaluating initial

bacterial adhesion, samples were incubated in an inoculum of 3 x 10^{11} CFU/mL *S. oralis* for 5 hours under constant agitation at 150 rpm.[36] Spike slips coated with Krytox 143 AZ (143 AZ), and Krytox GPL 104 (GPL 104) lubricants demonstrated the highest effects on limiting bacterial adhesion, as quantified by dividing the total surface area covered with bacteria by the mean surface area of a single bacterium in ImageJ.[36] These surfaces were further investigated to determine their long-term stability. Both samples could resist gravitational forces and ambient conditions for up to 15 days while maintaining biofilm repellant properties.[36] Cellular viability was quantified to determine cytocompatibility of 143 AZ and GPL 104 lubricants against human gingival fibroblasts. It was determined that metabolic activity was not significantly different from that of the control cells.[36] When lubricant concentration increased to greater than 5%, metabolic activity decreased.[36] Therefore, the authors concluded that the lubricant is not cytotoxic up to 5% concentration. Contrastingly, it should be noted that the LIS tested with a 0.3% concentration of lubricant exhibited no growth of fibroblasts or osteoblasts. Since the 0.3% lubricant was not proven to be toxic to fibroblasts, making these surfaces more appealing for biomedical applications where soft tissue sealing and osseointegration are not critical.[36] A later study by Doll et al. investigated antiadhesive mechanisms to repel *S. oralis* biofilms using LIS.[37] The LIS were fabricated similar to the previous study, except only spike structures were created on the surface using laser ablation, and only GPL 104 was used as the lubricant.[37] The LIS were tested against *S. oralis* and an oral multispecies composed of *S. oralis*, *Actinomyces naelundii* (*A. naelundii*), *Veillonella dispar* (*V. dispar*), and *Porphyromonas gingivalis* (*P. gingivalis*).[37] Biofilms were allowed to grow in a flow chamber system, and when tested against *S. oralis*, biofilm volume was reduced in the experimental Titanium LIS (biofilm volume = 2.9 x 106 μm^3 ± 1.9 x 106 μm^3) as opposed to the uncoated, unstructured Ti (biofilm volume = 3.8 x 107 μm^3 ± 2.3 x 107 μm^3).[37] Doll et al. furthered their research by testing SLIPS against multispecies communities that would be found in the oral cavity. The dominant species in the community was *S. oralis*, followed by *V. dispar*, while *A. naelundii* and *P. gingivalis* made up smaller portions.[37] The LIS samples experienced a biofilm reduction of approximately 60% when tested against the multispecies community compared to the Ti control group.[37] Bacterial adhesion forces were also reduced in LIS, as quantified by single bacterial cell force spectroscopy.[37] It should be noted that this study by Doll et al. did not further investigate coating cytocompatibility or toxicity. Therefore, it is still probable that the produced LISs are not appropriate for applications where osseointegration is critical. Conversely, recent literature has suggested that including chitosan in LISs can be beneficial in mitigating the lack of osseointegration promotion. A study by Villegas et al. created chitosan impregnated slippery LISs designed to facilitate cell adhesion and prevent biofilm formation.[7]

Chitosan is a natural, biodegradable biopolymer found in shellfish, which has been reported to promote the proliferation of osteoblasts and mesenchymal cells.[7] To create the coatings, titanium alloys (Ti6Al4V) were initially treated with oxygen-plasma to hydroxylate and sterilize the surface.[7] To create a stable interface in the coating, fluorinated silane Trichloro(1H,1H,2H,2H-perfluorooctyl) (TPFS) was selected, as it has a high affinity for perfluoroperhydro phenanthrene (PFPP) lubricant.[7] After disinfection, titanium samples were conjugated with chitosan in aseptic conditions to create covalent bonds between the biopolymer and the surface. This process, in further detail, is outlined schematically in Figure 2bi. The functional coatings were tested against MRSA (methicillin-resistant staphylococcus aureus) MW2 strain to determine their bacteria repellent properties.[7] Untreated titanium, conventional liquid-infused titanium (Ti-LIS), and chitosan-coasted titanium (Chitosan) were selected as control samples.[7] The experimental combined chitosan and liquid-infused coatings (Chitosan-LIS). Biofilm formation was quantized by crystal violet evaluation, where crystal violet values are proportional to biomass found on the surface.[7] A high value of normalized absorbance indicates large biomass; likewise, a low value indicates low biomass. Chitosan LIS reduced biofilm formation of MRSA up to 50% and 75% compared to untreated Ti and Chitosan Ti control groups. These results are summarized in Figure 2bii, and all data were normalized to the untreated Ti control group. Osteoblast-like SaOS-2 osteosarcoma cells were used to test cell adhesion and viability.[7] The control groups were untreated Ti, Ti-LIS, and Chitosan; the experimental group was Chitosan-LIS. As seen in Figure 2biii, after three- and seven-day cell cultures, Chitosan-LIS samples experienced high cell densities, superior to those of untreated Ti. This proves that the chitosan biopolymer increases mammalian cell adhesion and significantly promotes cell proliferation.[7] This study suggests that chitosan conjugated infused LISs have the potential to be beneficial in applications where bone ingrowth and tissue integration are critical.

Altogether, liquid-infused surfaces have great potential in the field of anti-adhesion coatings. Their simple fabrication steps and low sliding angles make them an efficient and inexpensive method to repel harmful bacteria and mitigate biofilm formation. Nevertheless, in the field of orthopedic and dental applications, it remains crucial for bioactive titania to present osteoconductive properties, and further research should be conducted into the cytocompatibility of liquid-infused surfaces. Although LISs have shown promise as antibacterial coatings, substantial progress must be made before these coatings can be applied clinically. Polymeric coatings are another alternative for anti-adhesion coatings. Like LISs, polymeric anti-adhesion coatings work to repel the surface attachment of bacteria. However, in place of a slippery surface, polymeric coatings repel bacterial attachment through polymer brushes

adhered to the surface. Polymeric coatings have demonstrated their efficiency in preventing biofouling and will be further investigated in this review.

2.3. Polymeric coatings

Polymeric coatings have been discovered to have anti-biofilm properties and have been increasingly used in orthopedic applications to prevent the build-up of bacteria. A polymer is commonly known as a material that consists of large molecules, where each molecule is comprised of repeating subunits. In general, polymers are suitable candidates to be involved with titanium coatings because of their stability, biocompatibility, and ability to prevent corrosion.[42,88] Polymeric coatings are usually characterized by the term polymer brush, which refers to a surface coating that contains polymers tethered to a surface.[88] This 'brushing' effect is created as the polymer molecules tend to repel the attachment surface due to steric repulsion and osmotic pressure, elongating the molecules near the attachment site and stretching them away from the surface.[88] The brushes are typically characterized by a high density of grafted polymer chains. They may either be in a solvated state, where the polymer layer consists of both solvent and polymer, or a melt state, where the polymer chains occupy the free space entirely.[88] These polymer brushes tend to have antifouling properties, but their effectiveness depends on the type of polymer and the bacteria strain present.[88] There are two main kinds of polymers, naturally occurring and synthetic. Researchers have taken a particular interest in the viability of silk-functionalized surfaces for natural polymers. For synthetic polymers, coatings on titanium comprised of polyethylene glycol (PEG) or polyethylene oxide (PEO) are the most common throughout the literature. Both categories of polymers and their efficacy will be addressed.

Silk is a natural polymer used as a component in titanium coatings to reduce bacterial adhesion. Raw silk is known to be produced in fiber form by various insects and spiders [38] Silk contains two different proteins: sericin and fibroin.[38] Sericin is preferred over fibroin because it is water soluble and easier to process. Furthermore, sericin is highly hydrophilic and is biocompatibile.[38,39] Zhang *et al.* produced a polymer using silk-sericin (SS) and polymethacrylic acid (PMAA) to promote osseointegration and inhibit bacterial adhesion on titanium implants.[38] The titanium surfaces were modified using surface-initiated atom transfer radical polymerization, allowing vastly different functions to be imparted on the same titanium surface.[38] The results from the study determined that the surface modified with PMAA and SS was effective at preventing the adhesion of bacteria, with this surface having significantly fewer *S. aureus* and *S. epidermidis* cells adhered compared to the control.[38] Additionally, the surface was still adhesive to osteoblast cells, and the coating did not prevent osseointegration, which was considered unique and

advantageous.[38] Next, Cheng *et al.* attempted to create an antifouling titanium surface through the co-deposition of natural tannic acid (TA) and SS.[39] The authors deposited the conjugated TA and SS on titanium surfaces via surface adhesive trihydroxyphenyl groups in TA, demonstrating a safe and environmentally friendly way of fabricating anti-adhesive coatings on a metallic surface. The titanium surfaces with co-deposited TA and SS showed a reduction in both *E. coli* and *S. aureus* compared to the controls, which had many viable bacterial cells adhered to the surface.[39] Most bacteria that had adhered to the modified surface were alive, and only a few were dead, confirming that the surface mainly had anti-adhesion abilities and negligible bactericidal properties.[39] Overall, it is clear that employing silk in titanium coatings is a promising approach to inhibit the attachment of bacteria. However, resources on this topic are limited and more research should be carried out with sericin, fibroin, and various derivatives to construct effective anti-adhesive surfaces.

Synthetic polymers are advantageous in tissue engineering applications because they have low toxicity and degradation rates.[44] Coatings comprised of PEG or PEO are characterized by flexible, highly hydrated chains of biocompatible polymers that hinder the attachment of bacteria through the water layer that covers the titanium surface and introduces a high activation barrier against bacterial adhesion.[44] PEG, in particular, has been used in polymer coatings for a plethora of reasons. PEG, specifically dense PEG brushes, are frequently involved in the preparation of coatings as they effectively decrease the number of proteins adsorbed on implant surfaces.[40] Furthermore, PEG can improve ductility and stop the coating from being brittle and fragile.[41] A unique PEG-based coating was used in a study by Harris *et al.*, where a poly(L-lysine)-*grafted*-poly(ethylene glycol) (PLL-*g*-PEG) coating was synthesized on titanium oxide surfaces, and the attachment of *S. aureus* was investigated.[89] Results of the study show that at the 24-hour mark, the PLL-*g*-PEG-coated surface reduced the amount of adherent *S. aureus* by 89–93% compared to the uncoated titanium control.[40] It was found that the bacteria-to-bacteria interactions were more substantial than the bacteria-to-surface interactions, as the small number of bacteria stuck on the polyethylene glycolated surface tended to clump together.[40] Next, Khoo *et al.* also tested the *S. aureus* resistance, but instead on titanium that was coated with multivalent PEGylated-peptides.[41] This study examined the ability of mono, di, and tetravalent titanium-binding peptides (TBPs) to resist bacterial adhesion.[41] Khoo *et al.* determined that all the PEGylated-peptide treated surfaces had considerably lower biofilm density than the uncoated titanium surfaces.[41] Furthermore, it was concluded that the performance of the coating improved with more TBP repeats, with the tetravalent coating showing a 90% reduction in *S. aureus* biofilm formation after 5 hours of incubation, while there was a 32% and 47% reduction, respectively

with the monomer and dimer.[41] In addition, Valliammai *et al.* evaluated the effectiveness of the synergistic combination of citral and thymol in a polymeric coating to inhibit the biofilm formation of methicillin-resistant *S. aureus* (MRSA).[42] PEG was used in the formulation of this coating to aid antibiofilm agents citral and thymol and to increase the plasticity of the coating, allowing the coating's outer surface to be smooth so that there were no ridges or crevices for the bacteria could adhere to.[42] After 24 hours of incubation, a robust MSRA biofilm was seen on the uncoated titanium, while on the coated specimen, there was no biofilm formation and the bacterial cells were sparse.[42] Similarly, Guo *et al.* investigated an antifouling polymeric coating created by mixing and co-depositing TA and PEG onto the titanium surface.[43] This study compared the effectiveness of a one-step simultaneous deposition process (Figure 2ci) against a two-step one at preventing adhesion of *S. aureus* and *E. coli*.[43] The anti-adhesion properties of the coating after 24 hours were observed through CLSM images, where green fluorescence highlighted the live bacteria with intact membranes and red fluorescence showed the bacteria that have damaged membranes.[43] Biofilm formation was evident on the pristine Ti surface, and on the TA-modified surface as a strong green fluorescence was present on both (Figure 2cii).[43] There was a low green fluorescence signal, implicating a minuscule bacteria presence seen on the surfaces that had the co-deposited polymer, both by the one-step (Ti-TA/PEG) and two-step (Ti-TA-PEG) deposition processes (Figure 2cii).[43] The same trend was observed for both *S. aureus* and *E. coli*.[43] The authors determined the mean fluorescent intensities of each image, confirming the antifouling properties of the Ti-TA/PEG surface. The results indicate that Ti-TA/PEG surfaces were 14.6% more effective at repelling bacteria than Ti-TA-PEG for *E. coli* and 12.4% for *S. aureus* (Figure 2ciii).[43] The effectiveness of PEG-based coatings is evident, but polymeric coatings consisting of PEO, particularly PEO nanofibers, have had promising anti-microbial effects. For one, Şimşek *et al.* examined a PEO coating created with sequential electrospinning and crosslinking processes.[44] Electrospinning involves utilizing electrical forces to make tiny polymeric fibers, which has been identified as a simple and cost-effective technique to create polymeric nanofiber coatings on metallic surfaces.[44] Moreover, electrospinning polymers do not require chemicals or high temperatures to coat a substrate, providing a highly functional and biocompatible coating.[44] It was determined that the PEO nanofiber coating significantly reduced the attachment of *S. epidermidis* after 24 hours of incubation, as a robust biofilm layer had formed on the bare titanium. However, the modified surfaces by the PEO nanofibers had a minimal amount of bacterial attachment and no proliferation or colonization.[44] The authors attributed this outcome to the PEO chains, which were very hydrophilic and flexible and could exert osmotic repulsion.[44] In a similar study, Boschetto *et al.* tested the performance of a distinctive coating for

titanium that involved electrospun chitosan mixed with PEO-based nanofibers and incorporated with bioactive glass particles.[45] Their analyses showed that substantially fewer *S. epidermidis* cells were found on the coated surfaces compared to the uncoated controls after 48 hours of incubation.[45] The coating showed a more effective action against bacteria than the uncoated titanium surfaces, likely because of the chitosan nanofibers that were a component in the coating.[45] Chitosan is a natural agent generally known to have bactericidal abilities.[45] Bactericidal coatings differ from anti-adhesion coatings as they represent a category of therapeutics that can kill any bacteria that populate on the surface instead of just decreasing and inhibiting the initial attachment of bacteria. This could provide a distinct advantage in orthopedic and dental applications as bactericidal coatings could continuously lyse bacteria on the implant and the peri-implant space. In contrast, anti-adhesion coatings have no bactericidal effects. Therefore, bacterial cells may eventually colonize the surface and form a biofilm. Bactericidal coatings, seen in various types and forms, will be discussed in the next section.

3. Bactericidal Coatings

Many antibacterial mechanisms exist to prevent bacterial adhesion, proliferation, and subsequent biofilm formation. Bactericidal coatings are active or passive coatings that can be applied onto a surface to kill bacteria.[90] In passive coatings, bactericidal surfaces disturb bacterial cells upon surface contact with the coating, which leads to cell death.[91] Conversely, active coatings represent bactericidal agents that are released from the surface coating to kill surface adhered bacteria and bacteria in the surrounding space.[90] There are many advantages and disadvantages to using passive and active coatings. A strong passive coating will inhibit bacterial adhesion to the surface and prevent biofilm formation without the release of antibacterial agents that could be toxic to the host.[90] Since antibacterial agents are not released, implant integration and osteogenic differentiation are not altered, and there is no cause for the development of bacterial resistance.[90] However, there is some risk for *in vivo* applications, as passive coatings can recruit plasma proteins from biological fluids that will enhance the colonization of bacteria.[90] Unlike passive coatings, active coatings have the ability to target bacteria in the peri-implant space, which can protect the implant effectively post implantation. However, active coatings must maintain a minimum inhibitory concentration (MIC) in order to be effective, and since the bactericide is released continuously, the long-term effectivity of the coating will eventually be compromised. Another consideration that needs to be addressed with active bactericidal coatings is the release dynamics. For example, large burst concentrations can be cytotoxic to the surrounding tissue, therefore preventing proper healing. In contrast, slow release over a long period of time of bactericides such as antibiotics has the potential

to cause bacterial resistance, which would promote additional infection.[90] For these reasons, bactericidal coatings continue to be heavily researched due to their great potential in combatting implant-associated infections (IAI). There are a plethora of bactericidal coatings include polymeric coatings,[92–94] antibacterial coatings,[17,62,63,95–100] bacteriophage containing coatings,[101] metallic coatings,[47,48,50,51,53,102,103] and more. Here, we will delineate the current state of these technologies.

3.1. Metallic Coatings

Among the many classes of bactericidal coatings, metal-based coatings exert their antibacterial effects through several metal-dependent methods. Silver (Ag) destroys the cell wall and cytoplasmic membrane to release silver ions which degenerate ribosomes and inhibit protein synthesis.[102] Silver ions are able to deactivate respiratory enzymes on the cytoplasmic membrane, which terminates ATP synthesis.[102] Reactive oxygen species (ROS) are produced as a cellular response to bacterial invasion in some habitats by abiotic processes.[104,105] ROS will cause membrane disruption and work alongside silver ions to bind to deoxyribonucleic acid (DNA) and prevent replication.[102] The silver nanoparticles will accumulate in the cell wall and migrate across the cytoplasmic membrane, causing perforation and degeneration, leading to organelle release and overall bacterial death. Similarly, the antimicrobial mechanism of copper (Cu) involves ROS generation and DNA degradation.[102] To exert antibacterial effects, zinc (Zn) aids in ROS generation and Zn^{2+} ion release, and strontium (Sr) works to inhibit bacterial cytoplasmic membrane permeability and cell metabolism.[102] Many different metals have been researched for their antibacterial effects as bactericides; including tantalum,[47] strontium,[48,50,51] copper,[103] silver,[51–53,102,103,106,107] and zinc[49,50,103] These metals have all been found to provide some degree of protection against various strains of bacteria as discussed below.

Metal-based coatings can be created through many different methods. Common methods include the use of metallic nanoparticles striking the surface, coating the surface using metal-infused thermal spraying techniques, micro-arc oxidation (MAO), magnetron sputtering, and complete surface incubation in calcifying solutions. A study conducted by Zhang *et al.* deposited tantalum-nitride (TaN) and titanium nitride (TiN) coatings onto commercial pure Ti through magnetron sputtering in a multi-functional coating rig.[47] Prior to coating, titanium was polished with a series of silicon carbide (SiC) abrasive papers and cleaned ultrasonically in acetone, anhydrous ethanol, and de-ionized (DI) water.[47] Magnetron sputtering was used to deposit a thin metallic film onto the Ti base. Ta and Ti of 99.99% purity were used as sputtering targets for deposition of TaN and TiN thin films onto the Ti substrate.[47] A significant reduction of biofilm formation ($p < 0.05$) against gram-positive (*A. viscosus and S. mutans*) and gram-negative (*P.*

gingivalis) bacteria was observed.[47] The titanium nitride (TiN) sample had a biofilm thickness of 17 µm after 14 days of incubation with mixed bacteria, in comparison to the TaN sample which had a reduction of 8 µm, proving that the addition of metallic tantalum was beneficial in reducing biofilm thickness. Nonetheless, although the biofilm thickness was reduced in the TaN sample, low levels of biofilm still pose a risk for future adverse events related to bacterial infection. Additionally, tantalum was not explicitly tested against mammalian cells, so there is some concern for cytotoxicity.

Single step micro-arc oxidation (MAO) is a common technique used to adhere metallic coatings to titanium, including strontium,[48] copper,[103] and strontium-silver bactericidal coatings.[51] MAO is an effective method of including metal substrates into porous coatings. Strontium is often chosen as an active agent for its osteogenic effects through the activation of calcium-sensing receptors and inhibition of bone resorption by increasing osteoprotegerin.[102] In addition, strontium can also inhibit bacterial cell metabolism by interfering with cytoplasmic membrane permeability.[102] In a study by Zhou *et al.*, MAO was used to create a microporous coating where strontium (Sr) was combined with cobalt (Co), fluorine (F), calcium (Ca), oxygen (O_2), and phosphorus (P).[48] The formulated coatings varied in strontium concentration and were tested *in vitro* for their osteogenic effects against mesenchymal stem cells (MSC).[48] The results indicated that the coatings including titanium, cobalt, phosphorus, calcium, and fluorine with varying percent weights of strontium (TiCPCF, TiCPCF-S6, TiCPCF-S11, and TiCPCF-S18) improved cell attachment and differentiation substantially in comparison to the Ti control group.[48] It was found that a Sr content of 11% weight in the TiO_2-based doped coating provided the best osteogenic activity *in vitro* as it was best able to stimulate MSC osteogenic differentiation.[48] After a 28-day incubation period with bacteria, all coatings had similar antibacterial removal rates of up to 92%, compared to the Ti control group (antibacterial rate of less than 10%).[48] The high antibacterial removal rate in the TiCPCF, TiCPCF-S6, TiCPCF-S11, and TiCPCF-S18 groups after 28 days indicates the potential for long-term antibacterial activity. Furthermore, the coatings tested by Zhou *et al.* showed no cytotoxic effects when tested against immature osteoblasts derived from mice (MC3T3-E1 cells). However, it is worth noting that other literature has proven that excessive concentrations of strontium can inhibit osteogenic differentiation and proliferation in osteoblasts.[108] This likely because at high concentrations Sr presents cytotoxic effects. Current evidence suggests that strontium is more efficient as a bone implant material than a bactericidal agent in terms of its abilities to promote osteogenic differentiation.[48,50,51,102] It is noteworthy that the TiCPCF coating without strontium

experienced similar antibacterial removal rates to the optimized TiCPCF-S11 coating, indicating that the strontium addition likely had negligible bactericidal effects.

Zinc has been proven to provide low cytotoxic risk, while also reducing biofilm formation against gram-positive (*S. aureus*) and gram-negative (*P. gingivalis and E. coli*) bacteria.[49,50,103] A 2017 study conducted by Aranya *et al.* created calcium phosphate (CaP) based zinc coatings to observe the antibacterial efficiency of zinc as a metallic bactericide.[49] Titanium alloy (Ti-6AL-4V, ASTM alloy standard Grade 5) disks were polished, rinsed with DI water, and air dried before applying the Zn-CaP coating. The Zn-CaP coating was tested against gram-negative bacteria (*P. gingivalis*) for three days. The results displayed a biofilm reduction of 89% compared to CaP coated Ti (55% reduction), indicating that the presence of zinc provided some degree of antibacterial protection.[49] When pairing zinc with strontium and calcium phosphate (SrCaP), the killing rates for SrCaPZn1 against *S. aureus* and *E. coli* reached 61.25% and 55.38%, respectively.[50] Increasing the concentration of zinc 4-fold (SrCaPZn4) had increased killing rates of 83.01% and 71.28% for *S. aureus* and *E. coli*, respectively.[50] The antibacterial results indicated a zinc-dependent relationship.[50] When testing the coatings with MC3T3-E1 cells *in vitro* and evaluating cell viability, the coatings displayed strong cytocompatibility for all zinc concentrations, indicating there was no excessive release of Zn^{2+}ions.[50]

The half maximal inhibitory concentration (IC_{50}) of a substance is the concentration of a substance that is required to inhibit a biological process by half.[109] A 2020 review conducted by Shimabukuro stated that the IC_{50} of silver, copper, and zinc for MC3T3-E1 cells, are 2.77 µM, 15.9 µM and 90 µM, respectively. These values indicate that silver is the most toxic element against osteoblast cells.[103] Silver has high cytotoxic activity because of its rapid burst releases of Ag^+ ions into the surrounding fluid.[51] This rapid release also leads to dramatically low silver content left in the coating, posing a risk for subsequent bacterial infections. A study conducted by Zhang *et al.* formulated silver strontium coatings and tested them using a bacterial inhibition zone (BIZ) assay against *S. aureus*.[51] The BIZ displayed a direct proportionality with Ag content (SrAg0.08 – 2.1 +/- 0.3mm vs. SrAg0.34 – 6.45 +/- 0.1mm, $p < 0.05$).[51] However, larger silver content also resulted in cytotoxic effects against MC3T3-E1 cells.[51] Silver has also been combined with calcium phosphates (CaP) to produce strong bactericidal effects against *S. aureus*, *E. coli*, and *Methicillin Resistant S. aureus* (MRSA) while providing an osteogenic effect .[52,106,107] For example, Ando *et al.'s* thermal spraying technique for Ag-CaP coated titanium completely inhibited MRSA adhesion (<10 colony forming units (CFU) after 10^2 CFU MRSA inoculation).[52] The thermal spraying powder was prepared by mixing 3 wt% of silver oxide and 97 wt% of hydroxyapatite (HA) and shaking them together to mix.[52] The coating was applied on the

sand-blasted surface of the disk using a flame spraying system.[52] This coating was proven to have strong antibacterial effects against *S. aureus*, *E. coli*, and MRSA in fetal bovine serum (FBS) and was not found to be cytotoxic against V79 Chinese hamster lung cells. Thukkaram *et al.* created a thin film amorphous hydrocarbon coating (a-C:H) with varying silver concentrations on medical grade Ti using a combination of gas aggregation source (GAS) and plasma-enhanced chemical vapor deposition (PE-CVD).[53] Coatings were bonded to the surface using the GAS system which lasted only 2.5 minutes. **Figure 3ai** highlights the fabrication process of the coating using the GAS PE-CVD system to create the Ag nanocomposite coating. The GAS system produced silver nanoparticles (AgNPs) with an average diameter of 24 +/- 6 nanometers (nm) embedded in a hydrocarbon matrix to prevent unwanted high burst release of silver ions into the body.[53] As seen in Figure 3ai, the matrix served as a reservoir for the continuous out-diffusion of silver ions.[53] Additional evidence by Thukkaram *et al*. demonstrated that coatings with a greater amount of AgNPs had a 6-log reduction in *E. coli* and a 4-log reduction in *S. aureus* after 24 hours of incubation.[53] Coatings with less AgNPs also provided strong antibacterial effects, however bactericidal efficacy was found to increase with silver content.[53] The a-C:H matrix was not found to induce any significant antibacterial activity in the absence of silver (Fig 3aii). Cytocompatibility testing with MC3T3 osteoblast cells was performed for a period of 7 days. The results indicated that the coated titania experienced a cell viability greater than 90% +/- 10%, in comparison, the uncoated Ti experienced a cell viability of about 70% +/- 10% (Fig 3aiii). It should be noted that lower Ag concentrations provided better MC3T3 cell viability, as increasing the silver content produced some cytotoxicity effects.[53]

The combined evidence suggests that the use of metallic elements in surface coatings have strong bactericidal activity against gram-positive and gram-negative bacteria. A critical strength that metallic coatings possess over other bactericidal coatings, such as bacteriophage or antibiotic coatings, is that they can be sterilized using standard procedures such as gamma irradiation,[110] alcohol disinfection,[110] and autoclave.[111,112] It should be noted that when many sterilization steps are used, the metal ion concentration may decrease. A study by DeVasConCellos *et al.* performed a rigorous sterilization process of their silver coatings, including autoclave, passivation, and ultrasonic cleaning.[113] The coating survived the sterilization process with fewer particles present on the surface, as observed by scanning electron microscopy (SEM).[113] The ability to ensure that the coating is sterile is beneficial in the prevention of bacteria formation. Nevertheless, it remains extremely important to optimize metal concentration to prevent metallic bactericidal coatings from becoming cytotoxic. Metallic coatings display several advantages, first, they are effective against a broad-range of bacteria. Secondly, metallic coatings are easily manufactured and easily tuned and combined

with other components. Lastly, metallic coatings can be sterilized through traditional means. One of the biggest disadvantages metallic coatings have is their lack of specificity, which can result in metallosis, or cytotoxicity toward the host's body. For this reason, the most common antibacterial coatings use molecules that can specifically target bacteria. In the next sections, we will review some common antibiotic coatings used for medical implants.

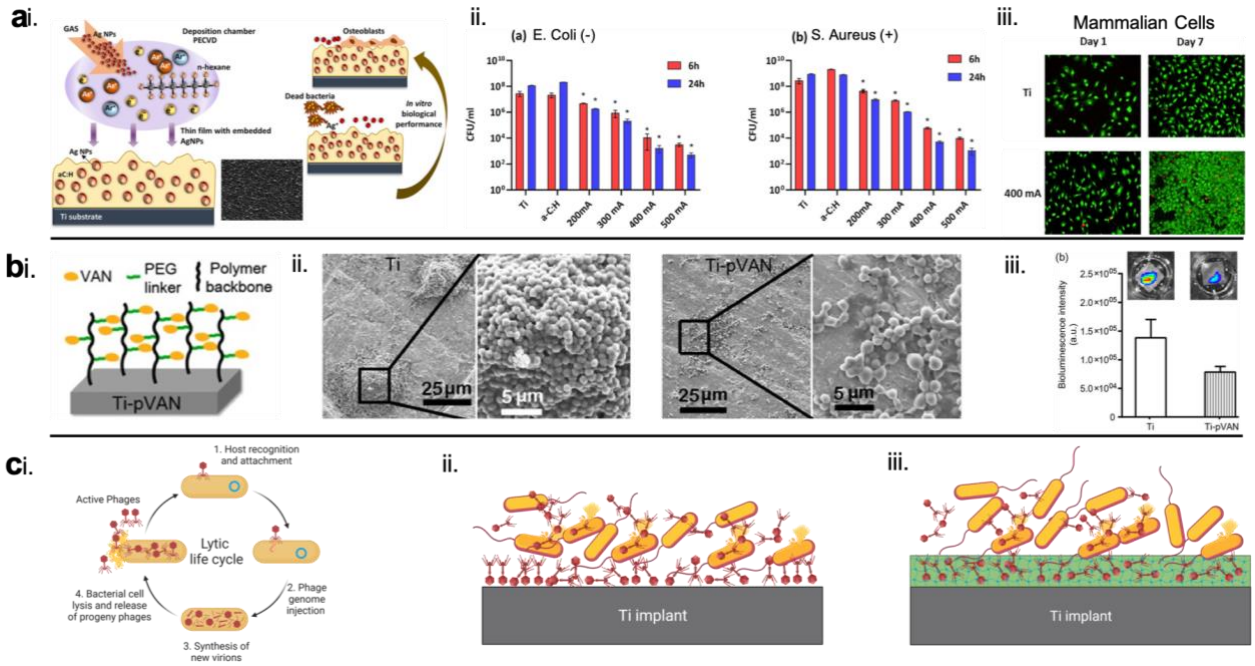

Figure 3. Bactericidal coatings. **ai**) Schematic representation of silver imbeded in a polymer coated on Ti. **aii)** Colony forming units (CFU) assay for *E. coli* and *S. aureus*. **aiii)** Fluorescent live/dead assay of osteoblast cells. Figure 3a was adapted with permission.[53]. **bi)** Schematic representation of vancomycin binded to polymer brush linker. **bii**) SEM images of *S. aureus* on Ti control and antibiotic coated surface. **biii)** Bioluminescent quantification of live bacteria. Figure 3b was adapted with permission. [114] **ci)** Schematic represtation of the bacteriophage's lytic life cycle. **cii)** Bacteriophages physcically or covalently bonded to Ti. **ciii)** Bacteriophages loaded into polymeric coating. Figure 3c was created in biorender.com.

3.2. Antibiotic Coatings

Antibiotics remain the most common prophylactic agents studied. Antibiotics delivered locally at the implant site could have an integral role post-surgery, since the periprosthetic tissue might be damaged, avascular or necrotic, limiting any systemic antibiotic and the immune system from reaching the implant zone,[115] which could lead to an increased risk of implant infection and biofilm formation.[26] The antibiotic for local delivery should be chosen properly based on target agents, and ideally should be broad-spectrum as the infections are usually polymicrobial.[115] To decrease the chance of antibiotic resistance occurrence, the use of at least two antibiotics from different families has been recommended.[115] In recent years, several antibiotic coatings have been created using polymers,[92–94]

ceramics,[58,116,117] polymer-ceramic composites,[58,118] and hydrogel coatings[119] to mitigate bacterial infections. Different methods have been implemented to apply antibiotic-loaded coating on Titanium-based dental/orthopedic implants, including electrospinning,[58,120] dip coating,[93] electrophoretic deposition,[118,121,122] plasma spray coating,[123] and sol-gel solutions.[124–126]

Antibiotics are applied onto an implant's surface in two general ways. The first one involves chemical immobilization on the surface, while the second one comprises of loading the antibiotics into the implant or into a porous sacrificial coating bonded on the implant's surface. The former method usually provides protection only at the surface of the implant since diffusion of the antibiotic is limited. On the other hand, loading the antibiotics provides the opportunity to have a controlled release of the antibiotics by fine-tuning the properties of the sacrificial layer (pore size and dissolvability). Another advantage of loading the antibiotics into a sacrificial layer is that the loading capacity is increased compared to coating the antibiotics directly onto the implant's surface.[115] The initial burst release of antibiotics is of great importance and should be higher than the minimum inhibition concentration (MIC) to protect the implant site against a bacterial infections. The subsequent release of antibiotic is relatively slow which is controlled by degradation rate of the coating or the elution of the antibiotics. The ideal long-term release of drug spans from several months to years post-surgery to prevent late infections.[127] It is worth noting the consequences of poorly designing the coating. If the initial burst release is too high, the coating could become depleted from antibiotics prematurely. Furthermore, high release of antibiotics could also cause negative effects toward the host's tissue.[128] If the coating releases the antibiotic is low, below the MIC, this could give bacteria the opportunity to create resistance to the antibiotics.

Physical adsorption of antibiotics directly onto the surface might not be suitable for long-term implantation due to the fast kinetics of drug release into the environment. Moreover, this approach is also limited by the amount of antibiotics being loaded, however, increasing the surface area through micro/nano structuring can improve the drug loading capacity. For example, the interconnected micropatterned Ti surface was shown to enhance the vancomycin loading through hole pattern structure.[129] To prevent antibiotic loss, covalent bonding onto the surfaces is preferred. This can be achieved through surface functionalization of titanium implants using linker such as silanes, catechol, and phosphor-based molecules.[130] For example, the covalent attachment of vancomycin to Ti surface was reported to reduce Staphylococcus aureus colony-forming units (CFU) by 88% ± 16% over 2 hours.[54] This was achieved by creating by coating Ti with 3-aminopropyltriethoxysilane (APTS; $NH_2PrSi(OEt)_3$), a hydrophilic flexible linker 8-

amino-3,6-dioxaoctanoate (aminoethoxyethoxy-acetate; AEEA) to extend the Vancomycin away from Ti surface to increase interactions with to the bacterial cell wall.[54] Covalent attachment of vancomycin to the surface of aminopropylated Ti alloy with aminopropyl-triethoxysilane and sequential coupling with two Fmoc-[2-(2-amino-ethoxy)-ethoxy]-acetic acid (AEEA) linkers has also shown to prevent *Staphylococcus epidermidis* biofilm formation.[131] In a different study, polydopamine (PD), a mussel-inspired molecule with excellent adhesive properties was used to graft Cefotaxime sodium (CS), to the surface of Ti implant. The interaction between the amino groups in CS and the catechol/quinone groups in PD through Michael addition and Schiff-base reactions provides the covalent grafting of the antibiotic to the surface.[55] The coating showed good hemocompatibility, no cytotoxicity and reduced gram-positive and gram-negative bacteria after 3 days.[55] In a different study, Zhang *et al*., formulated an infection-dependent drug-releasing surface.[56] In this method, vancomycin was covalently attached to the Ti surface through a tailor-made peptide which can be cleaved by a serine protease-like protease (SplB) secreted by S. aureus, providing an on-demand antibacterial response upon infection.[56]

Synergistic effect of antibacterial properties offered by both the loaded antibiotics and antibacterial properties of other coating components can enhance the coating performance. Drug-loaded biodegradable coatings can also provide the opportunity for dual functionality by promoting both antibacterial effect and osseointegration.[121,132] Min et al. reported a dual therapy nanolayered biodegradable polymeric coating on titanium implant using a layer-by-layer (L-b-L) deposition method containing gentamycin sulfate with bone morphogenetic protein (BMP-2), a prominent osteoinductive growth factor, to provide a bacteria killing and bone inducing microenvironment.[57] Rifampicin-loaded electrospun nanofibrous coating on titanium composed of hydroxyapatite (HA), a biocompatible osteoinductive ceramic and poly-caprolactone (PCL) polymer has shown an improved cell proliferation/adhesion and an effective antibacterial performance. This combination showed a 3-log reduction of *S. aureus* and *S. epidermidis*, and 2-log reduction of *P. aeruginosa* after a 24-hour incubation period. However, the long-term performance of these coating was not evaluated.[58] A composite chitosan-bioglass coating on titanium implant loaded with tetracycline and melittin, an antimicrobial peptide with a synergistic effect with antibiotics in killing drug resistant bacteria and prevent biofilm formation has been developed. A bacterial population decrease of >3-log has been reported after 6 hours for planktonic and adherent MRSA, confirming the synergistic effect of tetracycline and Melittin.[59] Choi et al. reported the development of a levofloxacin-loaded thermo-responsive poly (di(ethylene glycol) methyl ether methacrylate) (PDEGMA) brushes on titanium implants to disrupt bacterial colonization and biofilm formation. These polymeric

brushes were synthesized via surface-initiated activator regeneration by electron transfer atom transfer radical polymerization on the surface of titanium, followed by immersion in a levofloxacin solution, to load the polymer brushes with antibiotics. Increasing the temperature to 37°C and 45°C led to faster drug release rate up to 6 hours, confirming a controlled thermo-responsive drug release behaviour. These levofloxacin loaded polymeric brushes showed a 90% reduction of living bacteria after a 24-hour incubation *in vitro*, which was further tested *in vivo* in rats, showing an excellent antibiofouling properties with significantly lower amount of S. aureus after 7-days post innoculation.[60] A vancomycin-bearing polymer brushes on the surface of titanium alloy-based pins has also been prepared using surface-initiated atom transfer radical polymerization and copper-catalyzed azide-alkyne cycloaddition followed by vancomycin conjugation to azido-functionalized side chains of polymethacrylates. A flexible hydrophilic oligo (ethylene glycol) linker (PEG7) was also used to maintain the antibiotic activity of the covalently anchored vancomycin (Figure 3b-i). The treated titanium pins were able to successfully reduce the adherent bacteria by 20-fold compared to untreated control samples after 21 days post implantation in *S. aureus* infected mouse femoral canal.[61] Figure 3b-ii and iii shows the s. aureus adhesion to untreated and vancomycin treated surfaces *in vitro* after 5 hour incubation with 1.2×10^5 CFU/mL and quantification of bioluminescent signal after a 7-hour incubation with 2.3×10^6 CFU/mL.[61]

A lipid-based coating loaded with amikacin and/or vancomycin has been applied on titanium and successfully inhibited biofilm formation when exposed to S. aureus and P. aeruginosa for 24-hours, displaying a 5-log and 3-log bacterial reduction respectively. In this coating, the unloaded phosphatidylcholine-based material showed some antibacterial effect *in vitro*, although this inhibitory effect was not observed during the *in vivo* testing. This observation was interpreted to happen as a result of competing effects of protein and cell adhesion which shows the importance of *in vivo* studies in evaluating the coating's performance.[133]

Antibiotic coatings continue to be researched extensively as a prophylactic measure for medical implants. These technologies are of great value in dental and orthopedic applications as their use can reduce systemic dosing of antibiotics, protecting the medical surface from bacterial adhesion and biofilm formation and in some cases improve osseointegration. There are several limitations which need to be addressed before these coatings can be adapted in clinical settings. First, the long-term stability of the coating needs to be optimized. Although several studies show the efficacy of the coatings after 24-hours, with a few studies spanning 1-week to 1-month periods, ideally these coatings must be effective for longer periods (3 – months to several years). Secondly, the antibiotic coatings need to be design

with a dose profile which maintains the MIC, but doesn't cause any cytotoxicity. This Goldilocks' concentration can be challenging to maintain over the long-term, since the loading capacity is limited and the antibiotic concentration will be depleted with time. Lastly, with the use of antibiotics, especially when at low concentration, carry the risk of creating novel antibiotic resistant bacteria which could will be detrimental to the patient and could limit the efficacy of additional antibiotic treatments. Perhaps all of these downfalls can be circumvented though the novel use of bacteriophages, as discussed in the next section.

3.3. Bacteriophages

With high rates of antibiotic resistance among Gram-positive *S. aureus* and *S. epidermidis*, and increasing resistance among gram-negative bacteria such as *Enterobacter*, *Acinetobacter*, *Klebsiella*, and *Pseudomonas*, treating implant-associated infections are turning into a major challenge worldwide leading to increased rates of implanted device failure.[134,135] Bacteriophages, or phages for short, are bacterial viruses which have been around as antibacterial agents to treat bacterial infections for almost 100 years.[136] With increased prevalence of chronic bacterial infections due to spread of antimicrobial resistance as a global threat, phages have regained attention after being overshadowed for a long time by antibiotics' discovery in 1940s.[137,138] Phages are the most widespread entities on the planet earth with approximate population of 10^{31}, 10 times larger than bacterial population,[139] and they have different shapes and sizes. Morphologically, phages belong to two major categories including tailed (head-tail) and PFP (polyhedral, filamentous, or pleomorphic) and their size ranges from about 23 nm to filamentous phages with up to 2 μm length.[140] Phages are classified into two main categories including lytic or virulent, and lysogenic or temperate. In the lytic life cycle, having identified the host, phage inserts its genome into bacterial cell and takes control of the bacterial reproduction system and replicates itself. Newly formed phages, called progeny phages, are then released to the environment by lysis the bacterial cell. In the other hand, temperate phages incorporated its genome into the bacterial genome, a process known as prophage formation, and stays dormant until triggered by external factors such as UV exposure, heat, or chemicals, and as a result of this, the lysogenic life cycle is converted into lytic cycle.[141] For therapeutic applications, lytic phages are mostly preferred because of their immediate antibacterial effect as well as preventing the spread of horizontal gene transfer of virulence factor by temperate phages.[142]

Phages can attack bacterial cells and replicate themselves on-site by taking advantage of bacterial reproduction machinery.[143,144] This unique characteristic sets phage apart from the rest of antibacterial agents as phages can be implemented as self-sustained antibacterial agents. Another intriguing aspect of bacteriophages is their high

specificity in attacking bacterial species down to the strain level. Phage's specificity mitigates damage to the human microflora as opposed to antibiotics which can affect the good bacteria along with the troublesome ones.[145,146] In addition, phages can be potent biofilm eradicators due to their ability to produce endolysins and EPS depolymerases that can disrupt the biofilm matrix. This provides the opportunity for having access to biofilm depth and attacking the hidden bacteria.[147]

Although promising results have been obtained showing the success of phage therapy in treating bacterial infections, there are some challenges associated with its application in clinical settings. Bacteria can develop resistance against phage infections similar to resistance developed against antibiotics. On the other hand, unlike antibiotics, phages are smart antibacterial agents containing genomic materials enclosed in a proteinous capsid that enables them to fight against phage resistance by genetic mutations to circumvent bacteria defense mechanisms.[148] Phage resistance mechanism include preventing phage adsorption to bacterial cell receptors, preventing the phage DNA entrance, cutting phage nucleic acids and abortive phage infection. Preventing phage adsorption is the most common resistant mechanism which occurs by point mutations and changes in the expression of surface receptors.[149] However, there are reports that show phage resistance can lower fitness and/or reduce virulence which can lead to enhanced performance of the immune system in eradicating the invading bacteria.[150–152] Another limitation could arise from narrow host range of bacteriophages. Although the narrow spectrum is an important characteristic of phages which helps preserving the natural human microflora as opposed to antibiotics, this sometimes requires the application of phage cocktails which are designed to have synergistic effects in removing various strains of the same bacterial species or looking for phages with broad spectrum-strain lytic activity.[153] Application of phage cocktails, or combination therapy with phage and other antimicrobial agents such as antibiotics, antimicrobial peptides, biofilm disrupting enzymes could enhance the efficacy of phage therapy in treating bacterial infections.[154]

The ability of phage in bacterial biofilm eradication can be a game changer when it comes to treating chronic bacterial infections as biofilms are the most challenging forms of bacterial infections and they are very hard to treat. However, almost all of the studies included only a monomicrobial infection model to assess the ability of phage in biofilm eradication. Another area which requires more exploration is assessing the efficacy of phage therapy in treating polymicrobial biofilm infections.

There are several reports *in vitro* and *in vivo* studies supporting the application of phage therapy for treating patients with implant associated infections (IAI). Injectable phage delivering hydrogel based on poly(ethylene glycol)-

4-maleimide (PEG-4-MAL) hydrogel[155] have shown highly effective in reducing the P. aeruginosa population in both planktonic and biofilm states with a 4.7 fold less live bacteria in a mouse radial defect model. Alginate-based hydrogel systems[156,157] have been investigated for IAI treatment. Phage host range can be narrow down to strain levels, however, CRISPR-Cas9 technology can be implemented to modify the phage to have wider host range.[158] Moreover, this technology has been used to remove the staphylococcal cytotoxin and enterotoxin genes which can significantly enhance the safety of phage therapy.[159]

The use of phage as adjunct therapy for treating peri-prosthetic joint infections has been tested *in vivo* by implementing a combination of a phage cocktail with vancomycin against *S. aureus*. Titanium implants infected with *S. aureus* was press fit into a defect created in the distal femur of rats, and phage cocktail and/or vancomycin were administrated via the intraperitoneal (i.p.) route on day 21 to 27 post-surgery. Dual phage cocktail/antibiotic therapy showed the best results in treating infections and a 22.5-fold reduction was observed within joint tissue of animals with decreased swelling in the implanted knee. This supports the potential of phage therapy in combination with antibiotics to treat periprosthetic joint infections.[160] Local injection of *P. aeruginosa* and MRSA phages have been shown to successfully decrease the implant-related infections in a rat model, and when accompanied by appropriate antibiotic regimen, the biofilm of both bacteria was effectively eradicated.[161] Injection of a cocktail of highly lytic phages isolated form environmental sources, into the joint after replacement and joint closure accompanied with antibiotic treatment led to promising results and eradication of infection among patients with relapsing S. aureus prosthetic knee infection.[162]

Kaur et al. proposed a dual antibacterial using hydroxypropyl methylcellulose (HPMC) gel coating containing a broad-spectrum lytic phage against *S. aureus* strains, and linezolid (a bacteriostatic agent which inhibits the bacterial protein synthesis and creation of initiation complex in gram positive cocci including streptococci, enterococci, staphylococci) on K-wires which are commonly used in orthopaedic implant for pin fixation. The results confirmed a significant reduction in the adhered viable bacteria on implants and the surrounding tissue with no sign of resistant mutants arising in the phage and/or linezolid coated implants *in vivo*. The maximum bacterial reduction was observed when dual coated K-wires were used with a maximum decrease in associated inflammation. This result has been interpreted to happen due to the synergistic effect of phage and linezolid. Linezolid as a protein synthesis inhibitor prevents bacterial growth while boosting the phage assembly, production, lysis, and overall enhanced lytic activity of phage.[64,65]

In addition to use of whole phage as a bacteria devourer virus in treating bacterial infections, phage endolysins, or simply lysins, have been proposed as one of the interesting antibacterial agents. Phage-derived endolysins are peptidoglycan hydrolases used by bacteriophages towards the end of lytic cycle to rupture the peptidoglycan layer of the bacterial cell wall.[163] Currently, one endolysin (CF-301) has been used to treat bacteremia patients; however, there are no reports of antibacterial phage lysins for treating musculoskeletal infections.[152]

Overall, several successful cases of human clinical studies with phage monotherapy and phage-antibiotic combined therapy have been reported so far which along with several *in vivo* and *in vitro* studies demonstrates the significant potential application of phage therapy in treating orthopedic device-associated infections.[152] Similar to all pros and cons associated with use of other bactericidal agents, there are advantages and disadvantages to use of phages in treating bacterial infections which needs to be considered in designing phage-based coatings. This includes preserving phage infectivity (long term stability), narrow host range, phage resistance, and complicated interaction between phage-bacteria and human immune system. There are immediate needs of exploring phage pharmacodynamic and pharmacokinetics to shed more light on the efficacy of phage therapy in clinical settings by conducting more *in vivo* studies. Phage can be embedded into hydrogels and polymeric coatings or applied directly onto the implant surface, with or without other antibacterial strategies, to prevent implant-associated infections. Although there are no reports of incorporating phage coatings on titanium-based orthopedic and dental implants, there are evidence showing their potential applications in these applications, especially with alarming rates of global antibiotic.

4. Animal Studies

In vivo testing is a crucial step to promote these coatings along the commercialization pipeline and to translate these technologies from research to clinical use. Here, we will delineate some of the recent titanium-treated antibacterial coatings that have been tested under *in vivo* conditions.

When designing coatings for orthopedic and dental implants, osseointegration is a key factor to be considered, as proper integration is essential to produce a strong mechanical interlocking between the bone and the prosthetic device. This is especially important in load bearing prosthetics, as poor integration can cause loosening and failure of the device. As mentioned earlier, titanium surfaces are bioinert, and therefore provide a non-toxic and favorable surface for bone cells to grow onto. However, having a bioinert surface does not represent a surface with optimal conditions for bone to grow. For this reason, coatings have been developed with osteogenic molecules to enhance bone cell adhesion and differentiation to enhance bone integration. Among the osteogenic factors, the most used are

hydroxyapatite (HA) and calcium phosphate (CaP) coatings which have been shown to have osteoinductive and osteoconductive properties. It is worth noting that osteoinduction refers to the ability to stimulate immature host cells to develop into osteogenic cells, while osteoconduction refers to the ability to induce bone cell ingrowth and osteoid deposition.[164] Walter *et al*. coated titanium zirconium (TiZr) alloy discs with doxycycline using electrochemical deposition.[62] The coated samples were introduced onto rabbits near the bone marrow region of the tibia in the absence of bacteria. The samples were collected after 4 or 8 weeks of bone healing and the results demonstrated some positive trends. First, the doxy-coated titanium samples showed less cytotoxicity compared to TiZr control. Secondly, doxy-coated samples expressed upregulated genes for alkaline phosphatase (ALP), osteocalcin, and bone morphogenic protein-2 (BMP-2).[62] Lastly, an increased bone mineral density (BMD) and total bone volume was seen doxy-coated samples compared to uncoated devices.[62] All of these results put together indicate a positive osteoinductive effect for the doxycycline coating. A similarly coating was used by Rahmati *et al*. on TiZr alloy.[97] The antibiotic coating release profile was tested *in vitro* under acidic conditions to represent a bacterial infection.[97] The results indicated a neglegible antibiotic releaser for the first 24-hours, followed by an increased burst release concentration. Although physiological condtions were not tested, the researchers alluded that the coating should be more resistant at higher pH. The antibiotic coating was tested with two animal models (rabbit and dog). In the animal studies mirco-CT scans indicated a significat bone in-growth after an 8-week period. The researchers also performed histological studies, however, there was no statistical difference between uncoated and coated TiZr. Although doxycyclene did not show a great improvement in osseointegration, this data still proved that adding a layer of doxycyclene did not hinder any bone growth onto antibiotic coated devices. Although doxycyclene is a known antibiotic, no bacteria studies were performed *in vivo* or *in vitro*. Nie *et al*. also formulated an antibacterial coating using antibiotics (bacitracin).[63] The bacitracin was covalently bonded to dopamine-conjugated titanium rods via EDC/NHS chemistry and imbedded into the femur of Sprague-Dawley rats, in the presence or absense of *s. aureus*. After a 3-week period, the rats were tested using X-ray radiographic imaging which showed no signs of infection in the Ti-bacitracin (with s. aureus) group, or the Ti rod incubated in PBS. In contrast, the Ti sample imbeded with *s. aureus* did show signs of osteolysis. These results were also validated with micro-CT scans which showed a reduced bone volume in the infected device, while no bone loss was seen in the antibiotic coated implant.[63] Further testing was performed *ex vivo* using a spread plate assay, showing a 2.16 log CFU reduction compared to the infected Ti control. *In vivo* osseointegration studies were also performed using micro-CT testing in the absense of bacterial. Here, bone volume was enhanced on the antibiotic coated Ti

samples, compared to Ti, or dopanine coated Ti samples, therefore proving that bacitracin has an osteoconductive effect.[63] This study showed promising results regarding osseointegration and the antibacterial activity of their coating, however, neither this, nor their previous study[99] tested the longevity of their coating which is another important aspect to consider. Another commonly borad-spectrum antibiotic is gentamicin. In a study perfromed by Min *et al*., gentamicin and osteoconductive growth factor (BMP-2) were coated in a layer-by-layer (LbL) fashion onto Polyetheretherketone (PEEK) substrates.[57] The LbL approach provides the advantage of independently tune of multidrug release kinetics (**Figure 4ai**).[57] Therefore, these researchers envisioned a coating that releases antibiotics early on post-surgical implantation, followed by a slow release of BMP-2 to prommote osseointegration and to "win the race to the surface".[57] Their rat animal model consited of drilling small incision on the tibia into the medullary cavity, introducing an uncoated implants and then inoculating them with *s. aureus* ($5x10^5$ CFU) to produce an osteomyeletus model.[57] After a 7-day period, a revision surgery was perfromed and the infected implant was replaced with either coated implants or uncoated control.[57] The BMP-2 and gentamicin (BG) coated samples reduced the bacterial CFU by 2-3 orders of magnitude compared to uncoated control (Figure 4aii-4aiii).[57] Furthermore, when comparing for newly bone formation, uncoated samples showed significat bone destructuion due to long-term inflamation caused by the bacteria. On the other hand, gentamicin coated samples showed some bone growth, but samples containing gentamicin and BMP-2 displated increased bone formation.[57] In terms of the mechanical stability of the implant, the BG coated sample displayed 10-15 times shear strenght compared to uncoated control. This study demonstrated a novel coating with a unique osteomyletitis animal model. Although this study was not performed on titanium substrates, similar methods could be applied to create a layer-by-layer deposition of different bioactive drugs with independent release control on titanium, or other substrates.

Antibiotics are not the only methods to treat infected prosthetics. For example, Yuan *et al*. created a mesoporous polydopanine nanorparticle (MPDA) mesh loaded with photosensitizer Indocyanine Green (ICG) as shown in Figure 4b.[66] This coating provided photothermal (PTT) and photodynamic (PDT) therapeutic actions to lyse bacteria under near infrared (NIR) light. The MPDA were coated onto amino-modified titania, loaded with ICG, and further modified with RGD peptide to provide osteoconductive properties. *In vivo* testing was performed on a rat mode, where Ti rods were coated incubated with an *s. aureus* biofilm and then implanted into the femulr of the rats.[66] One day post-surgery, the rods were irradiated with an 808 nm laser to activate the PTT and PDT effects. After two weeks the rods were removed and the teste ex-vivo against bacteria. The results showed a reduction of bacteria up to

99.7% and the disruption of the biofilm compared to irradiate Ti (only 15% bacteria reduction). Figure 4bii and 5biii, clearly disply the a difference is CFU on a spread plate assay, as well as a disrupted biofilm (SEM ismages). The produced coating was also tested for cytocompatibily *in vitro*, which showed a greater mesenchymal stem cell (MSC) population compared to Ti samples due to the osteoconductive RGD proteins. Nevertheless, upon irradiating the samples containing MSCs with NIR light, the cell viability was reduced due to cytotoxic effects produced by PTT and PDT. All together, this technology can be proved to be beneficial in a medical prosthetic. Although the photothermal and photodynamic therapies can be cytotoxic towards the bone cells, these effects will only be seen upon NIR irradiation, which will be likely be administered through a controlled regiment in a clinical setting. On the other hand, this technology could treat established bacterial infections without requiring surgical precedure. One of the caveats for this technology is the possibility of overexposing the coating to NIR light with natural light. IR sources, such as the sun, could potentially create device losening due to PTT and PTD activation, therefore, extensive research into this technology is required. Yang *et al*., coated titanium with a hyperbranched poly-L-lysiene polymer (HBPL) which proved to have osseocoductive properties and excelent antibacteria properties *in vitro* against gram-positive and gram-negative bacteria (Figure 4ci-iv).[46] The HBPL was tested in an *in vivo* in a rat model by drilling holes into the tibia of the rat and introducing titanium screws in the presense of bacteria (*S. aureus*). After 3 days, the rats were euthanized and the screws and surrounding tissues were collected. Samples from the implant and the mdullary cavity were sonnicated then streak plated onto brain heart infusion agar paltes. As seen in figure 4civ, the Ti-HBPL coated samples significantly reduced bacterial colonies compared with the control groups. Histological staining also showed inflamation near the Ti-bone interface for the Ti and Ti-GPTMS groups but not on the HBPL coated samples. The HBPL coating was also tested against bone formation using micro-CT scanning and histological staining in the presence or absence of bacteria 4 weeks after implantation. In both tests, the Ti-HBPL samples showed increased bone formation compared to Ti control. Figures 5cii and 5ciii show the new bone formation on all three groups, and shows further proof that new bone formation was possible, even when the implant was infected with *s. aureus*. Although the bone volume / total volume (BV/TV) percentage growth is smaller in the noninfected rat model (figure 4ciii.), the absolute BV/TV did indicate that the infection did reduce bone formation.[46] This type of polymer is beneficial as an antibacterial coating, since it is not cytotoxic, had no proinflamatory response, enhanced osseointegration and reduced bacterial viability with *in vivo* and *in vitro*. This polymer's main antibacterial mode of action is through the production of ROS.

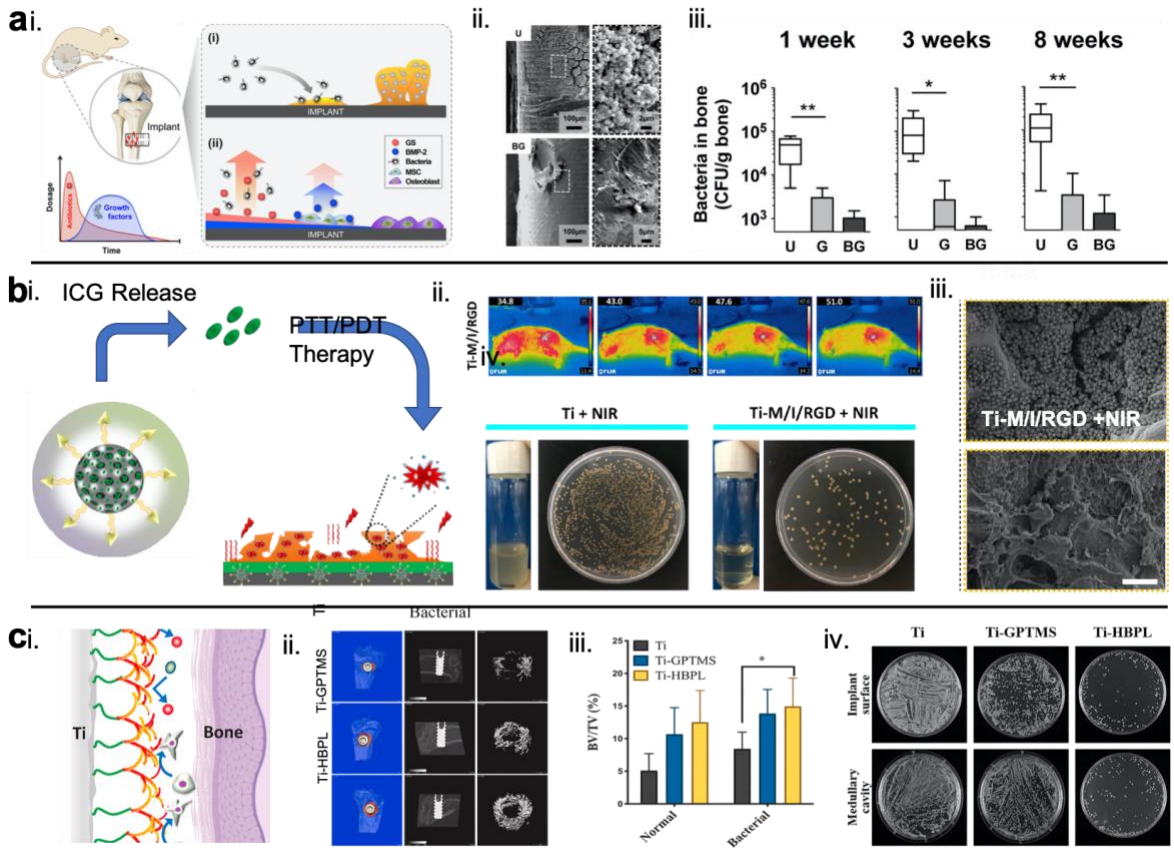

Figure 4. In vivo studies for antibacterial coatings. a) Doxycycline coating titanium was implanted into the tibia of a rabbit model and showed an increased bone formation toward the titanium screw using a 3D micro-CT technique. Figures 4a. were modified with permissions.[57] **b)** Mesoporous polydopamine nanoparticles were loaded with osteogenic peptide RGD and indocyanine green (ICG) to enhance osseointegration and eradicated biofilms upon near infrared (NIR) stimulus, respectively. When exposed to NIR, the ICG in the nanoparticles produced photothermal and photodynamic therapies to destroy biofilms and kill S. aureus with 99.7% efficiency. Scale bar in 5biii. represents 5 µm. Figures 5b. were modified with permissions.[66] **c)** Titanium coated with a hyperbranched poly-L-lysine promoted osseointegration (ii, iii) and lysed bacteria (iv) in a rat animal model. Figures 4c. were modified with permissions.[46]

5. Commercialization of antibacterial coatings.

Although several antibacterial coatings have been presented thus far, only a handful have been adopted in commercial and clinical settings. For example, the Defensive Antibacterial Coating (DAC) is a hydrogel composed of hyaluronic acid and poly-lactic acid, components which are naturally produced by the body.[165] This coating is meant to be applied onto the medical device at the time of operation, providing a hydrophilic physical barrier which prevents bacteria adhesion. Furthermore, this coating is resorbed by the body within 72 hours, detaching any bacteria into the planktonic phase in the process. The DAC coating can also be combined with active antibiotics, which would be released as the hydrogel dissociates. Although this coating provides an easy solution in the short-term, it is evident

that such coating would not provide protection days after the surgery. An antibiotic-tethered coating has also been approved for intermedullary nails for tibia injuries in Switzerland.[166] The current coating is designed with gentamicin, but this technology could be implemented with other antibiotics. As discussed earlier, antibiotic coatings can be cytotoxic in large concentrations or could lead to drug-resistant bacteria, therefore making this coating more difficult to be adopted worldwide. Moreover, antibiotics require a minimum inhibitory concentration (MIC) to be efficient against a pathogen, which limits the long-term results of such coating as these molecules tend to degrade over time. A different coating which has been approved in some European countries is Bactiguard.[167] This metal coating is composed of a gold, silver, and palladium, which created a small current through a galvanic effect to prevent bacterial adhesion.[167,168] Metallic coatings have the potential to prevent bacteria adhesion long-term, as the coating does not dissociate. Furthermore, these noble metals will not impede ossiointegration, so long as the concentrations of any ions produced remains low. Silver-containing hydroxy apatite has also been developed for use in orthopedic applications and for spinal surgeries.[102] These coatings have been shown to prevent bacteria postoperatively, and also provide exceptional osteoconductive properties due to the HA layer. Although these metallic coatings are becoming popular due to the low risk of bacterial resistance, there is still some skepticism behind metallic coatings, mainly, the potential toxicity of metal ions' release, or that the antibacterial pathways are still not clear.[169]

6. Discussion and Conclusion.

Implant-associated infections remain one of the major causes for medical device failure, especially in dental and orthopedic settings. This review highlights several strategies employed on titanium to prevent implant infections. These strategies include anti-adhesion coatings to repel bacteria or bactericidal coatings that lyse bacteria. In the later strategy, surfaces are typically modified to create a non-stick coating using polymers, superhydrophobic, liquid-infused or other strategies. Care must be employed when designing these coatings since they can also prevent host cell interaction which can impede newly bone formation and lead to aseptic loosening. However, through the combination of these surface coatings with osteogenic factors, proper osseointegration is possible. If the goal is to use these coatings in orthopedic or dental settings, these surfaces must be tested against bacteria attachment, but also proper bone integration. Furthermore, these coatings need to be tested for their long-term stability, as several studies showed these coatings to be effective after 24- or 48-hours, with few experiment spanning more than two weeks. Nevertheless, the multifunctional coatings do show a promising steppingstone to develop these technologies to prevent IAI long-term. The second major category of antibacterial coatings were composed of bactericidal agents. Metallic

coatings have been shown to have some of the greatest longevity because these coatings do not rely on molecules that degrade or elude from the surface to properly fend off against bacteria. However, metallic coatings have poor specificity, which increases the probability of cytotoxic interactions with the host and promote inflammation and prosthetic loosening. For this reason, these coatings must be further optimized to prevent these negative effects. Other bactericidal coating includes antibiotics and lytic proteins have been shown to be effective against bacteria with higher specificity. These molecules are either covalently attached onto the surface or are loaded into the structure, allowing it to elute from the surface. In some coatings, the structure material is biodegradable which allows for different dosing kinetics from the bactericidal agents. The advantages from the covalently bonded molecules, is that their availability is not depleted and lost systematically, while protecting the surface upon bacterial contact. The downside is that this strategy limits the interactions between these molecules and nearby bacteria.

Moreover, their long-term stability needs to be better understood, as lysed bacterial components could adhere to the surface, essentially blocking the interactions between the bactericidal agents and newly attached bacteria. The advantages of loading the structure with the bactericidal coatings include a higher loading capacity, and controlled dosing kinetics. Care must be employed, since large initial burst of the bactericidal molecules can be cytotoxic, or could deplete the coating from the bactericidal molecules. Moreover, unlike the metallic coatings, antibiotics might not be effective long-term and methods to prolong their stability needs to be investigated. A new strategy that could circumvent the issued discussed with antibiotics or metallic coatings can arise from the use of bacteriophages. Bacteriophages have been shown to be highly specific to a bacteria family, therefore reducing the chance for cytotoxicity. Furthermore, because bacteriophages reproduce after infecting the host bacteria, their availability is more abundant, and not limited by what was loaded onto the surface. Although this strategy is still in its infancy, it shows promising results for future study and for future commercial products.

Acknowledgements

References

1. López-Valverde, N., Macedo-De-Sousa, B., López-Valverde, A. & Ramírez, J. M. Effectiveness of antibacterial surfaces in osseointegration of titanium dental implants: A systematic review. *Antibiotics* **10**, (2021).

2. Sakka, S., Baroudi, K. & Nassani, M. Z. Factors associated with early and late failure of dental implants. *J Investig Clin Dent* **3**, 258–261 (2012).
3. *Early Revisions of Hip and Knee Replacements in Canada: A Quality, Productivity and Capacity Issue*. (2020).
4. D'Onofrio, K. Infection Most Common Culprit of Revision TKA Failure. https://www.aaos.org/aaosnow/2020/aaos-now-special-edition/research/557_knee/ (2020).
5. Trouillet-Assant, S. *et al.* Dual Impact of Live Staphylococcus aureus on the Osteoclast Lineage, Leading to Increased Bone Resorption. *J Infect Dis* **211**, 571–581 (2015).
6. Rasmussen, T. B. & Givskov, M. Quorum-sensing inhibitors as anti-pathogenic drugs. *International Journal of Medical Microbiology* **296**, 149–161 (2006).
7. Villegas, M. *et al.* Enhancing osseointegration and mitigating bacterial biofilms on medical-grade titanium with chitosan-conjugated liquid-infused coatings. *Sci Rep* **12**, 1–11 (2022).
8. Seth, A. K. *et al.* In vivo modeling of biofilm-infected wounds: A review. *Journal of Surgical Research* vol. 178 330–338 Preprint at https://doi.org/10.1016/j.jss.2012.06.048 (2012).
9. Zapotoczna, M., O'Neill, E. & O'Gara, J. P. Untangling the Diverse and Redundant Mechanisms of Staphylococcus aureus Biofilm Formation. *PLoS Pathogens* vol. 12 Preprint at https://doi.org/10.1371/journal.ppat.1005671 (2016).
10. Roy, R., Tiwari, M., Donelli, G. & Tiwari, V. Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. *Virulence* **9**, 522–554 (2018).
11. Lewis, K. Persister Cells. *Annu Rev Microbiol* **64**, 357–372 (2010).
12. Bhandari, M., Smith, J., Miller, L. E. & Block, J. E. Clinical and Economic Burden of Revision Knee Arthroplasty. *Clin Med Insights Arthritis Musculoskelet Disord* **5**, 89 (2012).
13. Zimmerli, W. Clinical presentation and treatment of orthopaedic implant-associated infection. *J Intern Med* **276**, 111–119 (2014).
14. Shi, Z., Neoh, K. G., Kang, E. T., Poh, C. & Wang, W. Bacterial adhesion and osteoblast function on titanium with surface-grafted chitosan and immobilized RGD peptide. *J Biomed Mater Res A* **86A**, 865–872 (2008).

15. Petrie, T. A., Reyes, C. D., Burns, K. L. & García, A. J. Simple application of fibronectin–mimetic coating enhances osseointegration of titanium implants. *J Cell Mol Med* **13**, 2602 (2009).

16. Vertesich, K. *et al.* Alendronate enhances osseointegration in a murine implant model. *J Orthop Res* **39**, 719 (2021).

17. Lee, D. W., Yun, Y. P., Park, K. & Kim, S. E. Gentamicin and bone morphogenic protein-2 (BMP-2)-delivering heparinized-titanium implant with enhanced antibacterial activity and osteointegration. *Bone* **50**, 974–982 (2012).

18. Saithna, A. The influence of hydroxyapatite coating of external fixator pins on pin loosening and pin track infection: A systematic review. *Injury* **41**, 128–132 (2010).

19. Chen, C., Li, H., Kong, X., Zhang, S. M. & Lee, I. S. Immobilizing osteogenic growth peptide with and without fibronectin on a titanium surface: effects of loading methods on mesenchymal stem cell differentiation. *Int J Nanomedicine* **10**, 283 (2015).

20. Eliaz, N. & Metoki, N. Calcium Phosphate Bioceramics: A Review of Their History, Structure, Properties, Coating Technologies and Biomedical Applications. *Materials* **10**, 334 (2017).

21. Wildemann, B., Lübberstedt, M., Haas, N. P., Raschke, M. & Schmidmaier, G. IGF-I and TGF-beta 1 incorporated in a poly(d,l-lactide) implant coating maintain their activity over long-term storage—cell culture studies on primary human osteoblast-like cells. *Biomaterials* **25**, 3639–3644 (2004).

22. Raines, A. L. *et al.* VEGF-A Regulates Angiogenesis during Osseointegration of Ti Implants via Paracrine/Autocrine Regulation of Osteoblast Response to Hierarchical Microstructure of the Surface. *J Biomed Mater Res A* **107**, 423 (2019).

23. Tan, G. *et al.* Antibacterial and Anti-Inflammatory Coating Materials for Orthopedic Implants: A Review. *Coatings 2021, Vol. 11, Page 1401* **11**, 1401 (2021).

24. Li, Q., Liang, B., Wang, F. & Wang, Z. Delivery of Interleukin 4 from a Titanium Substrate Coated with Graphene Oxide for Enhanced Osseointegration by Regulating Macrophage Polarization. *ACS Biomater Sci Eng* **6**, 5215–5229 (2020).

25. Vaidya, P., Mahale, S., Kale, S. & Patil, A. Osseointegration-A Review. *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) e-ISSN* **16**, 45–48 (2017).

26. Lu, X. *et al.* Multifunctional Coatings of Titanium Implants Toward Promoting Osseointegration and Preventing Infection: Recent Developments. *Front Bioeng Biotechnol* **9**, (2021).

27. Agarwal, R. & García, A. J. Biomaterial strategies for engineering implants for enhanced osseointegration and bone repair. *Advanced Drug Delivery Reviews* vol. 94 53–62 Preprint at https://doi.org/10.1016/j.addr.2015.03.013 (2015).

28. Zhao, T., Chu, Z., Ma, J. & Ouyang, L. Immunomodulation Effect of Biomaterials on Bone Formation. *Journal of Functional Biomaterials 2022, Vol. 13, Page 103* **13**, 103 (2022).

29. Kaufman, M. G., Meaike, J. D. & Izaddoost, S. A. Orthopedic Prosthetic Infections: Diagnosis and Orthopedic Salvage. *Semin Plast Surg* **30**, 66 (2016).

30. Villegas, M. *et al.* Antibiotic-Impregnated Liquid-Infused Coatings Suppress the Formation of Methicillin-Resistant Staphylococcus aureus Biofilms. *ACS Appl Mater Interfaces* **13**, 27774–27783 (2021).

31. Lin, C. *et al.* Effect of superhydrophobic surface of titanium on staphylococcus aureus adhesion. *J Nanomater* **2011**, (2011).

32. Souza, J. G. S. *et al.* Targeting Pathogenic Biofilms: Newly Developed Superhydrophobic Coating Favors a Host-Compatible Microbial Profile on the Titanium Surface. *ACS Appl Mater Interfaces* **12**, 10118–10129 (2020).

33. Montgomerie, Z. & Popat, K. C. Improved hemocompatibility and reduced bacterial adhesion on superhydrophobic titania nanoflower surfaces. *Materials Science and Engineering: C* **119**, 111503 (2021).

34. Bartlet, K., Movafaghi, S., Dasi, L. P., Kota, A. K. & Popat, K. C. Antibacterial activity on superhydrophobic titania nanotube arrays. *Colloids Surf B Biointerfaces* **166**, 179–186 (2018).

35. Fadeeva, E. *et al.* Bacterial retention on superhydrophobic titanium surfaces fabricated by femtosecond laser ablation. *Langmuir* **27**, 3012–3019 (2011).

36. Doll, K. *et al.* Development of Laser-Structured Liquid-Infused Titanium with Strong Biofilm-Repellent Properties. *ACS Appl Mater Interfaces* **9**, 9359–9368 (2017).

37. Doll, K. *et al.* Liquid-Infused Structured Titanium Surfaces: Antiadhesive Mechanism to Repel Streptococcus oralis Biofilms. *ACS Appl Mater Interfaces* **11**, 23026–23038 (2019).

38. Zhang, F., Zhang, Z., Zhu, X., Kang, E. T. & Neoh, K. G. Silk-functionalized titanium surfaces for enhancing osteoblast functions and reducing bacterial adhesion. *Biomaterials* **29**, 4751–4759 (2008).

39. Cheng, Y. F. *et al.* Tannic acid-assisted deposition of silk sericin on the titanium surfaces for antifouling application. *Colloids and Interface Science Communications* **35**, (2020).

40. Harris, L. G., Tosatti, S., Wieland, M., Textor, M. & Richards, R. G. Staphylococcus aureus adhesion to titanium oxide surfaces coated with non-functionalized and peptide-functionalized poly(L-lysine)-grafted- poly(ethylene glycol) copolymers. *Biomaterials* **25**, (2004).

41. Khoo, X. *et al.* Staphylococcus aureus resistance on titanium coated with multivalent PEGylated-peptides. *Biomaterials* **31**, 9285–9292 (2010).

42. Valliammai, A., Selvaraj, A., Mathumitha, P., Aravindraja, C. & Pandian, S. K. Polymeric antibiofilm coating comprising synergistic combination of citral and thymol prevents methicillin-resistant Staphylococcus aureus biofilm formation on titanium. *Materials Science and Engineering C* **121**, (2021).

43. Guo, L. L. *et al.* Simultaneous deposition of tannic acid and poly(ethylene glycol) to construct the antifouling polymeric coating on Titanium surface. *Colloids Surf B Biointerfaces* **200**, 111592 (2021).

44. Şimşek, M., Aldemir, S. D. & Gümüşderelioğlu, M. Anticellular PEO coatings on titanium surfaces by sequential electrospinning and crosslinking processes. *Emergent Mater* **2**, (2019).

45. Boschetto, F. *et al.* Antibacterial and osteoconductive effects of chitosan/polyethylene oxide (PEO)/bioactive glass nanofibers for orthopedic applications. *Applied Sciences (Switzerland)* **10**, (2020).

46. Yang, Z. *et al.* Covalent grafting of hyperbranched poly-L-lysine on Ti-based implants achieves dual functions of antibacteria and promoted osteointegration in vivo. *Biomaterials* **269**, 120534 (2021).

47. Zhang, Y. *et al.* Tantalum Nitride-Decorated Titanium with Enhanced Resistance to Microbiologically Induced Corrosion and Mechanical Property for Dental Application. *PLoS One* **10**, e0130774 (2015).

48. Zhou, J., Wang, X. & Zhao, L. Antibacterial, angiogenic, and osteogenic activities of Ca, P, Co, F, and Sr compound doped titania coatings with different Sr content. *Scientific Reports 2019 9:1* **9**, 1–11 (2019).

49. Kulkarni Aranya, A. *et al.* Antibacterial and bioactive coatings on titanium implant surfaces. *J Biomed Mater Res A* **105**, 2218–2227 (2017).

50. Zuo, K. *et al.* Zinc-Doping Induces Evolution of Biocompatible Strontium-Calcium-Phosphate Conversion Coating on Titanium to Improve Antibacterial Property. *ACS Appl Mater Interfaces* **14**, 7690–7705 (2022).

51. Zhang, Y. Y. *et al.* Evaluation of osteogenic and antibacterial properties of strontium/silver-containing porous TiO2 coatings prepared by micro-arc oxidation. *J Biomed Mater Res B Appl Biomater* **109**, 505–516 (2021).

52. Ando, Y. *et al.* Calcium phosphate coating containing silver shows high antibacterial activity and low cytotoxicity and inhibits bacterial adhesion. *Materials Science and Engineering: C* **30**, 175–180 (2010).

53. Thukkaram, M. *et al.* Investigation of Ag/a-C:H Nanocomposite Coatings on Titanium for Orthopedic Applications. *ACS Appl Mater Interfaces* **12**, 23655–23666 (2020).

54. Jose, B., Antoci, V., Zeiger, A. R., Wickstrom, E. & Hickok, N. J. Vancomycin covalently bonded to titanium beads kills Staphylococcus aureus. *Chem Biol* **12**, 1041–1048 (2005).

55. He, S. *et al.* Antibiotic-decorated titanium with enhanced antibacterial activity through adhesive polydopamine for dental/bone implant. *J R Soc Interface* **11**, (2014).

56. Zhang, Y. *et al.* Smart Titanium Coating Composed of Antibiotic Conjugated Peptides as an Infection-Responsive Antibacterial Agent. *Macromol Biosci* **21**, 1–8 (2021).

57. Min, J. *et al.* Designer Dual Therapy Nanolayered Implant Coatings Eradicate Biofilms and Accelerate Bone Tissue Repair. *ACS Nano* **10**, 4441–4450 (2016).

58. Kranthi Kiran, A. S. *et al.* Drug loaded electrospun polymer/ceramic composite nanofibrous coatings on titanium for implant related infections. *Ceram Int* **45**, 18710–18720 (2019).

59. Zarghami, V., Ghorbani, M., Bagheri, K. P. & Shokrgozar, M. A. Improving bactericidal performance of implant composite coatings by synergism between Melittin and tetracycline. *J Mater Sci Mater Med* **33**, (2022).

60. Choi, H. *et al.* Drug Release from Thermo-Responsive Polymer Brush Coatings to Control Bacterial Colonization and Biofilm Growth on Titanium Implants. *Adv Healthc Mater* **10**, 1–12 (2021).

61. Zhang, B., Braun, B. M., Skelly, J. D., Ayers, D. C. & Song, J. Significant Suppression of Staphylococcus aureus Colonization on Intramedullary Ti6Al4V Implants Surface-Grafted with Vancomycin-Bearing Polymer Brushes. *ACS Appl Mater Interfaces* **11**, 28641–28647 (2019).

62. Walter, M. S. *et al.* Bioactive implant surface with electrochemically bound doxycycline promotes bone formation markers in vitro and in vivo. *Dental Materials* **30**, 200–214 (2014).

63. Nie, B. *et al.* Immobilizing bacitracin on titanium for prophylaxis of infections and for improving osteoinductivity: An in vivo study. *Colloids Surf B Biointerfaces* **150**, 183–191 (2017).

64. Kaur, S., Harjai, K. & Chhibber, S. In Vivo Assessment of Phage and Linezolid Based Implant Coatings for Treatment of Methicillin Resistant S. aureus (MRSA) mediated orthopaedic device related infections. *PLoS One* **11**, 1–23 (2016).

65. Kaur, S., Harjai, K. & Chhibber, S. Bacteriophage Mediated Killing of Staphylococcus aureus In Vitro on Orthopaedic K Wires in Presence of Linezolid Prevents Implant Colonization. *PLoS One* **9**, (2014).

66. Yuan, Z. *et al.* Remote eradication of biofilm on titanium implant via near-infrared light triggered photothermal/photodynamic therapy strategy. *Biomaterials* **223**, 119479 (2019).

67. Zhang, X., Wang, L. & Levänen, E. Superhydrophobic surfaces for the reduction of bacterial adhesion. *RSC Adv* **3**, 12003–12020 (2013).

68. Gallardo-Moreno, A. M. *et al.* In vitro biocompatibility and bacterial adhesion of physico-chemically modified Ti6Al4V surface by means of UV irradiation. *Acta Biomater* **5**, 181–192 (2009).

69. Wenzel, R. N. Resistance of Solid Surfaces to Wetting by Water. *Ind Eng Chem* **28**, 988–994 (1936).

70. Cassie, A. B. D. & Baxter, S. Wettability of porous surfaces. *Transactions of the Faraday Society* **40**, 546 (1944).

71. Wang, Z. & Ren, B. Preparation of superhydrophobic titanium surface via the combined modification of hierarchical micro/nanopatterning and fluorination. *J Coat Technol Res* **19**, 967–975 (2022).

72. Hwang, G. B. *et al.* The Anti-Biofouling Properties of Superhydrophobic Surfaces are Short-Lived. *ACS Nano* **12**, 6050–6058 (2018).

73. Saulacic, N. & Schaller, B. Prevalence of Peri-Implantitis in Implants with Turned and Rough Surfaces: a Systematic Review. *J Oral Maxillofac Res* **10**, e1 (2019).

74. Peppou-Chapman, S., Hong, J. K., Waterhouse, A. & Neto, C. Life and death of liquid-infused surfaces: a review on the choice, analysis and fate of the infused liquid layer. *Chem Soc Rev* **49**, 3688 (2020).

75. Badv, M., Jaffer, I. H., Weitz, J. I. & Didar, T. F. An omniphobic lubricant-infused coating produced by chemical vapor deposition of hydrophobic organosilanes attenuates clotting on catheter surfaces. *Sci Rep* **7**, 11639 (2017).

76. Villegas, M., Cetinic, Z., Shakeri, A. & Didar, T. F. Fabricating smooth PDMS microfluidic channels from low-resolution 3D printed molds using an omniphobic lubricant-infused coating. *Anal Chim Acta* **1000**, 248–255 (2018).

77. Hosseini, A. *et al.* Conductive Electrochemically Active Lubricant-Infused Nanostructured Surfaces Attenuate Coagulation and Enable Friction-Less Droplet Manipulation. *Adv Mater Interfaces* **5**, 1800617 (2018).

78. Badv, M., Imani, S. M., Weitz, J. I. & Didar, T. F. Lubricant-Infused Surfaces with Built-In Functional Biomolecules Exhibit Simultaneous Repellency and Tunable Cell Adhesion. *ACS Nano* **12**, 10890–10902 (2018).

79. Osborne, M., Aryasomayajula, A., Shakeri, A., Selvaganapathy, P. R. & Didar, T. F. Suppression of Biofouling on a Permeable Membrane for Dissolved Oxygen Sensing Using a Lubricant-Infused Coating. *ACS Sens* **4**, 687–693 (2019).

80. Badv, M. *et al.* Biofunctional Lubricant-Infused Vascular Grafts Functionalized with Silanized Bio-Inks Suppress Thrombin Generation and Promote Endothelialization. *ACS Biomater Sci Eng* **5**, 6485–6496 (2019).

81. Badv, M., Weitz, J. I. & Didar, T. F. Lubricant-Infused PET Grafts with Built-In Biofunctional Nanoprobes Attenuate Thrombin Generation and Promote Targeted Binding of Cells. *Small* **15**, 1905562 (2019).

82. Shakeri, A. *et al.* Contamination and carryover free handling of complex fluids using lubricant-infused pipette tips. *Scientific Reports 2022 12:1* **12**, 1–10 (2022).

83. Villegas, M., Zhang, Y., Abu Jarad, N., Soleymani, L. & Didar, T. F. Liquid-Infused Surfaces: A Review of Theory, Design, and Applications. *ACS Nano* **13**, 8517–8536 (2019).

84. Wong, T.-S. *et al.* Bioinspired Selfrepairing Slip. *Nature* **477**, 443–447 (2011).

85. Mackie, G., Gao, L., Yau, S., Leslie, D. C. & Waterhouse, A. Clinical Potential of Immobilized Liquid Interfaces: Perspectives on Biological Interactions. *Trends in Biotechnology* vol. 37 268–280 Preprint at https://doi.org/10.1016/j.tibtech.2018.08.003 (2019).

86. Yousefi, H. *et al.* LISzyme Biosensors: DNAzymes Embedded in an Anti-biofouling Platform for Hands-free Real-Time Detection of Bacterial Contamination in Milk. *ACS Nano* **16**, 29–37 (2022).

87. Kasapgil, E. *et al.* Polysiloxane Nanofilaments Infused with Silicone Oil Prevent Bacterial Adhesion and Suppress Thrombosis on Intranasal Splints. *ACS Biomater Sci Eng* **7**, 541–552 (2021).

88. Szaraniec, B., Pielichowska, K., Pac, E. & Menaszek, E. Multifunctional polymer coatings for titanium implants. *Materials Science and Engineering C* **93**, (2018).

89. Harris, L. G., Tosatti, S., Wieland, M., Textor, M. & Richards, R. G. Staphylococcus aureus adhesion to titanium oxide surfaces coated with non-functionalized and peptide-functionalized poly(L-lysine)-grafted- poly(ethylene glycol) copolymers. *Biomaterials* **25**, (2004).

90. Qin, S., Xu, K., Nie, B., Ji, F. & Zhang, H. Approaches based on passive and active antibacterial coating on titanium to achieve antibacterial activity. *J Biomed Mater Res A* **106**, 2531–2539 (2018).

91. Olmo, J. A. Del, Ruiz-Rubio, L., Pérez-Alvarez, L., Sáez-Martínez, V. & Vilas-Vilela, J. L. Antibacterial Coatings for Improving the Performance of Biomaterials. *Coatings 2020, Vol. 10, Page 139* **10**, 139 (2020).

92. Guo, C. *et al.* Poly-l-lysine/Sodium Alginate Coating Loading Nanosilver for Improving the Antibacterial Effect and Inducing Mineralization of Dental Implants. *ACS Omega* **5**, 10562–10571 (2020).

93. Vester, H., Wildemann, B., Schmidmaier, G., Stöckle, U. & Lucke, M. Gentamycin delivered from a PDLLA coating of metallic implants: In vivo and in vitro characterisation for local prophylaxis of implant-related osteomyelitis. *Injury* **41**, 1053–1059 (2010).

94. Wu, S. *et al.* Long-lasting renewable antibacterial porous polymeric coatings enable titanium biomaterials to prevent and treat peri-implant infection. *Nat Commun* **12**, 1–14 (2021).

95. Xing, R. *et al.* Antibacterial effect of doxycycline-coated dental abutment surfaces. *Biomed Mater* **10**, (2015).

96. Ding, L., Zhang, P., Wang, X. & Kasugai, S. A doxycycline-treated hydroxyapatite implant surface attenuates the progression of peri-implantitis: A radiographic and histological study in mice. *Clin Implant Dent Relat Res* **21**, 154–159 (2019).

97. Rahmati, M. *et al.* Coating doxycycline on titanium-based implants: Two in vivo studies. *Bioact Mater* **5**, 787–797 (2020).

98. Song, W. *et al.* Doxycycline-loaded coaxial nanofiber coating of titanium implants enhances osseointegration and inhibits Staphylococcus aureus infection. *Biomedical Materials* **12**, 045008 (2017).

99. Nie, B., Ao, H., Zhou, J., Tang, T. & Yue, B. Biofunctionalization of titanium with bacitracin immobilization shows potential for anti-bacteria, osteogenesis and reduction of macrophage inflammation. *Colloids Surf B Biointerfaces* **145**, 728–739 (2016).

100. Shahi, R. G. *et al.* Novel bioactive tetracycline-containing electrospun polymer fibers as a potential antibacterial dental implant coating. *Odontology* **105**, 354–363 (2017).

101. Amankwah, S., Adisu, M., Gorems, K., Abdella, K. & Kassa, T. Assessment of Phage-Mediated Inhibition and Removal of Multidrug-Resistant Pseudomonas aeruginosa Biofilm on Medical Implants. (2022) doi:10.2147/IDR.S367460.

102. Morimoto, T. *et al.* Development of Silver-Containing Hydroxyapatite-Coated Antimicrobial Implants for Orthopaedic and Spinal Surgery. *Medicina 2022, Vol. 58, Page 519* **58**, 519 (2022).

103. Shimabukuro, M. Antibacterial Property and Biocompatibility of Silver, Copper, and Zinc in Titanium Dioxide Layers Incorporated by One-Step Micro-Arc Oxidation: A Review. *Antibiotics 2020, Vol. 9, Page 716* **9**, 716 (2020).

104. Ray, P. D., Huang, B. W. & Tsuji, Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal* **24**, 981–990 (2012).

105. Sen, A. & Imlay, J. A. How Microbes Defend Themselves From Incoming Hydrogen Peroxide. *Front Immunol* **12**, 1104 (2021).

106. Honda, M. *et al.* Bactericidal and Bioresorbable Calcium Phosphate Cements Fabricated by Silver-Containing Tricalcium Phosphate Microspheres. *International Journal of Molecular Sciences 2020, Vol. 21, Page 3745* **21**, 3745 (2020).

107. Noda, I. *et al.* Development of novel thermal sprayed antibacterial coating and evaluation of release properties of silver ions. *J Biomed Mater Res B Appl Biomater* **89B**, 456–465 (2009).

108. Zhang, W. *et al.* Effects of strontium in modified biomaterials. *Acta Biomater* **7**, 800–808 (2011).

109. Aykul, S. & Martinez-Hackert, E. Determination of half-maximal inhibitory concentration using biosensor-based protein interaction analysis. *Anal Biochem* **508**, 97–103 (2016).

110. Kuehl, R. *et al.* Preventing Implant-Associated infections by silver coating. *Antimicrob Agents Chemother* **60**, 2467–2475 (2016).

111. Mokabber, T., Cao, H. T., Norouzi, N., Van Rijn, P. & Pei, Y. T. Antimicrobial Electrodeposited Silver-Containing Calcium Phosphate Coatings. *ACS Appl Mater Interfaces* **12**, 5531–5541 (2020).

112. Ziąbka, M. *et al.* Antibacterial composite hybrid coatings of veterinary medical implants. *Materials Science and Engineering: C* **112**, 110968 (2020).

113. Devasconcellos, P., Bose, S., Beyenal, H., Bandyopadhyay, A. & Zirkle, L. G. Antimicrobial particulate silver coatings on stainless steel implants for fracture management. *Materials Science and Engineering C* **32**, 1112–1120 (2012).

114. Zhang, B., Braun, B. M., Skelly, J. D., Ayers, D. C. & Song, J. Significant Suppression of Staphylococcus aureus Colonization on Intramedullary Ti6Al4V Implants Surface-Grafted with Vancomycin-Bearing Polymer Brushes. *ACS Appl Mater Interfaces* **11**, 28641–28647 (2019).

115. Esteban, J., Vallet-Regí, M. & Aguilera-Correa, J. J. Antibiotics-and heavy metals-based titanium alloy surface modifications for local prosthetic joint infections. *Antibiotics* **10**, (2021).

116. Bargavi, P. *et al.* Drug infused Al2O3-bioactive glass coatings toward the cure of orthopedic infection. *Prog Biomater* **11**, 79–94 (2022).

117. Stigter, M., Bezemer, J., De Groot, K. & Layrolle, P. Incorporation of different antibiotics into carbonated hydroxyapatite coatings on titanium implants, release and antibiotic efficacy. *Journal of Controlled Release* **99**, 127–137 (2004).

118. Ballarre, J. *et al.* Versatile bioactive and antibacterial coating system based on silica, gentamicin, and chitosan: Improving early stage performance of titanium implants. *Surf Coat Technol* **381**, 125138 (2020).

119. Garg, D., Matai, I. & Sachdev, A. Toward Designing of Anti-infective Hydrogels for Orthopedic Implants: From Lab to Clinic. *ACS Biomater Sci Eng* **7**, 1933–1961 (2021).

120. Zhang, L. *et al.* Electrospun vancomycin-loaded coating on titanium implants for the prevention of implant-associated infections. *Int J Nanomedicine* **9**, 3027–3036 (2014).

121. Djošić, M., Janković, A. & Mišković-Stanković, V. Electrophoretic deposition of biocompatible and bioactive hydroxyapatite-based coatings on titanium. *Materials* **14**, (2021).

122. Song, J. *et al.* Electrophoretic Deposition of Chitosan Coatings Modified with Gelatin Nanospheres to Tune the Release of Antibiotics. *ACS Appl Mater Interfaces* **8**, 13785–13792 (2016).

123. Rajesh, K. *et al.* Surface Modified Metallic Orthopedic Implant for Sustained Drug Release and Osteocompatibility. *ACS Appl Bio Mater* **2**, 4181–4192 (2019).

124. Aguilera-Correa, J. J. *et al.* A New Antibiotic-Loaded Sol-Gel Can Prevent Bacterial Prosthetic Joint Infection: From in vitro Studies to an in vivo Model. *Front Microbiol* **10**, 1–14 (2020).

125. Jaafar, A., Hecker, C., Árki, P. & Joseph, Y. Sol-gel derived hydroxyapatite coatings for titanium implants: A review. *Bioengineering* **7**, 1–23 (2020).

126. Nichol, T. *et al.* The antimicrobial activity and biocompatibility of a controlled gentamicin-releasing single-layer sol-gel coating on hydroxyapatite-coated titanium. *Bone and Joint Journal* **103 B**, 522–529 (2021).

127. Zhang, L. *et al.* Polymeric nanoarchitectures on Ti-based implants for antibacterial applications. *ACS Appl Mater Interfaces* **6**, 17323–17345 (2014).

128. Ferreira, M. B., Myiagi, S., Nogales, C. G., Campos, M. S. & Lage-Marques, J. L. Time- and concentration-dependent cytotoxicity of antibiotics used in endodontic therapy. *Journal of Applied Oral Science* **18**, 259 (2010).

129. Zhang, H. *et al.* Vancomycin-loaded titanium coatings with an interconnected micro-patterned structure for prophylaxis of infections: An: in vivo study. *RSC Adv* **8**, 9223–9231 (2018).

130. Chouirfa, H., Bouloussa, H., Migonney, V. & Falentin-Daudré, C. Review of titanium surface modification techniques and coatings for antibacterial applications. *Acta Biomater* **83**, 37–54 (2019).

131. Antoci, V. *et al.* The inhibition of Staphylococcus epidermidis biofilm formation by vancomycin-modified titanium alloy and implications for the treatment of periprosthetic infection. *Biomaterials* **29**, 4684–4690 (2008).

132. Mattioli-Belmonte, M. *et al.* Characterization and cytocompatibility of an antibiotic/chitosan/cyclodextrins nanocoating on titanium implants. *Carbohydr Polym* **110**, 173–182 (2014).

133. Jennings, J. A. *et al.* Novel Antibiotic-loaded Point-of-care Implant Coating Inhibits Biofilm. *Clin Orthop Relat Res* **473**, 2270–2282 (2015).

134. Gibb, B. P. & Hadjiargyrou, M. Bacteriophage therapy for bone and joint infections an instructional review. *Bone and Joint Journal* **103 B**, 234–244 (2021).

135. Li, B. & Webster, T. J. Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. *Journal of Orthopaedic Research* **36**, 22–32 (2018).

136. Abedon, S. T., Kuhl, S. J., Blasdel, B. G. & Kutter, E. M. Phage treatment of human infections. *Bacteriophage* **1**, 66 (2011).

137. Merril, C. R., Scholl, D. & Adhya, S. L. The prospect for bacteriophage therapy in Western medicine. *Nat Rev Drug Discov* **2**, 489–497 (2003).

138. Kortright, K. E., Chan, B. K., Koff, J. L. & Turner, P. E. Phage Therapy: A Renewed Approach to Combat Antibiotic-Resistant Bacteria. *Cell Host Microbe* **25**, 219–232 (2019).

139. Mushegian, A. R. Are There 1031 Virus Particles on Earth, or More, or Fewer? *J Bacteriol* **202**, 2192–2197 (2020).

140. Fard, R. M. N. A Short Introduction to Bacteriophages. *Trends in Peptide and Protein Sciences* **1**, 7–13 (2016).

141. Bayat, F., Didar, T. F. & Hosseinidoust, Z. Emerging investigator series: bacteriophages as nano engineering tools for quality monitoring and pathogen detection in water and wastewater. *Environ Sci Nano* **8**, 367–389 (2021).

142. Melo, L. D. R., Oliveira, H., Pires, D. P., Dabrowska, K. & Azeredo, J. Phage therapy efficacy: a review of the last 10 years of preclinical studies. *Crit Rev Microbiol* **46**, 78–99 (2020).

143. Petrovic Fabijan, A. *et al.* Phage therapy for severe bacterial infections: a narrative review. *Medical Journal of Australia* **212**, 279–285 (2020).

144. Tian, L. *et al.* Phage display for the detection, analysis, disinfection, and prevention of Staphylococcus aureus. *Smart Medicine* **1**, e20220015 (2022).

145. Xie, Y., Wahab, L. & Gill, J. J. Development and validation of a microtiter plate-based assay for determination of bacteriophage host range and virulence. *Viruses* **10**, (2018).

146. Tian, L. *et al.* Self-assembling nanofibrous bacteriophage microgels as sprayable antimicrobials targeting multidrug-resistant bacteria. *Nature Communications 2022 13:1* **13**, 1–12 (2022).

147. Akanda, Z. Z., Taha, M. & Abdelbary, H. Current review—The rise of bacteriophage as a unique therapeutic platform in treating peri-prosthetic joint infections. *Journal of Orthopaedic Research* **36**, 1051–1060 (2018).

148. Kalatzis, P. G., Castillo, D., Katharios, P. & Middelboe, M. Bacteriophage interactions with marine pathogenic vibrios: Implications for phage therapy. *Antibiotics* **7**, 1–23 (2018).

149. Onsea, J. *et al.* Bacteriophage therapy as a treatment strategy for orthopaedi c-devi ce-related i nfecti ons: Where do we stand? *Eur Cell Mater* **39**, 193–210 (2020).

150. León, M. & Bastías, R. Virulence reduction in bacteriophage resistant bacteria. *Front Microbiol* **6**, 1–7 (2015).

151. Oechslin, F. Resistance development to bacteriophages occurring during bacteriophage therapy. *Viruses* **10**, (2018).

152. Onsea, J. *et al.* Bacteriophage therapy as a treatment strategy for orthopaedi c-devi ce-related i nfecti ons: Where do we stand? *Eur Cell Mater* **39**, 193–210 (2020).

153. Gibb, B. P. & Hadjiargyrou, M. Bacteriophage therapy for bone and joint infections an instructional review. *Bone and Joint Journal* **103 B**, 234–244 (2021).

154. Morris, J. L. *et al.* Evaluation of bacteriophage as an adjunct therapy for treatment of peri-prosthetic joint infection caused by Staphylococcus aureus. *PLoS One* **14**, 1–18 (2019).

155. Wroe, J. A., Johnson, C. T. & García, A. J. Bacteriophage delivering hydrogels reduce biofilm formation in vitro and infection in vivo. **108**, 39–49 (2020).

156. Barros, J. A. R. *et al.* Encapsulated bacteriophages in alginate-nanohydroxyapatite hydrogel as a novel delivery system to prevent orthopedic implant-associated infections. *Nanomedicine* **24**, 102145 (2020).

157. Cobb, L. H. *et al.* CRISPR-Cas9 modified bacteriophage for treatment of Staphylococcus aureus induced osteomyelitis and soft tissue infection. *PLoS One* **14**, 1–17 (2019).

158. Cobb, L. H. *et al.* CRISPR-Cas9 modified bacteriophage for treatment of Staphylococcus aureus induced osteomyelitis and soft tissue infection. *PLoS One* **14**, 1–17 (2019).

159. Park, J. Y. *et al.* Genetic engineering of a temperate phage-based delivery system for CRISPR/Cas9 antimicrobials against Staphylococcus aureus. *Sci Rep* **7**, 1–13 (2017).

160. Morris, J. L. *et al.* Evaluation of bacteriophage as an adjunct therapy for treatment of peri-prosthetic joint infection caused by Staphylococcus aureus. *PLoS One* **14**, 1–18 (2019).

161. Yilmaz, C. *et al.* Bacteriophage Therapy in Implant-Related Infections. *The Journal of Bone and Joint Surgery-American Volume* **95**, 117–125 (2013).

162. Ferry, T. *et al.* Phage Therapy as Adjuvant to Conservative Surgery and Antibiotics to Salvage Patients With Relapsing S. aureus Prosthetic Knee Infection. *Front Med (Lausanne)* **7**, 1–9 (2020).

163. Fischetti, V. A. Bacteriophage endolysins: A novel anti-infective to control Gram-positive pathogens. *International Journal of Medical Microbiology* **300**, 357–362 (2010).

164. Turnbull, G. *et al.* 3D bioactive composite scaffolds for bone tissue engineering. *Bioactive Materials* vol. 3 278–314 Preprint at https://doi.org/10.1016/j.bioactmat.2017.10.001 (2018).

165. DAC® | What is DAC®. http://www.dac-coating.com/dac/what-is-dac/.

166. EXPERT™ Tibial Nail and EXPERT™ Tibial Nail PROtect™. https://www.jnjmedtech.com/en-EMEA/product/expert-tibial-nail-etn.

167. The Bactiguard technology - Bactiguard. https://www.bactiguard.com/about-bactiguard/the-bactiguard-technology/.

168. Zimmer Natural Nail System. https://znnbactiguard.com/wp-content/uploads/2021/06/3405.1-EMEA-en-ZNN-with-Bactiguard-Brochure.pdf.

169. Zhang, E. *et al.* Antibacterial metals and alloys for potential biomedical implants. *Bioact Mater* **6**, 2569–2612 (2021).

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M. Villegas, F. Bayat, T. Kramer, E. Schwarz, D. Wilson, Z. Hosseinidoust*, T. F. Didar*

Strategies to Prevent Bacterial Infections on Titanium-Based Orthopedic and Dental Implants

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