

Review

Antibacterial Coatings on Titanium Implants

Lingzhou Zhao,¹ Paul K. Chu,² Yumei Zhang,¹ Zhifen Wu¹

¹ School of Stomatology, The Fourth Military Medical University, Xi'an 710072, People's Republic of China

² Department of Physics and Materials Science, City University of Hong Kong, Kowloon, Hong Kong, People's Republic of China

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Abstract: Titanium and titanium alloys are key biomedical materials because of their good biocompatibility and mechanical properties. Nevertheless, infection on and around titanium implants still remains a problem which is usually difficult to treat and may lead to eventual implant removal. As a result, preventive measures are necessary to mitigate implant-related infection. One important strategy is to render the implant surface antibacterial by impeding the formation of a biofilm. A number of approaches have been proposed for this purpose and they are reviewed in this article. © 2009 Wiley Periodicals, Inc. *J Biomed Mater Res Part B: Appl Biomater* 91B: 470–480, 2009

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INTRODUCTION

Titanium and titanium alloys are widely used in orthopedic and dental implants, but infection associated with these implants still poses serious threat leading to possible complications^{1–6} such as prolonged hospitalization, complex revision procedures, implant failure, complete removal, patient suffering, financial burden, and even death.⁷ As titanium implants are getting more popular in the biomedical industry, the prevalence of infection rises. To prevent such infections, one approach is to improve the antibacterial ability of the materials. This article reviews the current status of antibacterial coatings on titanium, describes their advantages and disadvantages, and discusses potential of further studies.

INFECTION ASSOCIATED WITH TITANIUM IMPLANTS

The implant surface is susceptible to infection because of two main reasons, namely formation of a surface biofilm and compromised immune ability at the implant/tissue interface. The biocompatibility of titanium implant can be attributed to a surface protein layer formed under physiological conditions. This protein layer actually makes the

surface suitable for bacterial colonization and biofilm formation.^{8–10} Biofilms are defined as a microbially derived sessile community characterized by cells irreversibly attached to a substratum, interface or to each other, embedded in a matrix of extracellular polymeric substances that they have produced, and exhibiting an altered phenotype with respect to growth rate and gene transcription.¹¹ Concerning the pivotal role that biofilm plays in implant-associated infections, the process of biofilm formation has been well documented,^{8–11} which will not be detailed here. The biofilm protects adherent bacteria from the host defense system and bactericidal agents via several proposed mechanisms.^{10–12} The host immunity ability on the implant is consequently impaired. In the early phase after implantation, the local defense system is severely disturbed by the surgical trauma, and so it is the most dangerous time for infection. Even after completion of tissue integration, the defense ability at the implant/tissue interface is still compromised on account of the small number of blood vessels in this zone. The reduced defense mechanism facilitates colonization of bacteria and infection may result.

Although various measures such as thorough disinfection and stringent aseptic surgical protocols have been proposed to mitigate bacterial contamination, there is still evidence that bacterial invasion usually occurs after surgery.⁶ Bacterial contamination can also arise from hematogenous sources at a later time.² Percutaneous and transmucosal implants such as external fixation pins and dental implant are even more vulnerable to bacterial contamination^{13,14} as

Correspondence to: Y. Zhang (e-mail: wqtzym@fmmu.edu.cn) or Z. Wu (e-mail: wzfxy@fmmu.edu.cn)

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the bacteria on the skin, mucosa, and implant surface can invade the peri-implant soft tissue, eventually causing deep peri-implant bone tissue infection. Besides, soft tissue bonding with percutaneous/transmucosal implants is still not very satisfactory and it is another concern for bacterial invasion. The incidence of infection has been reported to be as high as 50%, especially pin track infection on percutaneous fracture fixators.^{15,16}

Because bacteria in the biofilm are more resistant to treatment with antimicrobial agents than their planktonic counterparts,¹⁵⁻¹⁷ routine antibiotic treatments are usually incapable of reducing implant-associated infection. There is still no good means to eliminate the infection after it has occurred, and implant removal is usually the only effective way to eradicate the problem. Collectively, formation of the biofilm and the compromised immunity at the implant/tissue interface render the titanium implant susceptible to bacterial colonization and further infection, but because it is impossible to completely eliminate bacterial contamination, it is imperative to explore effective ways to prevent implant-associated infection. In the pathogenesis of infection around implants, initial adhesion of bacteria onto biomaterial surfaces is believed to be a critical event¹⁸ and so an important strategy is to prevent initial bacterial adhesion onto the implant surface.

ANTIBACTERIAL COATINGS ON TITANIUM

Titanium implants are widely used in many biomedical applications such as dental and orthopedic implants. Formation of biofilms on titanium implants is a complex issue because of the diversity of bacterial ecosystems and so antibacterial coatings should be tailored to tackle different bacterial species in different environments. Antibacterial coatings have been traditionally designed to prevent initial adhesion of bacteria onto the implant surface without much attention to the mini-environment encountered after implantation. Many surface coatings such as adhesion-resistant coatings and coatings containing or releasing antimicrobial agents shown in Table I are used to inhibit initial attachment of bacteria to titanium. One essential requirement is that it should not hamper tissue-integration and in fact, it will be better if the coating can benefit tissue integration.

Coatings Loaded With Antibiotics

Systemic antibiotic prophylaxis is routinely applied to patients who receive implantation to prevent postsurgical infection.^{19,20} However, systemic administration of antibiotics has many disadvantages such as relatively low drug concentration at the target site and potential toxicity. Thus, topical application of antibiotics has attracted much attention. Since the early 1970s, antibiotics has been incorporated into bone cement to give local antibiotic prophylaxis in cemented total joint arthroplasty.²¹ Clinical investigation has shown that antibiotic prophylaxis in bone cement can depress the revision, aseptic loosening, and deep infection

rates of cemented total hip arthroplasties when combined with systemic administration.²² At present, cementless bone implants are mostly used instead of the cement counterparts as the cementless ones can produce better early and intermediate-term results compared with cemented ones.²³ Hence, researchers are trying to develop antibiotic-loaded coatings on the titanium implants.

Gentamicin belongs to the family of aminoglycoside antibiotics which has a relative broad antibacterial spectrum. Furthermore, gentamicin is one of the rare kinds of thermostable antibiotics and so it is one of the most widely used antibiotics in antibiotics-loaded coatings on titanium implants.^{24,25} Besides, other antibiotics with broad antibacterial spectra, for instance, cephalothin, carbenicillin, amoxicillin, cefamandol, tobramycin, and vancomycin have been used in coatings on bone implants.²⁵⁻²⁷

Calcium phosphates, which are known to be biocompatible and osteoconductive have been verified to be potential vectors of bioactive molecules.^{28,29} Antibiotics have been loaded into porous hydroxyapatite coatings on titanium implants.^{19,22} The antibiotic-HA-coatings exhibit significant improvement in infection prophylaxis compared with standard HA coatings *in vivo*,²⁴ but some problems still exist. The antibiotics cannot be incorporated into the calcium phosphate coatings during its formation because of the extremely high processing temperature such as that encountered in plasma spraying. Moreover, physical absorption of these drugs onto the surface of calcium phosphates limits the loaded amount and release characteristics.^{27,30} It has been reported that loading by a dipping method leads to burst release of the antibiotics, that is, more than 80-90% of the antibiotics released from the calcium phosphates coating within the first 60 min. Application of a lipid layer which serves as a hydrophobic barrier can retard the drug release from the calcium phosphate surfaces, but only up to 72 h *in vitro*.^{27,30} A biomimetic method for coating medical devices with carbonated HA and other calcium phosphate phases has been developed by which calcium phosphate can be deposited on the surface of titanium implants by immersion into a supersaturated solution of calcium phosphate at ambient temperature.³¹ The antibiotics are added to the supersaturated solutions and gradually coprecipitates with the calcium phosphate crystals forming a layer on the titanium implants.^{25,26} By means of this method, a larger amount of antibiotics can be integrated into the biomimetic calcium phosphate coating (10 folds) than by simple physical adsorption onto the plasma-sprayed coating whereas release of the antibiotics is not slowed down too much.^{25,26}

In addition to calcium phosphate, biodegradable polymers and sol-gel coatings are also utilized to form controlled-release antibiotic-laden coatings on titanium implants. A new biodegradable gentamicin-loaded poly(D,L-lactide) (PDLLA) coating has been developed to prevent implant-related osteomyelitis in rats.³² The release of the antibiotics from PDLLA seems to be slower than that from calcium phosphates with ~80% of the antibiotics

TABLE I. Representative Antibacterial Coatings on Titanium Implants

Type of Coating	Fabrication Method	Test Condition	Effect	Experimental Model	References
Antibiotic loaded coatings	Immersion	<i>In vitro</i>	Effective bacterial inhibition up to 72 h	<i>Staphylococcus aureus</i>	27
Vancomycin-loaded calcium phosphate coatings					
Antibiotics incorporated carbonated hydroxyapatite coatings	Biomimetic coprecipitation	<i>In vitro</i>	Inhibit bacterial growth and the release rate of antibiotics is related to the structure of the antibiotics	<i>Staphylococcus aureus</i>	25, 26
Gentamicin-loaded titania nanotubes	The nanotubes were filled via a simplified lyophilization method	<i>In vitro</i>	Reduce bacterial adhesion and enhance osteoblast differentiation	<i>Staphylococcus epidermidis</i> and MC3T3-E1	36
Gentamicin-loaded poly(D,L-lactide) coating		<i>In vivo</i>	Reduce implant-related infection	Rat acute osteomyelitis model induced by <i>Staphylococcus aureus</i> inoculation	32
Vancomycin-loaded sol-gel films	Dipping	<i>In vitro</i>	Zero-order release of vancomycin up to 2 wk		34
Vancomycin-bonded surfaces	Covalent Bonding	<i>In vitro</i>	Reduce bacterial colonization even upon repeated challenge	<i>Staphylococcus aureus</i> and <i>Staphylococcus epidermidis</i>	38–40
Chlorhexidine incorporated hydroxyapatite coating	Surface-induced mineralization	<i>In vitro</i>	Large inhibition zone	<i>Staphylococcus aureus</i>	48
Chlorhexidine-containing Poly(lactide) coating on anodized surface	Spraying deposition	<i>In vitro</i>	Large inhibition zone		50
Polymer and calcium phosphate coatings with chlorhexidine	Impregnation	<i>In vitro</i>	Inhibit the growth and viability of bacteria tested, show cytotoxicity to fibroblasts	<i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i> and hTERT human fibroblasts	51
Coatings with the antiseptic combination of chlorhexidine and chloroxylenol	Unclear	<i>In vitro</i>	Produce zones of inhibition against bacteria tested	<i>Staphylococcus epidermidis</i> , <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> and <i>Candida albicans</i>	54

released during the first 48 h.³³ An optimized multilayered vancomycin-incorporated silica sol-gel film shows zero release of vancomycin up to 2 wk.³⁴ Recently, it has been reported that titania nanotubular surfaces fabricated using an anodization technique can improve the functions of bone cells^{35–37} and are potentially useful in implants. Loading of gentamicin into the nanotubes is effective in minimizing initial bacterial adhesion without adverse influence on the good cytocompatibility of the nanotubes.³⁶ However, elution of gentamicin is still too fast with all the drugs delivered within 50–150 min in phosphate-buffered saline (PBS).³⁶

Besides antibiotic-releasing coatings, there is interest in covalent bonding of drugs to the implant surface to realize long lasting antibacterial ability. Vancomycin has been successfully covalently bonded to titanium and its antibacterial activity is retained even after incubation in PBS for at least 11 mo. Unlike a noncovalent coating that slowly releases bactericide, covalently bonded vancomycin is not delivered from the surface during incubation with the bacteria.^{38–40} This may be beneficial to the confinement and reinforcement of the antibacterial ability to the surface whereas simultaneously eliminating side effects caused by drugs released into the body fluids. However, as a protein layer is quickly deposited onto the implant surface after implantation into the body, it is questionable if the covalently bonded antibiotics can exert an effect through this protein layer and the *in vivo* effects of the antibiotics covalently bonded to implants are still in doubt.

Before antibiotic-loaded coatings can be applied clinically, there are still many outstanding issues. First of all, the susceptibility of the bacteria in the vicinity of the implant to antibiotics is a problem and drug resistance of bacteria isolated from orthopedic implants has been reported.⁴¹ Choosing the effective antibiotics to incorporate into the coating is thus crucial. Secondly, it is challenging to produce an antibiotics-laden coating with a relative long antibiotics delivery time at effective concentrations. Thirdly, it has been reported that some drug carriers release antibiotics at concentrations lower than the minimum inhibitory concentration for an indefinite period. For example, gentamicin can be detected from tissues adjacent to prosthesis anchored with gentamicin-loaded bone cement 5.5 yr after surgery.⁴² This raises the risks by new antibiotic-resistant bacteria. Consequently, it has been proposed that the ideal antibiotics-delivering coatings should release antibiotics at optimal effective levels for a sufficiently long period of time, which is enough to prevent potential infection and then release of the antibiotics should cease quickly to eliminate the risk of developing resistant bacteria. Lastly, although antibiotics is considered highly biocompatible, there are references documenting that some types of antibiotics may harm cell functions.^{43–47} In future studies, the effects of antibiotics on tissue integration around the implants should be considered.

Coatings Containing Nonantibiotic Organic Antimicrobial Agents

Regarding the risk of antibiotic resistance associated with the application of antibiotics-containing coatings, nonantibi-

otic organic antimicrobial agents such as chlorhexidine, chloroxylenol, and poly(hexamethylenbiguanide)^{48–54} may be good alternatives.⁴⁹ They are widely used in daily life for their broad spectrum of antimicrobial action and lower risk of drug resistance, especially chlorhexidine which is well known for its extensive application in dentistry such as the use gelatine for the treatment of periodontal infection⁵⁵ and in mouthwash. The investigation of chlorhexidine adsorption onto the unmodified titanium surface is clinically meaningful as it is easy to carry out. Studies have shown that chlorhexidine can adsorb to the TiO₂ layer on the titanium surface and desorb gradually over a period of several days.^{52,53} The adsorption and leaching kinetics of chlorhexidine to TiO₂ are related to the surface roughness, crystal structure of TiO₂, and buffer used.⁵³

To contain a larger amount of nonantibiotic organic antimicrobial agents and achieve better elution kinetics, many complex coatings are also utilized. Harris et al. have compared several kinds of coatings fabricated on titanium with and without chlorhexidine adsorption, and their conclusion is that PDLLA and politerefate have the best potential as coatings on implants for drug delivery because they are cytocompatible, elute chlorhexidine effectively with relatively slower drug release kinetics, and have satisfactory mechanical properties.⁵¹ A surface-induced mineralization technique has been utilized to produce hydroxyapatite coatings on external fixation pins incorporating chlorhexidine.⁴⁸ The chlorhexidine release pattern is similar to that of the antibiotics from antibiotic-laden coatings with an initially rapid release rate followed by a period of slower but sustained release.⁴⁸ Chlorhexidine can also adsorb onto the titanium implant surface modified by the covalent coupling of collagen on a polyanionic acrylic acid overlayer via the ionic interaction between the cationic chlorhexidine and polyanionic collagen surface.⁴⁹ Other alternative methods have also been employed to form coatings comprising non-antibiotic organic antimicrobial agents on titanium.⁵⁴

On account of a lower risk of drug resistance, the non-antibiotic organic antimicrobial agents may be applied *in vivo* for a relatively long period of time. However, several reports have pointed out that the nonantibiotic organic antimicrobial agents may cause cell damage.^{44,51} Thus, more comprehensive studies are needed to clarify its biocompatibility to tissues adjacent to the implant. Furthermore, similar to the antibiotic-loaded coatings, suitable coating materials that can load a satisfactory amount of these nonantibiotic organic antimicrobial agents and release them in a controlled fashion are urgently needed.

Coatings Containing Inorganic Antimicrobial Agents

Inorganic antimicrobial agents are very attractive alternatives from the perspective of doping of biomaterials because they possess many advantages such as good antibacterial ability, excellent biocompatibility, and satisfactory stability. Among the various dopants, silver is the most

well known. The merits of silver in antibacterial doping applications are listed in the following.

1. Its broad antibacterial spectrum to both gram-positive and gram-negative bacteria and some drug-resistance bacteria⁵⁶ at very low ppb concentrations.⁵⁷
2. Silver doping can also inhibit bacterial attachment onto biomaterials.⁵⁸
3. The antibacterial effect of silver is long lasting.
4. Although the underlying mechanisms are still not well known, silver is less prone to resistance development.⁵⁹
5. *In vitro* studies have demonstrated that silver coatings possess excellent biocompatibility without genotoxicity or cytotoxicity⁶⁰ and *in vivo* studies have indicated that silver coatings have no local or systemic side-effects.^{61,62}
6. Because silver is relatively stable, it can be introduced by many well established techniques such as plasma immersion ion implantation (PIII),⁶³ pulsed filtered cathodic vacuum arc deposition,⁶⁴ physical vapor deposition (PVD),⁶⁵ magnetron sputtering,⁶⁶ and so on.
7. Silver can be readily used to dope a myriad of biomaterials including polymers,^{63,67} diamond-like carbon,⁶⁴ bioactive glass and ceramics,^{68,69} metals,⁶⁰ and so forth.

Owing to these advantages as a bactericide, silver has also been introduced into titanium to enhance the bactericidal ability. For example, silver has been ion implanted into titanium and Ti-Al-Nb alloy to improve their antibacterial characteristics and wear performance.⁷⁰ Additionally, a titanium/silver hard coating has been deposited on titanium by PVD⁶⁵ and a silver-containing hydroxyapatite coating has been produced on titanium by magnetron sputtering.⁶⁶ Silver doping can effectively inhibit bacterial adhesion and growth without compromising the activity of osteoblasts and epithelial cells.^{65,66} The cells cultured on the silver-coated materials even exhibit better spreading and the cell count is higher compared with the uncoated materials.^{60,63} Though the underlying mechanism is not yet fully understood, there is evidence that silver-containing coatings are promising in titanium implant applications. Furthermore, the antibacterial ability of silver can be augmented by other elements such as nitrogen.⁶³

One interesting phenomenon is that silver ions generated at the anode electrode with a weak direct current can inhibit bacterial growth effectively.⁷¹ The inhibitory concentrations of the electrically induced silver ions are ~100 times lower than that in silver sulfadiazine.⁵⁶ The results show that anodization can yield extra antibacterial ability although the reason is still unclear. It has been reported that silver-coated titanium screws can prevent implant-associated deep bone infection when they are anodically polarized.⁷² We believe that anodization of silver-coated implants may be of special interest for percutaneous implants or dental implants. It is feasible to coat them with silver and anodize them through the part outside the body. Consequently, their antibacterial ability can be tunable with

and without anodization to address different degrees of infection.

Silver is thus very attractive as an antibacterial dopant in titanium implants, but further clarification of its bactericidal mechanism is needed to broaden clinical applications. Besides silver, other inorganic antimicrobial agents such as copper,^{70,73} fluorine, calcium, nitrogen, and zinc^{74–76} have been introduced into titanium but they have not been as widely investigated as silver. The possible reasons for the enhanced properties are also not clearly known and there may be side effects. For instance, copper ion implantation compromises the physical properties of titanium such as its corrosion resistance.^{70,73}

Adhesion Resistant Coatings

The surface characteristics of implants such as surface roughness and chemistry,⁷⁷ hydrophilicity, surface energy, surface potential, and conductivity^{78–84} play crucial roles in the initial adhesion and subsequent growth of bacteria on the surface and the subsequent cell action and response. These surface characteristics can influence the amount and/or the conformation of adsorbed proteins thereby influencing ensuing bacterial adhesion and biofilm formation. A bacteria adhesion resistant surface may be achieved by altering these surface characteristics.

Modification of Physical and Chemical Surface Properties. Surface modification to alter the physico-chemical surface properties is a relatively simple and economic way to repel bacteria colonization. For example, ultraviolet (UV) light irradiation can lead to a “spontaneous” wettability increase on titanium dioxide.⁸⁵ *In vitro* experiments have indicated that UV treatment of Ti6Al4V inhibits bacterial adhesion without compromising the good response of human bone-forming cells to this alloy.⁷⁸ *In vitro* and *in vivo* experiments have also shown that UV light pretreatment of titanium substantially enhances its osteoconductive capacity, possibly in association with UV-catalyzed progressive removal of hydrocarbons from the TiO₂ surface.⁸⁶ These data show that UV irradiation is a potential facile means to render titanium implant antibacterial.

An antiadhesion surface can also be achieved by changing the crystalline structure of the surface oxide layer. It has been shown that a crystalline anatase-type titanium oxide layer can reduce significantly bacterial attachment without negatively affecting the cell metabolic activity. Moreover, this anatase-type oxide layer can stimulate precipitation of apatite in simulated body fluids⁸⁷ suggesting good osteoconductive ability. The antibacterial property of anatase-type titanium oxide may arise from its photocatalytic ability.⁸⁸

Korner et al. have demonstrated that bacterial adhesion to conducting surfaces is relevant to the resistivity of the substrate. In this respect, TiNOX coatings can be applied to metals to alter the surface conductivity. A surface resis-

tivity of around $10^4 \mu\Omega \text{ cm}$ is found to show the minimal bacterial adhesion onto TiNOX for all strains observed,⁸³ suggesting a new direction for producing adhesion-resistant surfaces.

Anti-Adhesive Polymer Coatings. Some polymer coatings such as the hydrophilic poly(methacrylic acid)⁸⁹ and protein-resistant poly(ethylene glycol)⁹⁰ can be produced on titanium by simple methods. These coatings can significantly reduce the adhesion of *Staphylococcus aureus* and *Staphylococcus epidermidis*.^{89,90} However, osteoblast functions on these surfaces are impaired simultaneously, but fortunately, the impaired cell functions can be restored and even improved by immobilization of bioactive molecules such as sericin and arginine-glycine-aspartic acid (RGD) motif whereas maintaining good antibacterial ability.^{89,90} The principal function of these adhesion-resistant coatings is to prevent adhesion of bacteria around the implant to impede the development of an antibiotic/immune resistant biofilm that can otherwise counteract the host defensive mechanism. The situation must also be considered from the viewpoint of tissue integration. The ideal scenario is that satisfactory adhesion-resistant ability can be attained by simply altering the structure of the surface oxide layer on the titanium surface, but no such surface has heretofore been reported.

Biofunctionalization With Antibacterial Bioactive Polymers

Some bioactive molecules such as chitosan^{91,92} and hyaluronic acid^{93,94} possess the ability to inhibit bacterial adhesion and/or kill them. Chitosan, with a chemical structure similar to hyaluronic acid, is obtained from deacetylation of chitin and is found in the exoskeletons of insects and marine invertebrates and the cell walls of certain fungi. Chitosan is noted for various biological properties including biocompatibility, biodegradability into harmless products, nontoxicity, physiological inertness, remarkable affinity to proteins, and antibacterial, hemostatic, fungistatic, antitumoral, and anticholesteremic properties.⁹⁵ Chitosan leads to differentiation of osteoprogenitor cells⁹⁶ and improves the attachment, growth, viability, alkaline phosphatase (ALP) activity, and phenotypic expression of the osteoblast cells.^{97–99} Additionally, chitosan has a broad antibacterial spectrum and hence, it is widely used in bone substitutes, wound dressing, tissue engineering scaffolds for different tissues, and carriers for various active agents.^{95,100}

Chitosan has been bonded to titanium via a layer of linking molecules.^{94,101,102} Martin et al.^{97,101} have produced chitosan films on titanium via a three-step process involving deposition of 3-aminopropyltriethoxysilane (APTES) in toluene, a reaction between the amine end of APTES with gluteraldehyde, and finally a reaction between the aldehyde end of gluteraldehyde and chitosan. A two-step process that involves the deposition of triethoxysilylbutyaldehyde

(TESBA) in toluene followed by a reaction between the aldehyde of TESBA with chitosan has also been proposed.¹⁰² Chua et al.⁹⁴ have used a three-step process similar to that in Ref. 101 to graft chitosan onto the titanium surface, and the main difference is that they use dopamine instead of APTES. The chitosan films improve the attachment and growth of osteoblasts.^{94,97} However, there is still insufficient *in vivo* evidence illustrating that chitosan films offer better osseointegration compared with other coatings such as calcium phosphate.¹⁰³ The bonding strength of chitosan (1.5–1.8 MPa) which is smaller than that reported for calcium-phosphate coatings (6.7–26 MPa) may still be not good enough.⁹⁷ The antibacterial ability of these chitosan coatings has not been assessed in details but their antibacterial ability is generally accepted.

A hybrid calcium phosphate/chitosan coating has been formed on Ti6Al4V by electrodeposition. The chitosan alters some of the physical properties of the calcium phosphate coating but without compromising its good adhesive strength.¹⁰⁴ The calcium phosphate/chitosan coating has been shown to provide a favorable surface for attachment of bone marrow stromal cells¹⁰⁴ and proliferation and differentiation of osteoblasts.¹⁰⁵ Unfortunately, the antibacterial properties of the hybrid coating have not been evaluated in details.

To accomplish long-lasting antibacterial ability, polyelectrolyte multilayers consisting of chitosan and hyaluronic acid have been fabricated on titanium via layer-by-layer self-assembly.⁹⁴ The polyelectrolyte multilayered films can indeed reduce bacterial adhesion by about 80% compared with pristine Ti. However, adhesion of osteoblasts is impaired because of the presence of the hyaluronic acid chains.⁹⁴ It should be noted that with additional immobilized cell-adhesive RGD moieties, osteoblast adhesion can be improved significantly. The density of the surface-immobilized RGD peptide has a significant effect on osteoblast proliferation and ALP activity, and both functions can be increased 100–200% over that on the pristine titanium substrates whereas retaining high antibacterial efficacy.⁹⁴ Chitosan coatings are thus very attractive because they can simultaneously enhance the antibacterial and tissue integration ability of the implants. However, there is still room for improvement before chitosan coatings can be applied clinically. For instance, the *in vivo* performance is not yet well known.

Coatings Delivering Nitrogen Monoxide (NO)

Nitric oxide (NO) is a common molecule in many biological processes such as neural transmission, vasodilatation, angiogenesis, wound healing, and phagocytosis.¹⁰⁶ It has been demonstrated that low concentrations of NO are bacteriostatic to log-phase cultures of some bacteria including *Staphylococcus aureus*.¹⁰⁷ Raulli et al.¹⁰⁸ have also verified the wide-range antibacterial properties of NO in a series of solution-based *in vitro* assays, showing NO-mediated

inhibition of a wide variety of gram-negative and gram-positive bacteria. NO also acts as an important mediator produced by macrophages and plays crucial roles in the natural immune response to bacterial infection.¹⁰⁹ Exogenous NO may be used to prevent the survival of pathogenic bacteria on the implant surface because of the direct bactericidal effect and/or by augmenting the natural antimicrobial ability of the immune system.

Nablo and coworkers¹¹⁰ have investigated the development of sol-gel coatings capable of NO release from implants for potential antibacterial coatings applications. The local surface flux of NO generated from these sol-gel materials significantly reduces the adhesion of three common opportunistic pathogens *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*.^{110–113} The NO-releasing xerogels have also been shown to kill adherent bacteria cells¹¹³ and used on orthopedic implants.¹¹² *In vitro* biomaterials comparisons suggest that the application of a NO-releasing xerogel layer may dramatically improve the bacterial-adhesion resistance of medical-grade stainless steels.¹¹² Application of NO-releasing xerogels on titanium has not been reported, but it is expected to be a viable method.

Although the antibacterial ability of NO-releasing xerogels has been studied extensively, there are still many outstanding issues. Firstly, the bonding strength between the xerogels and metal implant is not well known. It is unclear whether the xerogel coatings are strong enough to withstand the stress during surgery and normal functions *in vivo*. Secondly, the interactions between the xerogel coatings and bone tissue have not been revealed. Thirdly, as NO exhibits a broad range of physiological effects, prolonged exposure to NO at elevated concentrations has been associated with many detrimental physiological conditions including septic shock, apoptosis, cytotoxicity, DNA damage, and carcinogenesis.¹⁰⁶ Consequently, the tissue compatibility to NO-releasing materials is a concern. Fourthly, delivering of NO from these xerogels is in a burst mode with a large initial NO flux followed by a sharp exponential decrease during the initial 24 h. The reduction in the NO flux observed after 24 h is ~90%.^{106,112,114} Further studies are still needed to deliver NO in a controlled fashion. Last but not the least, the *in vivo* antibacterial ability of NO-releasing coatings is still controversial.¹¹⁵

ISSUES ABOUT PASSIVE OR ACTIVE COATINGS

Depending on whether there are antibacterial agents delivered, coatings can be categorized as passive or active. Passive coatings do not release bactericidal agents to the surrounding tissues. Instead, they just inhibit bacterial adhesion and/or kill bacteria upon contact. The typical means is to modify the physico-chemical surface properties such as the surface hydrophilicity and crystal structure. These coatings are highly preferred as long as their antibacterial ability is strong enough to prevent biofilm formation. The

reasons are that these coatings can be put inside the body for a relatively long period without local and general side effects and their antibacterial ability can be sustained.

In comparison, active coatings release preincorporated bactericidal agents such as antibiotics, antiseptics, silver, and NO. The most attractive advantage is their obvious antibacterial effects. However, there is biological safety concern and these coatings can only release antibacterial agents for a limited period of time after implantation. This is especially true for coatings that release antibiotics, antiseptics, and NO, and delivery of bactericides at effective concentrations can be maintained only for several days. Hence, these coatings may only prevent early postsurgical infection caused by surgical contamination. With regard to prophylaxis of some later infection caused by hematogenous spread or direct or contiguous spread,^{2,116} these coatings may not be very useful. Furthermore, how to fabricate a coating that can load enough bactericides and release them in a controlled fashion throughout the lifetime of the implant is still problematic. The development of smart coatings which can deliver bactericidal agents only when bacteria invasion occurs may be a good research direction.^{117,118}

CONCLUSION

Infection around titanium implants continues to be a concern in clinical research. Good progress has been made in recent years but antibacterial coatings are still not widely used clinically. *In vivo* information on these antibacterial coatings is still scarce. It is generally agreed that the host defense ability around the implant is the ultimate means to prevent infection. Hence, how to improve tissue integration and immune ability on the implant is of paramount importance in preventing infection. In future studies, surfaces with both excellent tissue-integration ability and good antibacterial properties should be explored.

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