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Review

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Polymeric Nanoarchitectures on Ti-Based Implants for Antibacterial Applications

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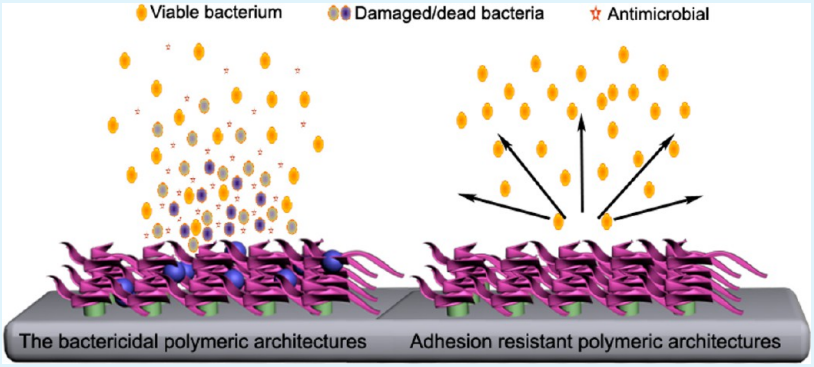

ABSTRACT: Because of the excellent mechanical properties and good biocompatibility, titanium-based metals are widely used in hard tissue repair, especially load-bearing orthopedic applications. However, bacterial infection and complication during and after surgery often causes failure of the metallic implants. To endow titanium-based implants with antibacterial properties, surface modification is one of the effective strategies. Possessing the unique organic structure composed of molecular and functional groups resembling those of natural organisms, functionalized polymeric nanoarchitectures enhance not only the antibacterial performance but also other biological functions that are difficult to accomplish on many conventional bioinert metallic implants. In this review, recent advance in functionalized polymeric nanoarchitectures and the associated antimicrobial mechanisms are reviewed.

KEYWORDS: *polymeric nanoarchitecture, antibacterial properties, biomedical implants, titanium, surface modification*

1. INTRODUCTION

The aging population spurs increasing demand for orthopedic devices and implants. For instance, about 69.4 million people over the age of 50 suffer from osteoporosis in China and there are more than 687 000 hip fracture and 1.8 million vertebral fracture incidents per year.[1] Since the use of pure titanium in bone implants in vivo in the 1930s,[2] different Ti-based alloys including first-generation pure titanium products and new-generation Al- and V-free titanium alloys have been developed to repair or substitute for damaged hard tissues. Ti-based materials offer advantages such as good biocompatibility, corrosion resistance, and wear resistance, suitable Young's modulus, as well as high strength compared to other metals.[3−5]

Unfortunately, bacterial infection is a serious complication during and after surgery jeopardizing long-term fixation of the metallic implants and causing premature failure.[6−8] For example, the Norwegian Arthroplasty Register has reported infection rates of about 17 and 11% for total hip arthroplasty and total knee arthroplasty in 2009, respectively,[8] and as a result, the need for recurring surgery is rapidly rising. Clinical analysis shows that the infection rate after a follow-up surgery is higher (5−40%) than that after the first one.[9] Although infection is generally less likely to happen than aseptic failure, it can cause complications with high morbidity and incur substantial cost.[10] To prevent infection, it is essential to endow metallic implants with the self-antibacterial ability. The coating strategy has been proven to be effective to enhance the antimicrobial properties of Ti-based implants[11,12] and various

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Table 1. Representative Functionalized Polymeric Architectures on Ti-based Metallic Implants with Enhanced Antibacterial Properties

antibacterial modes	types of coating		fabrication methods	test condition	the role of polymers	antibacterial mechanism	antibacterial efficiency	experimental model	ref
bactericidal properties	antibiotic- loaded polymeric architectures	(vancomycin and cefuroxime)-loaded PLGA coatings	plasma-sprayed	in vitro	PLGA: encapsulated antibiotics and extended antibiotic release up to 2.5 weeks	the inhibition of bacterial cell wall synthesis	bactericidal effect lasts 17 days	*S. aureus*	54
		penicillin/ streptomycin--loaded PPy coatings	electrodeposited	in vitro	PPy can release drugs upon electrical stimulation; biocompatibility	the inhibition of bacterial cell wall synthesis		fibroblast; osteoblast	55
		GS-functionalized NPs coatings	NPs: ROMP; by grafting GS-functionalized NPs onto titanium surfaces	in vitro	NPs allows a higher drug density and realizes the release of pH-controlled of GS	interrupting protein synthesis	the release of GS is pH-controlled with an adjustable period of time from 1 to 2 years; full bactericidal levels (100%) after 96 h release	*S. aureus*	56, 57
		vancomycin-PEG - acrylate coatings	photochemical polymerization	in vitro	increase the load density of vancomycin	the inhibition of bacterial cell wall synthesis	a well-defined zone of inhibition	*S. epidermidis*	58, 59
		P(HEMA-SA-GE/ Col) - Ti	surface-initiated atom transfer radical polymerization (ATRP)	in vitro	as a carrier of antibiotic and biomacromolecule.	interrupting protein synthesis	almost complete inhibition of S. aureus growth	*S. aureus*; fibroblast; osteoblast	60
		GS/BMP-2/ heparinized -DOPA-Ti	immersion; covalent bonding	in vitro	heparin allows the efficient bonding of GS/BMP-2; controlling release of BMP-2 and GS in a sustained and prolonged manner	interrupting protein synthesis	inhibit the growth of bacteria cells and improve osteoblast functions	*S. aureus*	61−63
	nonantibiotic bactericide loaded polymeric architectures	chlorhexidine-loaded polybenzyl acrylate coatings	solvent casting methodologies	in vitro	antiadhesive properties; resistance to enzymatic hydrolysis (dental implants); As a drug delivery systems	interactions with the cell membrane	significant decrease (80% of control) in the bacterial cell number	*Streptoccoccus*; UMR106 cells	16
		chlorhexidine-loaded polylactide coatings	spraying deposition	in vitro	effectively control the release of CHX	interactions with the cell membrane	large inhibition zone; the antibacterial effect of the polymer coating was increased with the decreased concentration of the polymer	*S. aureus*	64
		silver-loaded hydroxyapatite (HAP) /lignin coatings	electrophoretic deposition (EPD)	in vitro	lignin as a component of HAP composite can provide better interconnected porous structure and thus prompt osteogenesis; lignin possesses antioxidant and antimicrobial properties	interactions with the cell membrane; interact with nucleic acids; inhibit respiratory enzymes; accelerate the generation of reactive oxygen species.	diminish the growth of bacteria strain *S. aureus* (percentage of cell reduction was 98.17% after just 1h)	*S. aureus*; Peripheral blood mononuclear cells	65
		silver nanoparticle-loaded alginate/ chitosan coatings	layer-by-layer technique	in vitro	good biocompatibility; antibacterial properties	interactions with the cell membrane and nucleic acids, etc.	large inhibition zone; the ZOI is 2.85 ± 0.24 mm and 2.13 ± 0.15 for two bacteria, respectively	*Escherichia coli*; *S. aureus*; L929 cells	66
		antimicrobial peptide Tet213-bonded poly(DMA-*co*-APMA) copolymer brushes coatings	surface-initiated atom transfer radical polymerization (SI-ATRP)	in vitro and in vivo	as a carrier of Tet213; the graft density of Tet213 is closely related to the compositions of brush	interactions with the cell membrane	inhibit bacterial growth and biofilm formation; CFUs of bacteria were decreased by at least 85% in vivo	*P. aeruginosa*; *S. aureus*; MG-63	67, 68
		cecropin B-loaded polydopamine coatings	immersion; covalent bonding	in vitro	as an intermediate linker for further anchoring of Cec B	interactions with the cell membrane	inhibit the bacteria adhesion and biofilm formation	*P. aeruginosa*; *S. aureus*; Osteoblasts	69
antiadhesion property	adhesion-resistant polymeric architecture	P(MAA-Silk)-Titanium	surface-initiated atom transfer radical polymerization (ATRP)	in vitro	antiadhesion property of PMAA	electrostatic interactions	inhibit bacterial adhesion	*S. aureus*; *S. epidermidis*; osteoblast	17

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Table 1. continued

antibacterial modes	types of coating		fabrication methods	test condition	the role of polymers	antibacterial mechanism	antibacterial efficiency	experimental model	ref
		PPy/PEG coatings; polypyrrole and polyethylene glycol	electrodeposited	in vitro	polypyrrole is a excellent conducting polymers; PEG can enhance the mobility of ions, improve stability, and enhance antibacterial properties	surface hydrophobicity; surface charge and electrostatic interaction	the percentage of bacteria growth inhibition increases to 88%	*Escherichia coli*	70
		PLL-*g*-PEG/ PEG-RGD coatings	immersion	in vitro	PEG can decrease the amount of adsorbed proteins	surface charges; surface topography	decrease the amount of adherent bacteria by 69%	*S. aureus*; fibroblast; osteoblast	71
	antibacterial polysaccharides polymeric architecture	ulvan polysaccharides-Ti	covalent binding	in vitro	antiadhesive properties of ulvan polysaccharides	surface hydrophobicity; surface charge and electrostatic interaction	decrease bacterial adhesion, with a reduction in the initial adhesion up to 96%.	*P. aeruginosa*; *S. epidermidis*	72
antibacterial properties	antibacterial polysaccharides polymeric architecture	RGD-Hyaluronic acid/chitosan coatings	the layer-by-layer (LbL) electro-static deposition	in vitro	good biocompatibility; Antibacterial properties	chitosan acts as a chelating agent; interacts with the membrane etc.	exhibiting about 80% reduction in bacterial adhesion	*S. aureus*; osteoblasts	37, 73−75
		CH-LA-PDA-Ti	immersion; covalent bonding	in vitro	PDA: as an intermediate linker for further anchoring of CH-LA	interactions with the cell membrane etc.	The antibacterial rate of 93.3% against P. eruginosa and 95.6% against S. aureus	*P. eruginosa; S. aureus*; osteoblast	76
		O-CMCS-PDA-Ti	immersion; covalent bonding	in vitro	PDA: as an intermediate linker for further anchoring of O-CMCS	interactions with the cell membrane etc.	the *O*-CMCS coating results in 75−85% reduction in adherent bacteria	*S. epidermidis*; osteoblast	77

[a]Abbreviations: PLGA, poly(lactic-*co*-glycolic acid); *S. aureus, Staphylococcus aureus*; *S. epidermidis, Staphylococcus. epidermidis; P. aeruginosa, Pseudomonas aeruginosa;* MG-63, osteoblast-like cells from human osteosarcoma; PPy, polypyrrole; NPs, spherical nanoparticles; ROMP, ring-opening metathesis copolymerization; GS, gentamicin sulfate; HEMA, 2-hydroxyethyl methacrylate; SA, Succinic Anhydride; GE, gentamicin; Col, collagen; PEG, polyethylene glycol; DA, dopamine; PDA, polydopamine; CHX, Chlorhexidine; CH-LA, chitosan-lauric acid; O-CMCS, *O*-carboxymethylated chitosan; ZOI, zone of inhibition; CFUs, colony-forming unit.

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kinds of coatings have been developed, for example, antibiotics loaded coatings,[13] inorganic bactericide doped coatings,[14] bioactive antibacterial polymerization,[15] nonantibiotic organic bactericide loaded coatings,[16] and adhesion-resistant coatings.[17] Among them, antibacterial polymeric coatings possess distinct advantages such as tunable physical and chemical properties, diverse functionalities, simple structure, low cost, and easy fabrication.[18,19] However, because of the nature of most polymers, these polymeric coatings do not possess some special surface properties such as hydrophilicity, roughness, and surface polarity thereby hampering clinical applications.[20,21] In this respect, a functionalized polymeric nanoarchitecture on the materials surface not only preserves the favorable bulk attributes of the polymer, but also introduces other unique functions such as drug delivery, modulation of cell adhesion, cell differentiation, as well as inhibition and killing of bacteria. This paper aims to review the recent research and development of antibacterial polymeric nanoarchitectures on Ti-based biomedical implants.

2. BACTERIAL INFECTION AND ANTIBACTERIAL MECHANISM

2.1. Biofilm-Induced Infection. Implant-associated infection occurs frequently during and after surgery and is caused mainly by staphylococci and streptococcus. Complications can lead to implant failure necessitating removal by a second surgery. Formation of a biofilm is one of the main reason for infection. A biofilm that develops quickly after bacteria attach onto the implant surface often contains a macromolecular layer formed under physiological conditions.[22] The macromolecular layer constitutes the so-called "conditioning film" rendering the surface more hospitable for bacteria to colonize.[11,22] Various kinds of proteins from surrounding biological fluids like blood, interstitial fluids, salivary proteins, and plasma proteins adsorb on the implants before the first germs appear.[22,23] However, when the conditioning film is formed, bacteria can adhere and subsequently colonize to form a biofilm that possesses a complex architecture by accumulation.[22,24] A biofilm can resist the host immune response via host defense mechanism consequently destroying the host immunity ability on the metallic implant.[25,26] Furthermore, it can withstand antimicrobial challenge in two ways.[25] The first one is failure of the antimicrobial agent that penetrates into the biofilms due to neutralization reactions between the antibacterial agent and some components in the biofilms.[25,27−29] The second one is reduced susceptibility of the biofilm to antibacterial agents, resulting in loss of activity of antibiotics against some slow-growing bacteria in the biofilm.[25,30]

Although contamination during surgery can induce infection, most infection occurring on Ti-based metallic implants is not related to surgeries.[31,32] In the early stage after implantation, the local immune system is affected by the surgical trauma, and consequently bacterial infection often occurs during this stage.[33] Even after tissue integration, the local host immune ability is poor and restricted by a small quantity of blood vessels at the interface between the implant and surrounding tissues. If biofilm-related infection becomes chronic, implant failure normally ensues because both surgical debridement and conventional systemic antibiotic therapy have no effects at that time.[34] Hence, it is critical to prevent the formation of biofilms by inhibiting initial bacterial adhesion or killing bacteria directly[35,36] and a good understanding of the general antimicrobial mechanism is crucial to the fabrication of functionalized antibacterial polymeric nanostructures on Ti-based metallic implants.

2.2. Antimicrobial Mechanism. Generally, there are two types of antibacterial modes including the bactericidal and antiadhesion way. The former is to kill bacteria directly by bactericides on the implants,[13] whereas the latter is to prevent adhesion of microorganisms through antiadhesive agents on the surface.[37,38] Although the precise antibacterial mechanism is still not very clear at present, according to the reaction between antibacterial agents and bacterial cells, it can be generally divided into four main antimicrobial mechanisms as follows.

2.2.1. Inhibiting the Synthesis of Bacterial Cell Wall. The antibacterial activity of penicillin and other antibacterial agents such as vancomycin and cefuroxime can directly inhibit the synthesis of bacterial cell wall leading to the death of bacteria.[39,40]

2.2.2. Disruption of Protein Synthesis. Protein is often synthesized directly from genes by translating mRNA.[41] If inaccurate mRNA translation occurs in this process, the functions of the protein can be changed and as a result, the primary enzymes needed for cell survival cannot be synthesized thereby causing death of the bacteria. Some antibiotics like aminoglycosides and chloramphenicol operate in this way. For example, gentamicin acts by irreversibly binding the 30S subunit of the bacterial ribosome to disturb protein synthesis.[42]

2.2.3. Interaction with Cell Membrane. Some antibacterial agents interact with the plasma membrane of bacteria to disrupt the integrity of membranes and impact the membrane permeability[43] to kill them. Most antimicrobial peptides can achieve this effect through interaction with the phospholipid component of the cell membrane.[43−45] However, there is an increasing number of peptides found to proceed under other mechanisms such as inhibition of cell wall or protein synthesis or interaction with DNA or RNA.[46]

2.2.4. Inhibiting Transcription and Replication of Nucleic Acid. Some antibacterial agents can inhibit the transcription and replication of DNA, thus influencing the synthesis of necessary enzymes and cell division. For example, silver ions interact with nucleic acids and DNA molecules become condensed and lose their replication ability after treatment with silver ions.[47] It has also been shown that silver ions can inhibit respiratory enzymes,[48] accelerate the generation of

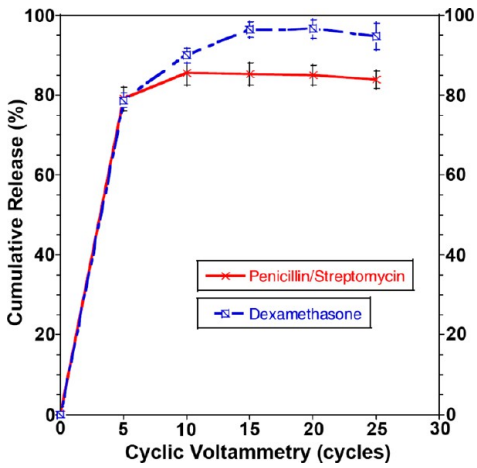

Figure 1. Cumulative concentrations of P/S and Dex after electrical stimulation (scanning rate = 100 mV s^{-1}). Reprinted with permission from ref 55. Copyright 2011 IOP Publishing.

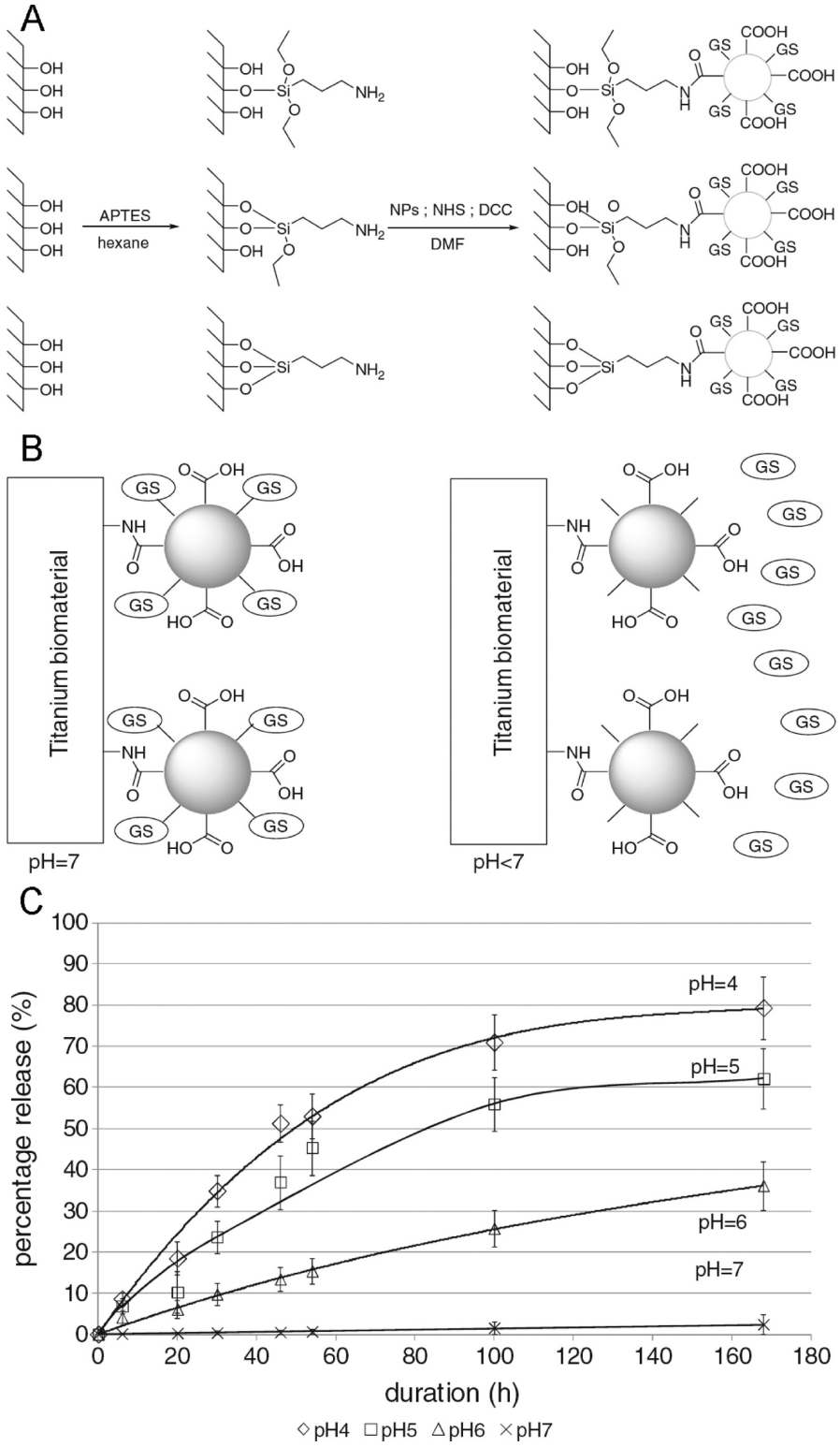

Figure 2. (A) Grafting of NPs onto titanium. (B) Representation of the bioactive materials at neutral and acidic pH. (C) Release kinetic profiles of titanium functionalized with FITC-labeled GS as a function of pH for GS grafted to titanium, DGS = 1.0 ± 0.3 $\mu g/cm^2$. Reprinted with permission from ref 56. Copyright 2012 Elsevier.

reactive oxygen species,[49] and interfere with the membrane permeability consequently leading to bacteria death.[48,50]

On the basis of the aforementioned possible antibacterial mechanisms, while some antibacterial agents work on the cell membrane of the bacteria, others are taken inside the cells. The former antibacterial agents can be easily fixed on polymeric nanoarchitectures by chemical reactions. The polymeric nanoarchitecture with a sustainable high concentration of antibacterial agents can kill microorganism effectively and decreases the amount of drug-resistant bacteria.[51] The latter antibacterial agents can be released controllably from polymeric nanoarchitectures to avoid possible cytotoxicity and generation

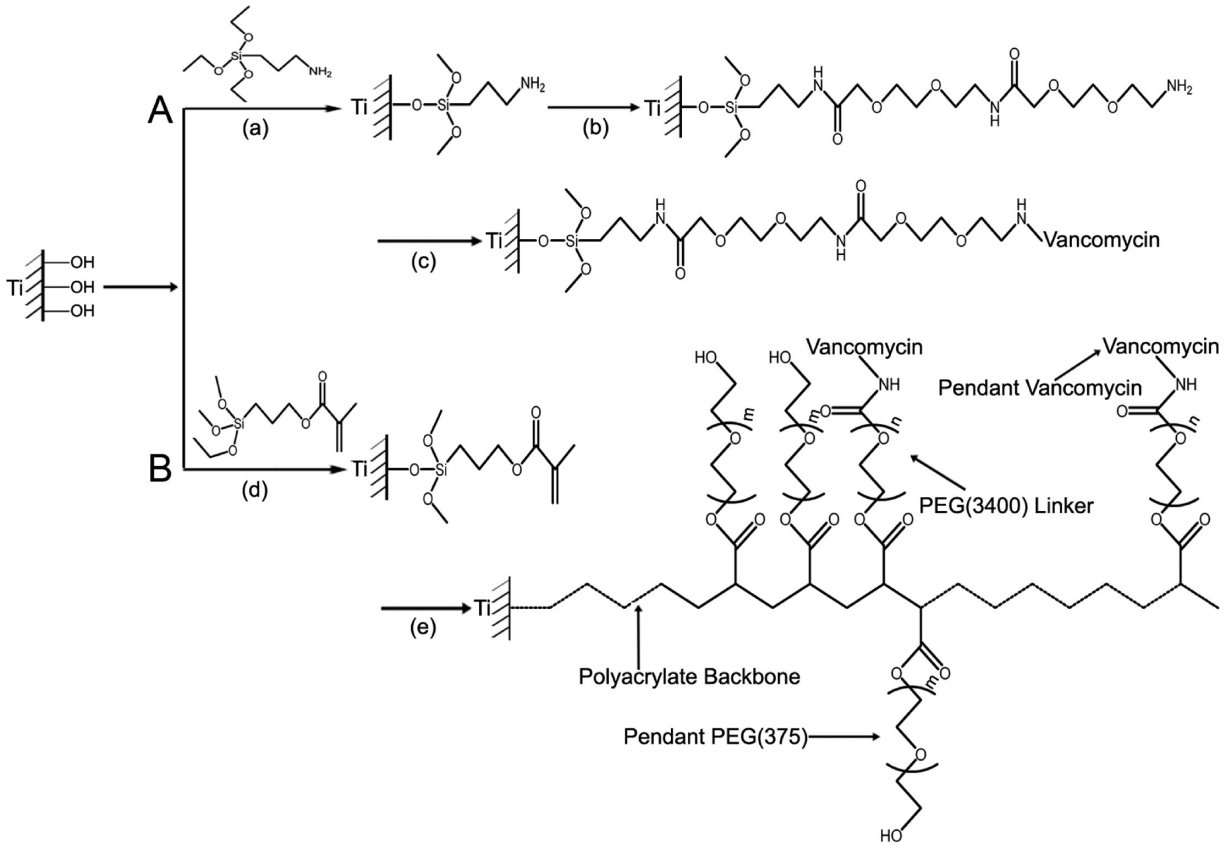

Figure 3. (A) Reaction schematic illustrating the synthesis of VAN bonded to Ti: (a) toluene at room temperature; (b) (i) Fmoc-AEEA, HATU, Me_2NCHO, and *N*-methylmorpholine at room temperature; (ii) piperidine/Me_2NCHO; (iii) Fmoc-AEEA, HATU, Me_2NCHO, and *N*-methylmorpholine at room temperature; (iv) piperidine/Me_2NCHO; (c) VAN, HATU, Me_2NCHO, and *N*-methylmorpholine at room temperature. (B) Schematic of the Ti surface modification procedure. (d) Anhydrous toluene at room temperature. This reaction places a layer with methacrylate groups on the surface to take part in free radical polymerization and anchor the polymer chains to the surface. (e) UV light, monomer solution, photoinitiator. The methacrylated surface is covered by a monomer solution containing a polymerizable antibiotic (VPA[3400] in this case), another polymerizable species (PEG[375]-acrylate), and a photoinitiator followed by exposure to ultraviolet radiation resulting in polymerization. A fraction of the polymer chains is covalently attached to the surface, albeit in a geometrically structure more complex than that shown here. In this reaction scheme, a polyacrylate backbone is formed with pendant VPA(3400) and PEG(375) assuming ideal polymerization, $m = 9$ and $n = 80$. Reprinted with permission from refs 59 and 87. Copyright 2010 Springer and 2005 Elsevier.

of drug-resistant bacteria.[52,53] In addition, some polymeric nanoarchitectures have a suitable chemical composition to body and good biocompatibility. Owing to the versatility and advantages, functionalized polymeric nanoarchitectures with unique antibacterial functions have been developed on Ti-based metallic implants. Table 1 summarizes the fabrication methods and corresponding antibacterial activity of typical functionalized polymeric nanoarchitectures, which can be categorized into two groups including the bactericidal and adhesion resistant ones.[16,17,37,54−77] Of course, it is possible to combine the two modes, for instance, antibacterial polysaccharides consisting of chitosan (bacteriostatic/bactericidal) and hyaluronic acid (antiadhesion), the so-called antibacterial architecture.

3. BACTERICIDAL POLYMERIC NANOARCHITECTURES

3.1. Antibiotic-Loaded Architectures. Conventional systemic antibiotic therapy may cause side effects and drug interactions.[78] Furthermore, antibiotic prophylaxis cannot achieve the expected therapeutic effect on periprosthetic infection because of the low vascularity at the site of new metallic implants. In contrast, the application of topical antibiotics that can avoid these potential hazards though local release of antibacterial agents has attracted growing attention. Recently, there is more interest in applying this technique to enhancing the antibacterial properties of Ti-based metallic implants by introducing antibiotic-loaded biocompatible polymers such as poly(lactic-*co*-glycolic acid) (PLGA),[54] *α*-*ω*-functionalized poly(ethylene oxide) nanoparticles (NPs),[56] PEG-acrylate,[58,59] poly(2-hydroxyethyl methacrylate) (P-(HEMA)),[60] and heparin-dopamine.[61,62]

Various kinds of antibiotics such as gentamicin sulfate, vancomycin, penicillin, and cefuroxime have been incorporated into polymers by encapsulation[54] and covalent bonding[56,60] to introduce bactericidal functions on Ti-based metallic implants because of their broad spectrum being effective against infection caused by pseudomonas, proteus organisms, staphylococci, and *Staphylococcus aureus*.[54,56,61,79,80]

The effectiveness of an antibiotic-releasing architecture depends strongly on the rate and manner in which the drug is released, which is determined mainly by the properties of the polymeric architectures and antibiotic type. PLGA is often used as a carrier of these antibiotics on account of its excellent biocompatibility and biodegradability. The antibiotics encapsulated in PLGA is released at a sustained rate either by diffusion of the drug or through degradation of the polymer. For example, Yeh et al. have prepared a vancomycin and cefuroxime

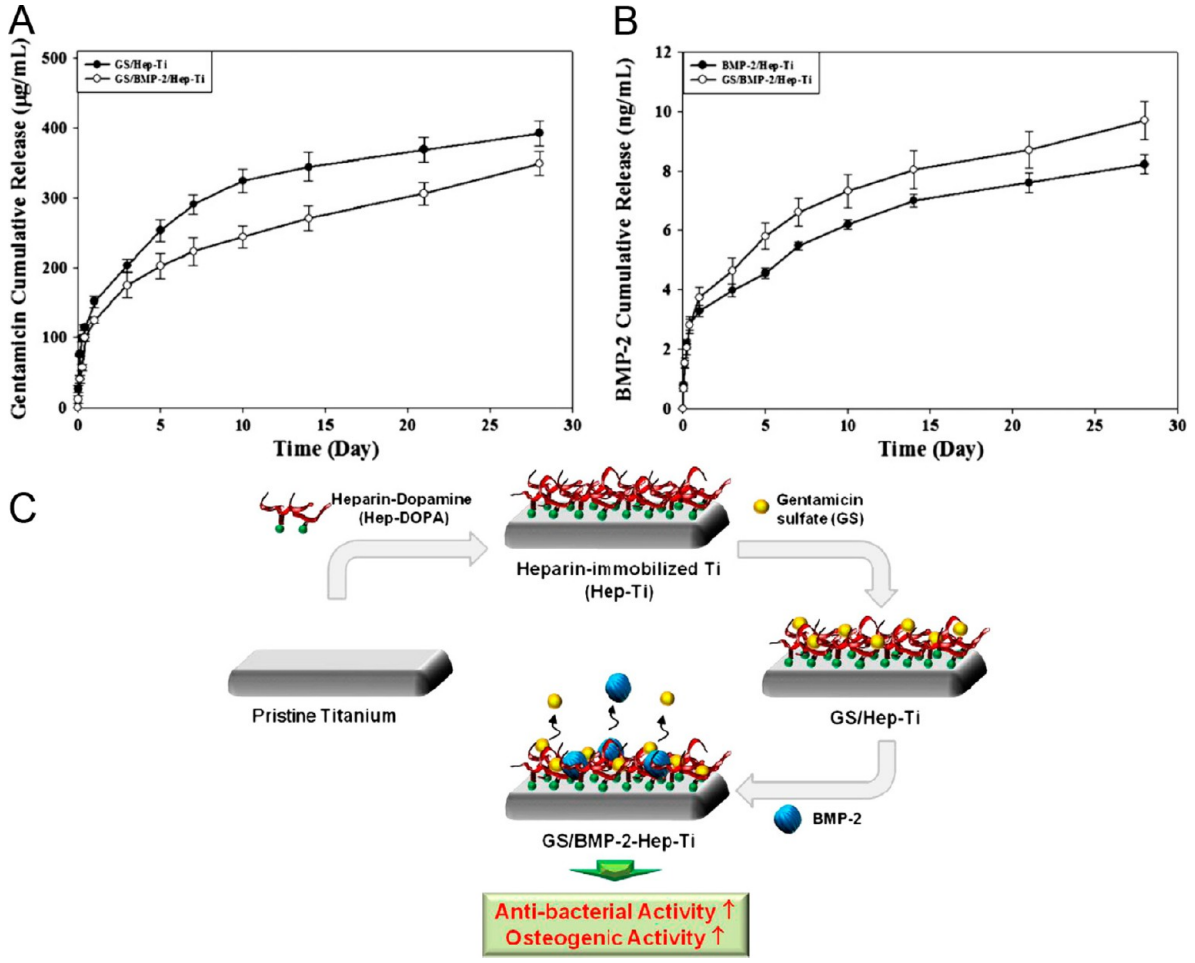

Figure 4. In vitro release of gentamicin or BMP-2 from the Ti substrate. (A) Gentamicin release from GS/Hep-Ti (●) and GS/BMP-2/Hep-Ti (○). (B) BMP-2 release from BMP-2/Hep-Ti (●) and GS/BMP-2/Hep-Ti (○). (C) Schematic diagram showing immobilization of gentamicin sulfate and bone morphogenic protein-2 (BMP-2) on the heparinized-Ti substrate. Reprinted with permission from ref 61. Copyright 2012 Elsevier.

loaded PLGA layer on Ti6Al4 V by plasma spraying and the release rate is determined by the antibiotics concentration and number of coating layers of the PLGA encapsulation.[54] In fact, the optimal PLGA capsulation architecture shows a suitable initial burst release (about 8% release in 24 h) which is a good feature because the relatively high dose initially released to protect against bacterial infection during the most critical period after implantation, followed by relatively slow and continuous release of cefuroxime over a period of 17 days with an effective inhibitory effect.[54,81] Subsequent antibiotics release over the minimum inhibition concentration (MIC) can prevent bacterial infection at the implant site during healing. The initial burst release can be explained by diffusion of the antibiotics on the surface or near the surface of the polymer coating, while subsequent release is attributed to the diffusion process influenced by the degradation rate of the polymer.[81] The hydrophilicity of the polymer and antibiotics affects the release profile. For example, the total amount of released vancomycin is about 95% of the loaded amount and larger than that of cefuroxime (about 65%).[54] However, the duration of effective release of vancomycin is 5 days shorter than that of cefuroxime. This release phenomenon can be explained by the reduced affinity between the hydrophilic vancomycin and hydrophobic PLGA after degradation of the initial hydrophilic poly glycolic acid (PGA).[54] Another reason is that the hydrophilic property of vancomycin makes it easier to dissolve in the PBS solution and release from the PLGA matrix.

Polypyrrole (PPy), an intrinsic conductive polymer, has been used in controlled drug delivery and some unique characteristics.[82] For example, the antibiotics that are encapsulated in PPy on titanium can be released on demand to potentially combat bacterial infection by applying a voltage.[55] As shown in Figure 1, 80% of the drugs are released at a constant rate on demand when the voltage is applied for five cycles at a scanning rate of 0.1 V s^{-1}.[55]

Although the controlled drug delivery system is an excellent way to improve the lifetime of orthopedic implants, there are some shortcomings such as the demand for detection of infection beforehand and lack of suitable early burst release. The ideal drug delivery systems (DDS) should be able to release drugs over a period of several months to years in order to prevent "late" infection that can occur 24 months later postsurgery while improved efficacy, reduced toxicity and resistance, improved patient compliance, and convenience are attained.[83,84] Pichavant et al. have developed a drug delivery system with automatic capacity on Ti6Al4 V by surface silanization, in which pH-sensitive functionalized nanoparticles (NPs) are formed by ring-opening metathesis copolymerization in a dispersed medium of norbornene with $\alpha-\omega$-functionalized macromonomers ended with gentamicin sulfate (GS). This process is schematically illustrated in Figure 2A.[56]

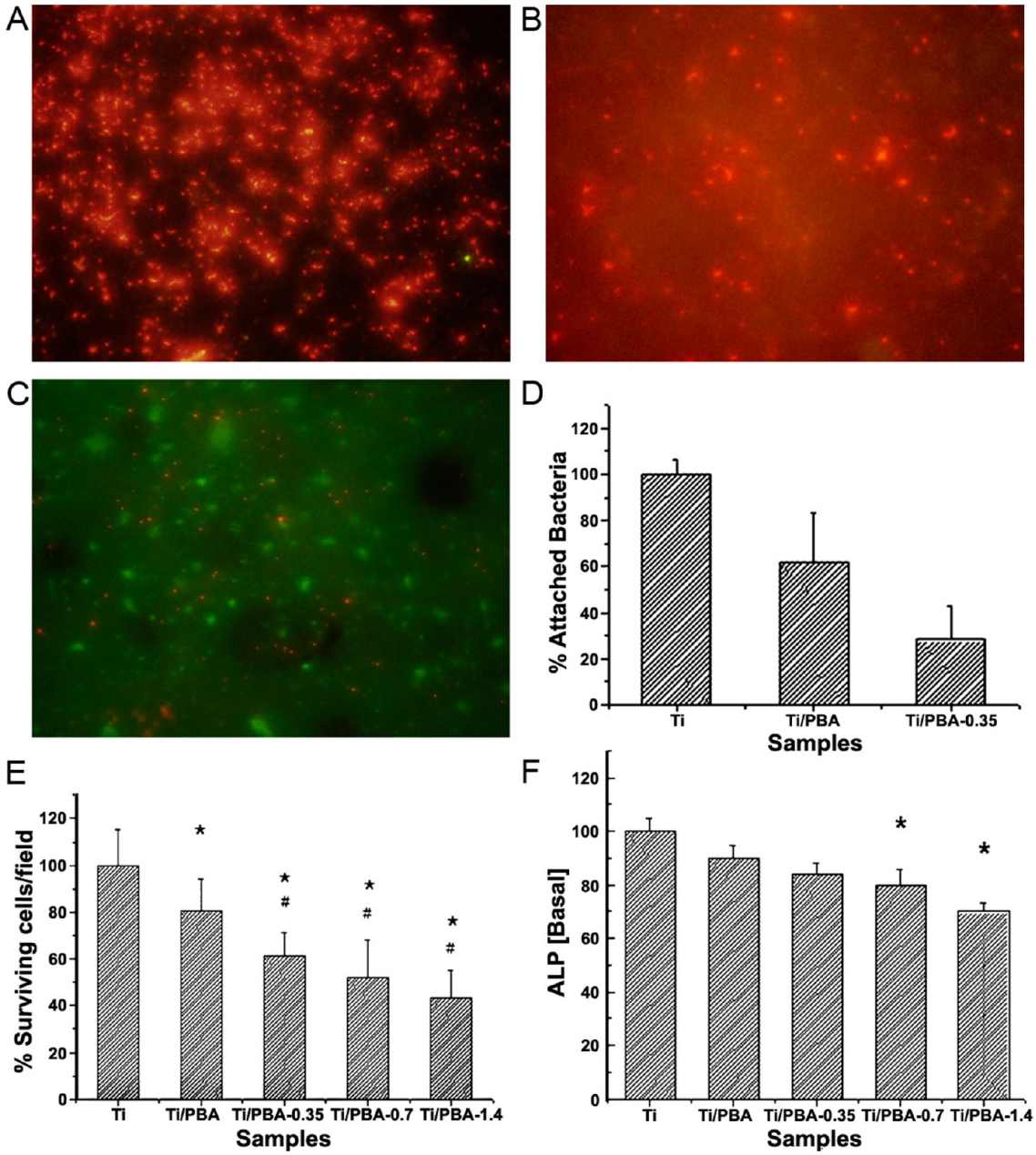

Figure 5. Bacterial adhesion on (A) Ti, (B) Ti/PBA, and (C) Ti/PBA-0.35. Percentage of bacterial adhesion to (A) Ti, (B) Ti/PBA, and (C) Ti/PBA-0.35. (D) Ti is the control surface (100%). Effects of the substrate on (E) the number of surviving cells and (F) ALP activity. The UMR106 cells are cultured on different substrates for 24 h. The values are shown as the means. $^{*}p < 0.05$ for Ti sample and $^{\#}p < 0.05$ for Ti/PBA. Reprinted with permission from ref 16. Copyright 2012 Elsevier.

The GS-loaded NPs-APTES (aminopropyltriethoxysilane) polymeric architecture allows local release of GS only under acidic conditions (as shown in Figure 2B, C) because the bond of GS-NPs is pH sensitive and broken gradually as the pH decreases.[55,57] In view of the decrease of pH (between 6.9 and 6.0) due to inflammation during surgery, about 10% of the initial GS are released and 90% of the GS remain.[56] In general, bacterial infection and inflammation typically occur at pH as low as 5.5−7.0 and local acidosis occurs.[56,85,86] Therefore, controlled release of GS at the site of surgical implantation has an advantage of adjustable time period and can minimize the occurrence of antibiotic-resistant bacteria for a long time. In practice, GS-loaded NPs functionalized titanium implants significantly inhibit the growth of *Staphylococcus aureus* (*S. aureus*) after the release of GS at pH 4, 5, and 6 after 48 and 96 h *in vitro*.[56,57]

Unlike architectures that can release antibiotics continuously, some polymeric architectures immobilize antibiotic macromolecules on Ti-based metallic implants by means of intermediate polymeric functional groups. It is schematically illustrated in Figure 3.[59,87] In these cases, a silane coupling agent is often tethered to the Ti-based metallic implants by Si−O−Ti covalent bond to form stable self-assembled monolayers (SAMs) through cross-linking,[88−90] which acts as a "molecular bridge" between the metal and polymeric macromolecules. 8-amino-3,6-dioxaoctanoate (aminoethoxyethoxyacetate; AEEA) and PEG-acrylate are often used to anchor organic biocides tightly and increase the amount of anchoring organic

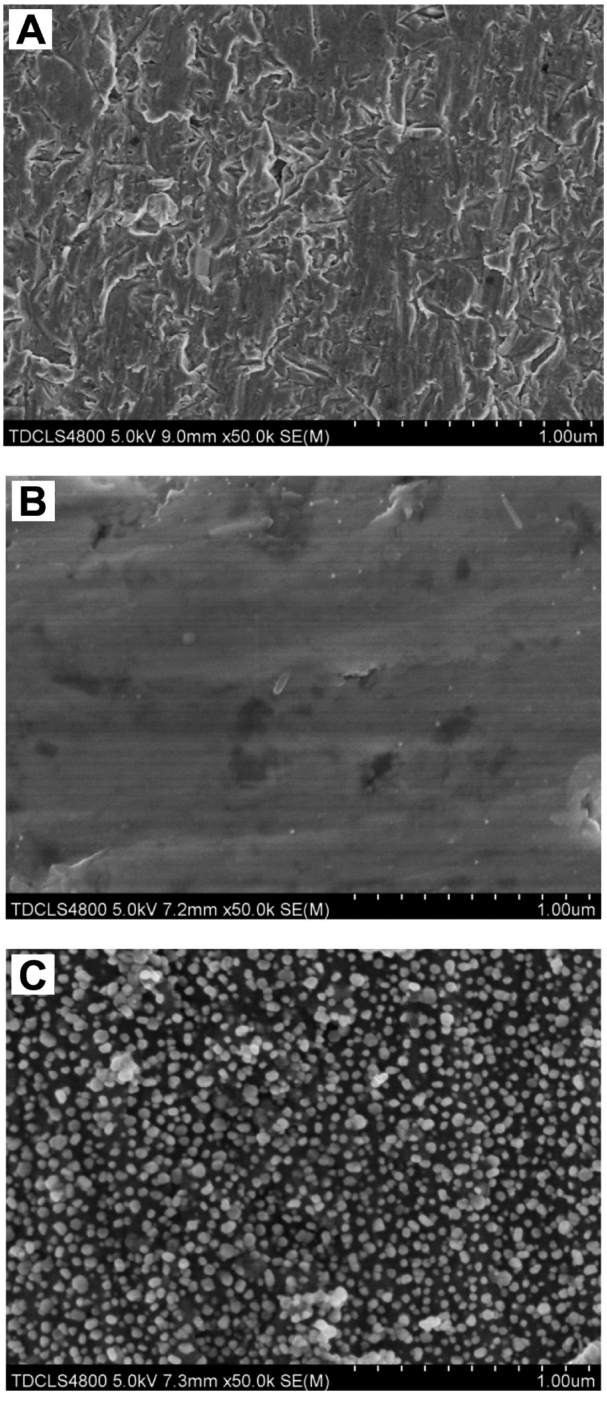

Figure 6. Scanning electron microscopy images: (A) original titanium surface, (B) DAL/CHI multilayer coated surface, and (C) AgNP-DAL/CHI multilayer coated surface. Reprinted with permission from ref 66. Copyright 2013 Elsevier.

biocides.[58,59,88] The covalent bonds between them are not influenced by the biological conditions such as pH and composition of the electrolyte. Hence, the surface-tethered metallic implants exhibit stable antibacterial activity as well as good cytocompatibility and tissue compatibility.[80,87,88] For example, Jose and Wickstrom et al. show that vancomycin covalently attached to the Ti alloy surface (Vanc-Ti) effectively prevent colonization of *Staphylococci epidermidis* (*S. epidermidis*) and formation of biofilms even in continuous culturing or when challenged seven times by *S. epidermidis*.[79] This strategy has been reported to have the same effects on killing *S. aureus* with 88% ± 16% reduction over 2 h and meanwhile, Vanc-Ti can retain the antibacterial activity after repetitive challenges.[87]

Besides conventional radical polymerization, intermediate polymeric macromolecules that are utilized to anchor antibiotics can be grafted onto Ti-based metallic implants by atom transfer radical polymerization (ATRP).[60,91] In this process, the properties of the intermediate polymers such as molecular weight, dispersion effectiveness, composition, and functionalities can be precisely controlled regardless of the shape and composition of the substrate.[92,93] Therefore, the content and type of the antibiotics immobilized on the surface can be modulated by controlling the chain length and end group of the intermediate polymers. For instance, P(HEMA) has been grafted onto Ti by surface-initiated ATRP.[60] Besides favoring surface silanization on the titanium substrate, the pendant hydroxyl end groups of P(HEMA) can be converted into carboxyl groups and introduce amine groups allowing covalent anchoring of penicillin on titanium.[60,94,95] The sustainable high concentration of antibiotics almost completely inhibits the growth of *S. aureus* on the antibiotics-P(HEMA)-Ti surface for a long period.[60] However, the bactericidal effect of the antibiotic-tethered architecture depends on the amount of antibiotics that may be lost during implantation. When the amount of the antibiotics falls below MIC, prolonged exposure increases the bacterial resistance.

In practice, biocides like gentamicin sulfate are often incorporated into polymeric architectures on Ti-based implants together with bone morphogenic protein-2 (BMP-2), growth factors like transforming growth factor-β (TGF-β), and vascular endothelial growth factor (VEGF), which not only prevent bacterial infection but also promote bone integration between implants and surrounding tissues.[96−98] Titanium implants functionalized by dual drugs (gentamicin sulfate and BMP-2)-eluting exhibit excellent antibacterial activity and enhanced differentiation of osteoblasts attributable to the controlled release of BMP-2 and gentamicin sulfate from the heparinized-Ti surface in a sustained and prolonged manner (shown in Figure 4A, B).[61,62] The functionalized polymeric architecture is composed of heparin, dopamine, gentamicin sulfate, and BMP-2. Because of the abundant sulfate and carboxylic groups as well as high binding affinity, heparin, a highly sulfated glycosaminoglycan, is often used as the intermediate polymeric bridge between the implants and antibiotics as well as BMP-2. The preparation process of this complex is presented in Figure 4C.[61,99]

All in all, antibiotics-loaded polymeric architectures have large potential in enhancing the self-antibacterial properties of metallic implants. However, the architecture may induce antibiotics-resistant bacteria and causes implant failure. Some death-causing microorganisms such as the superbug NDM-1 and the Ebola virus have developed resistance against known antibiotics.[100,101] To solve the problem, the antibiotic-resistance mechanism must be better understood. A good way may be the combined use of various antibiotics and inorganic nanoparticles such as ZnO and silver nanoparticles that are primary inorganic bactericides against a broad spectrum of antibiotic-resistant bacteria.

3.2. Nonantibiotic-Loaded Architectures. *3.2.1. Chlorhexidine.* Besides antibiotics, nonantibiotic agents such as chlorhexidine (CHX), Ag nanoparticles, and antimicrobial peptides can be introduced to Ti-based metallic implants by the surface polymeric nanoarchitecture.[16,64−68] Chlorhexidine

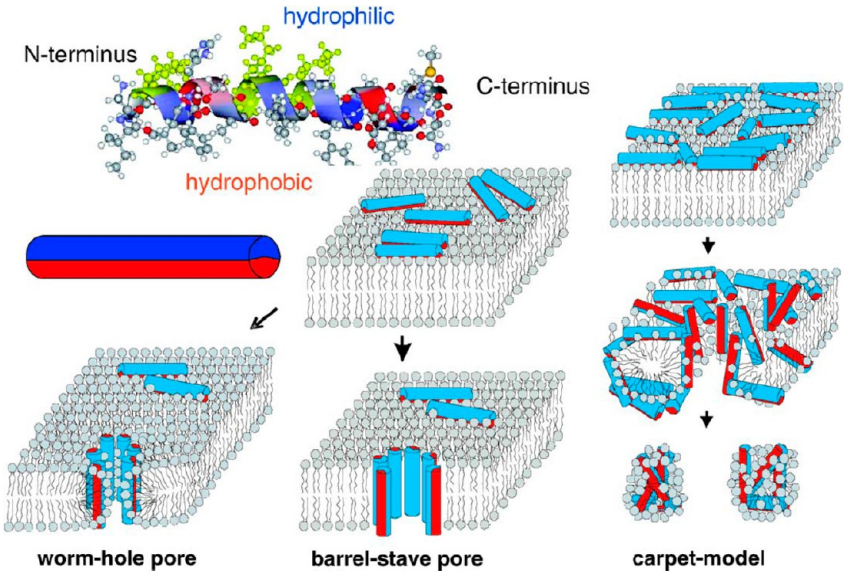

Figure 7. Sketch of different models describing the mechanism and structure of antimicrobial peptides interacting with lipid bilayers, as discussed in the literature. (Top left) α-helical conformation of magainin with its hydrophilic and hydrophobic sides of the helix, as quantified by helical wheel analysis, along with the representation as an amphiphilic cylinder. (Bottom left) Wormhole pore model as proposed for magainin. (Top center and right) Surface (S) state of antimicrobial peptides with the hydrophobic side groups anchored on the hydrophobic core of the bilayer. (Bottom center) Barrel-stave pore model as proposed for alamethicin. (Bottom right) Carpet model: antimicrobial peptides crowding in the S state leading to micellation. Reprinted with permission from ref 43. Copyright 2006 Elsevier.

(CHX), a symmetrical molecule with two ionizable guanidine moieties, is effective against Gram-positive/negative bacteria and fungi. In addition, it can be retained by dentinal hard tissues. CHX is thus often to combat bacterial infection by immobilizing it on the surface of titanium implants.[102−106] Incorporation of CHX into some biocompatible polymers such as cyclodextrin andits derivatives (*b*-cyclodextrin (CD), maltodextrin (MD), methylated-*b*-cyclodextrin (MBCD), hydroxypropyl-*b*-cyclodextrin (HPBCD)), PLGA, polylactide (PLA), and poly(ethylene-*co*-vinyl acetate) copolymer can achieve controllable and continuous release of CHX.[16,64,107−109] For example, recent studies show that CHX can be released from CHX-PLA-modified titanium and the release rate is determined by the degradation rate of PLA.[64,110] In the process, the titanium substrate is anodized and then coated in a mixed solution composed of PLA and CHX by spraying.[64]

The cytotoxicity of CHX should be considered when this biocide is used to prevent bacterial infection because it can induce direct cytotoxicity in human alveolar osteoblasts, human alveolar bone cells, and stem cells from exfoliated deciduous teeth (SHED).[111−116] The best way is to optimize the content of CHX to achieve a satisfactory balance between cytocompatibility and antibacterial properties. Cortizo et al. have prepared an effective antibacterial architecture on titanium dental implants coated with polybenzyl acrylate (PBA) that is loaded with different contents of CHX (labeled as Ti, Ti/PBA, Ti/PBA-0.35, Ti/PBA-0.70, Ti/PBA-1.4).[16] When 0.7 and 1.4% CHX are loaded, some harmful signals indicating cytotoxicity are found, for instance, morphological changes, shrinkage, smaller and pyknotic cells, and loss of cytoplasmic processes. Conversely, assays with 0.35% CHX do not show such effects. In comparison with 0.7% and 1.4% CHX loading in the polymeric architecture, the lowest CHX concentration of 0.35% results in better comprehensive performance, i.e., higher antibacterial efficiency and greater osteogenic activity (shown in Figure 5).[16] This CHX-loaded PBA architecture exhibits both bactericidal effect and antiadhesion function simultaneously in the early stage. The former is ascribed to CHX that inhibits the gycosydic and proteolytic activity to reduce the proteinase activity in most oral bacteria and fungi,[117,118] whereas the latter is attributed to the nonadhesive properties of PBA suppressing hydrolysis of enzymes.[16] However, the development of suitable drug carriers for prolonged controllable release of CHX still faces many challenges because of the cytotoxicity of CHX, and good stability of the architecture is needed considering that CHX is mainly used in dental implants.

3.2.2. Silver. Silver is a well-known primary inorganic bactericide against antibiotics-resistant bacteria.[119,120] Biomaterials loades with nano silver particles exhibit higher and long-term antibacterial efficiency, excellent biocompatibility, as well as low cytotoxicity and genotoxicity.[121,122] In the case of Ti-based metallic implants, coatings incorporated with silver nano particles are often introduced.[65,66] In comparison with inorganic coatings such as Ag/hydroxyapatite (HA), Ag/TiO_2, Ag/TaN, and Ag/titanate,[14,65,66,123−127] the Ag/ceramics/polymeric structure has better properties such as higher toughness, better biocompatibility, and controllable release of Ag ions.[65,66,128] For example, the Ag/HA/lignin complex has been prepared by Erakovic et al. and immediate and continuous release of Ag ions from this complex is responsible for the reduction of *S. aureus*.[65,128] In comparison with Ag/HA coatings, the lignin-modified nanoarchitecture is homogeneous without fractures.[65,129] Similarly, Zhang et al. have combined silver nanoparticles (AgNPs) with the dopamine-modified alginate/chitosan (DAL/CHI) polyelectrolyte multilayer. The materials inhibit the growth of *S. aureus* and *Escherichia coli* (*E. coli*) because of the synergistic effects of CHI and embedded AgNPs.[66] Furthermore, incorporation of AgNPs into the polymeric multilayer (Figure 6) can avoid cell and tissue toxicity induced by the released AgNPs.[66]

In conclusion, nano silver-loaded polymeric architectures can regulate the release of silver ions and provide multiple

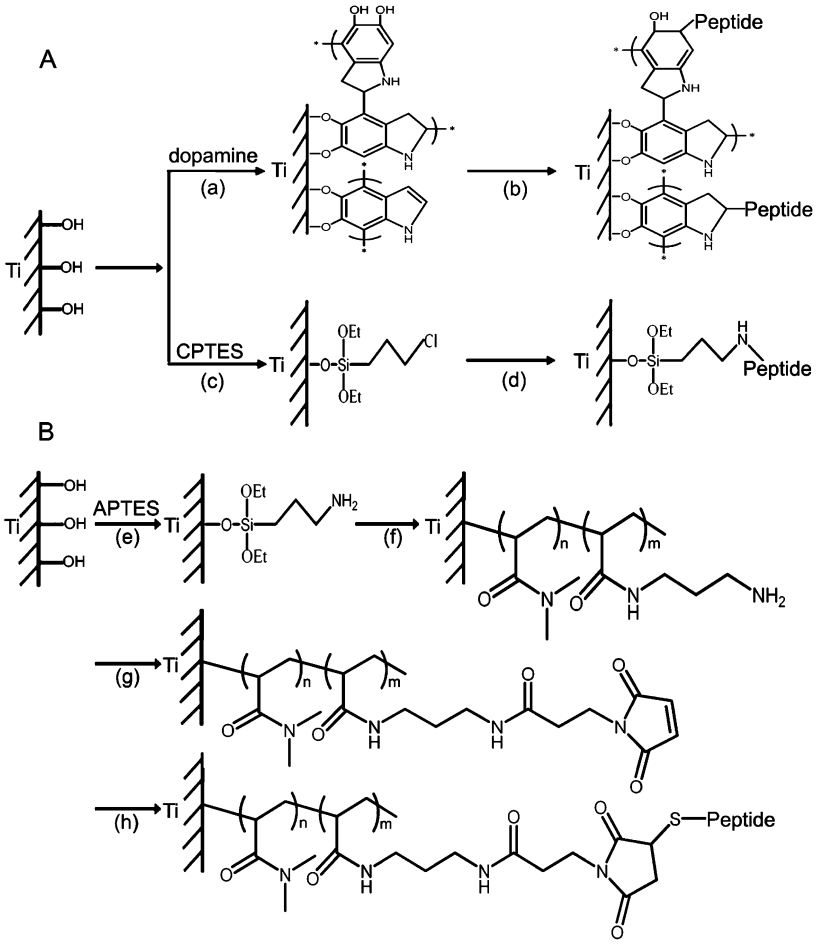

Figure 8. Schematic illustrating immobilization of antimicrobial peptides (AMPs) on the biomaterials surface though intermediate polymeric complexes. (A) Schematic of the chemical surface modification methodology using dopamine or a silane linker (CPTES) to immobilize cecropin B, peptides GL13K, and GLK7-NH2 to Ti. (a) Popamine, Tris buffer (pH 8.5) at room temperature. (b) Cecropin B, Tris buffer (pH 8.5) at room temperature. (c) Anhydrous pentane, 3-(chloropropyl)-triethoxysilane (CPTES), diisopropylethylamine (DIPEA), N_2 atmosphere. (d) Na_2CO_3, peptides GL13K and GLK7-NH2. (B) Schematic of the synthetic route for copolymer brushes and peptide conjugation. (e) Toluene at room temperature. (f) 2-Chloropropionyl chloride, DMA/APMA by ATRP, and Et3N. (g) 3-Maleimidopropionic acid *N*-hydroxysuccinimide, and acetonitrile. (h) Peptide-SH. Reprinted with permission from refs 68, 69, and143. Copyright 2011 Elsevier, 2013 Elsevier, and 2013 Elsevier.

biological functions on titanium implants. However, the detailed antibacterial mechanism of AgNPs is not fully understood and more comprehensive research should be carried out to obtain a better understanding of the interactions between bacteria and AgNPs.

3.2.3. Antimicrobial Peptides. Natural antimicrobial peptides that are secreted by living organisms such as mammalians and amphibians have become promising bactericides due to their broad-spectrum antibacterial property and lower toxicity.[130−134] Furthermore, as parts of the innate immune system of multicellular organisms, they rarely induce bacterial resistance on account of the complex mechanism.[46,67,130−132,134−137] Antimicrobial peptides (AMPs) achieve bactericidal effects mainly by disrupting the integrity of microbial membranes and subsequent cell lysis (shown in Figure 7).[43,44] However, strong membrane binding and insertion of AMPs to bacteria may cause danger to human red blood cells and induce lysis via a similar membrane-disruption mechanism.[138,139] There are also shortcomings such as pH sensitivity, susceptibility to proteolysis, peptide self-aggregation, and so on.[133,140,141] The cytotoxic effects become apparent when higher titers are employed to avoid these problems but nevertheless, covalent immobilization of AMPs on titanium surface may be a good way to overcome these difficulties.

There are so far more than 750 kinds of AMPs but only a few are used to modify metallic implants, for example, cecropin B, antimicrobial peptide Tet-213, GL13K, Magainin2, and E14LKK.[67,68,142−144] Cecropin B, Tet213, and GL13K are three common cationic AMPs composed of 35, 10, and 13 amino acids, respectively.[67,69,143,145,146] Generally, these AMPs are immobilized on the biomaterials surface through intermediate polymeric complexes such as copolymer brushes, polydopamine, 3-(chloropropyl)-triethoxysilane (CPTES), poly(ethylene), poly(styrene), 2-(2-methoxyethoxy) ethyl methacrylate, and hydroxyl-terminated oligo(ethylene glycol) methacrylate[67−69,143,147−149] by two routes. The first way is direct grafting of AMPs onto polydopamine or silane coupling agent that connects the substrate on the other side utilizing a reaction with hydroxide radicals on the surface (depicted in Figure 8A).[69,143] For example, cecropin B has been immobilized on titanium disks by conjunction with intermediate polydopamine.[69] This polymeric architecture not only shows enhanced cytocompatibility (shown in Figure 9), but also efficiently inhibits bacterial adhesion as well as biofilm formation. In comparison with pure titanium or polydopamine

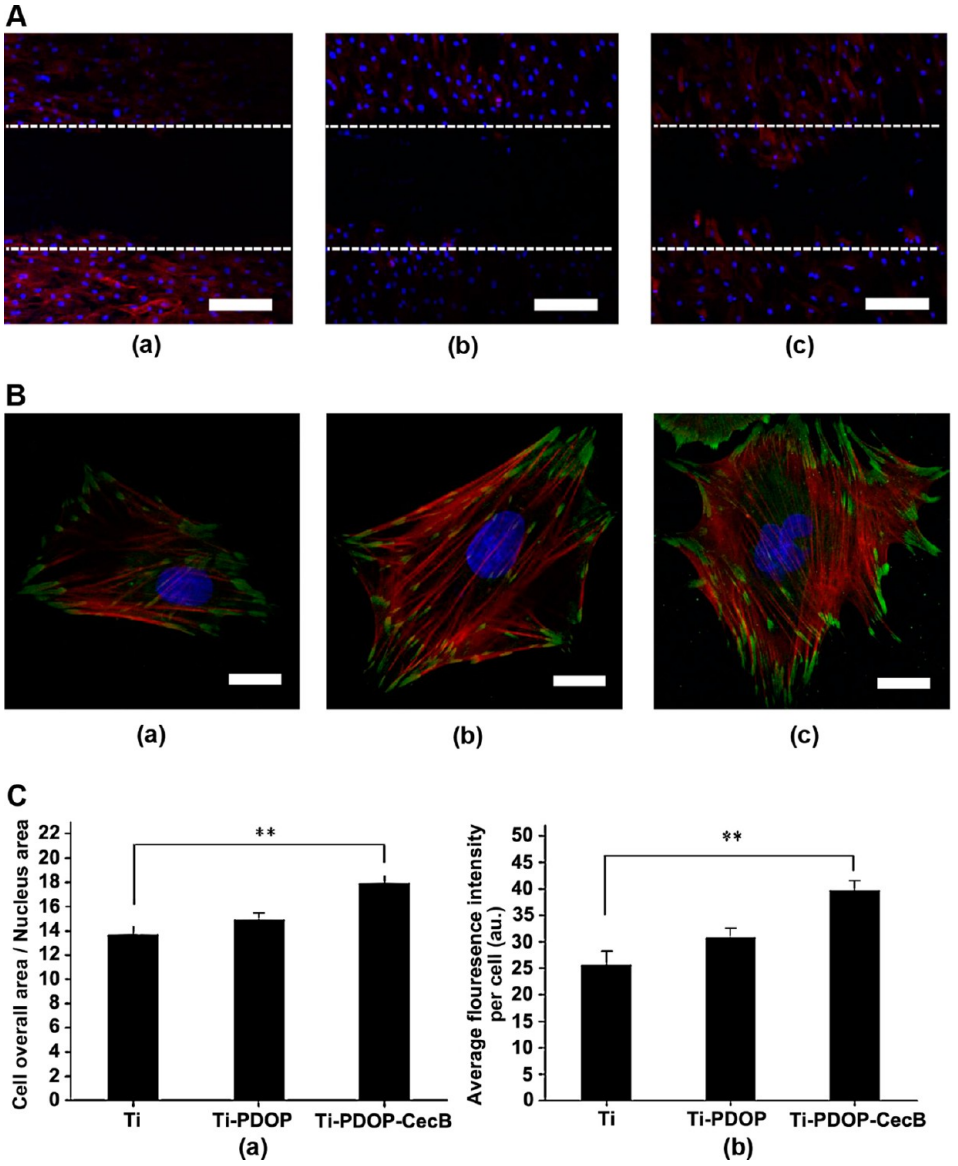

Figure 9. (A) Motogenic response of osteoblasts adhered to different substrates after culturing for 12 h: (a) Ti, (b) Ti-PDOP, and (c) Ti-PDOP-CecB. The initial seeding density is 6 × 104 cells/cm^2. The scale bar in the images is 200 μm. Cell morphology observation of osteoblasts adhered to different substrates: (B) CLSM images of (a) Ti, (b) Ti-PDOP, and (c) Ti-PDOP-CecB. The cells are stained with actin filaments (red), cell nuclei (blue), and vinculin (green). The scale bar in the CLSM images is 20 μm. (C) (a) Image analysis of the cell morphology of osteoblasts adhered to different substrates (n = 50); and (b) fluorescence intensity analysis of the vinculin expression of osteoblasts cultured on different substrates (n = 50), $^{**}p < 0.01$. Reprinted with permission from ref 69. Copyright 2013 Elsevier.

modified titanium, the viability of four kinds of bacteria, i.e. *S. aureus*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *E. coli*, and *Bacillus subtilis* (*B. subtilis*) is reduced nearly by 50% or more.[69]

The other route typically involves three steps as illustrated in Figure 8B.[68] In the first step, the intermediate copolymer brushes are synthesized by surface initiated ATRP and immobilized on the dopamine modified or silanized titanium. The next step is functionalization of the brush with the maleimide group for specific site linkage of AMPs in the final step. The advantage of this method is that the type, density, and content of AMPs can be controlled by adjusting the terminal group as well as distribution and length of intermediate copolymer brushes. As a result, specific biological functions such as excellent biocompatibility, modulation of particular cell behavior, and high antibacterial efficiency are achieved. Gao et al. have synthesized and immobilized N,N-dimethylacrylamide (DMA)/N-(3-aminopropyl) methacrylamide hydrochloride (APMA)copolymer brush on titanium using APTES, and ensuing functionalization of DMA/APMA using amine and maleimide favors conjunction of various kinds of AMPs such as Tet-213, 1010cys, Tet-20, Tet-21, Tet-26, HH2, and MXX226.[68] Surface peptide conjugation and the related antibacterial efficiency depend upon the grafting density, thickness of the DMA/APMA brushes, as well as DMA/APMA molar ratio (Figure 10A, B). A surface concentration as high as 23.9 peptides/nm^2 has been achieved.[67,68] All the polymer brush tethered AMPs display excellent and broad-spectrum antimicrobial activity as well as biofilm resistance in vitro, and the efficiency depends on the type of AMPs used (shown in Figure 10C). For example, Ti-DMA/APMA-brush-Tet-20 yields 100% antibacterial effects against *P. arruginosa* and *S. aureus* after culturing for 1 h.[68] The AMPs-fixed architecture is not toxic to mammalian

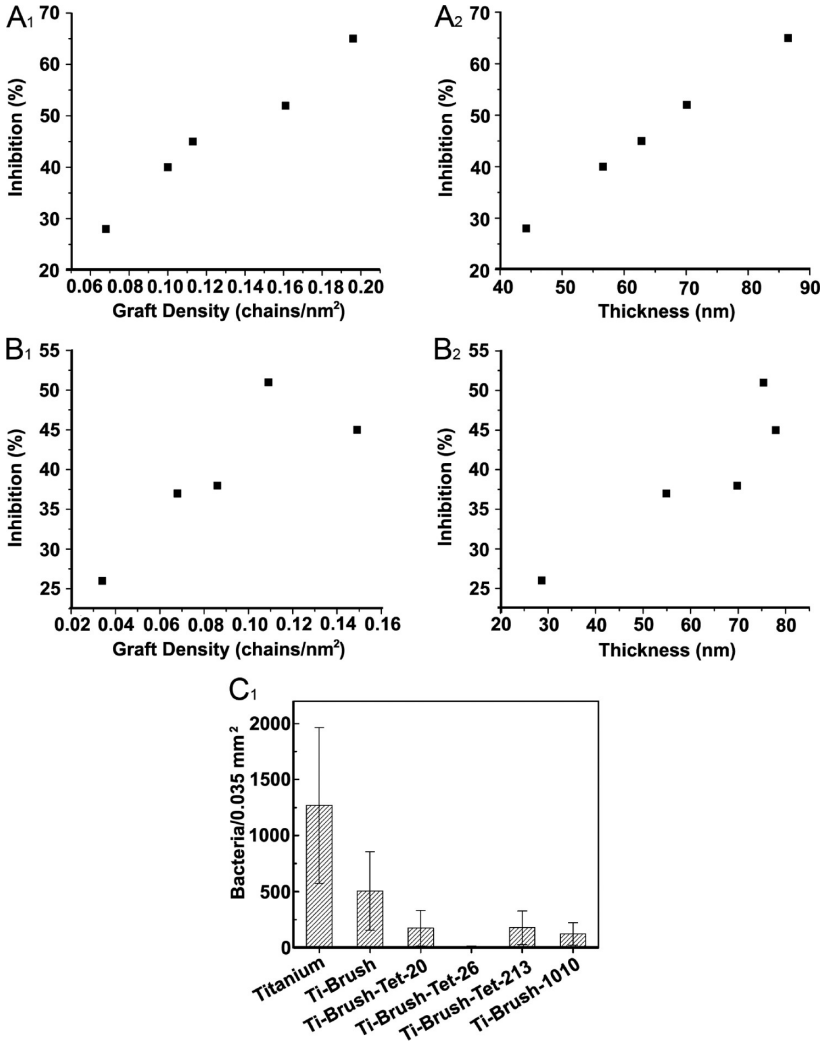

Figure 10. Effects of grafting density and thickness of the peptide grafted brush on bacterial growth inhibition. The compositions (DMA/APMA) 5/1 (A_1+A_2) and 4/1 (B_1+B_2) are shown. The error estimated for % inhibition of luminescence is ±5. (C_1) Comparison of biofilm formation on the AMPs conjugated copolymer brushes on titanium surface. Reprinted with permission from refs 67 and68. Copyright 2011 American Chemical Society and 2011 Elsevier.

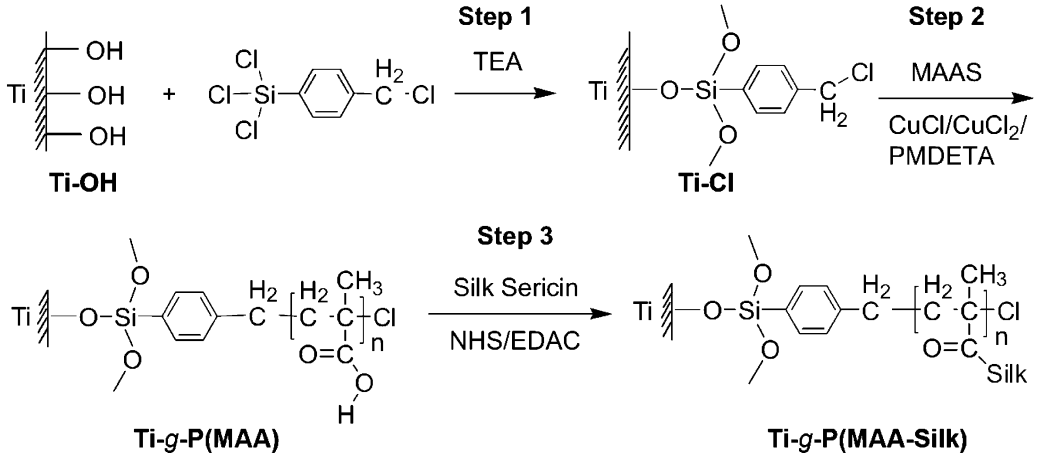

Figure 11. Schematic diagram illustrating the process of silanization of the Ti−OH surface to prepare the Ti−Cl surface, surface-initiated ATRP of MAAS chains from the Ti−Cl surface, and immobilization of silk sericin on the Ti-polymer hybrid [TEA, triethylamine; PMDETA, N, N, N′, N″, N″-pentamethyldiethylenetriamine; MAAS, methacrylic acid sodium salt; EDAC, 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide hydrochloride; NHS, *N*-hydroxysuccinimide]. Reprinted with permission from ref 17. Copyright 2008 Elsevier.

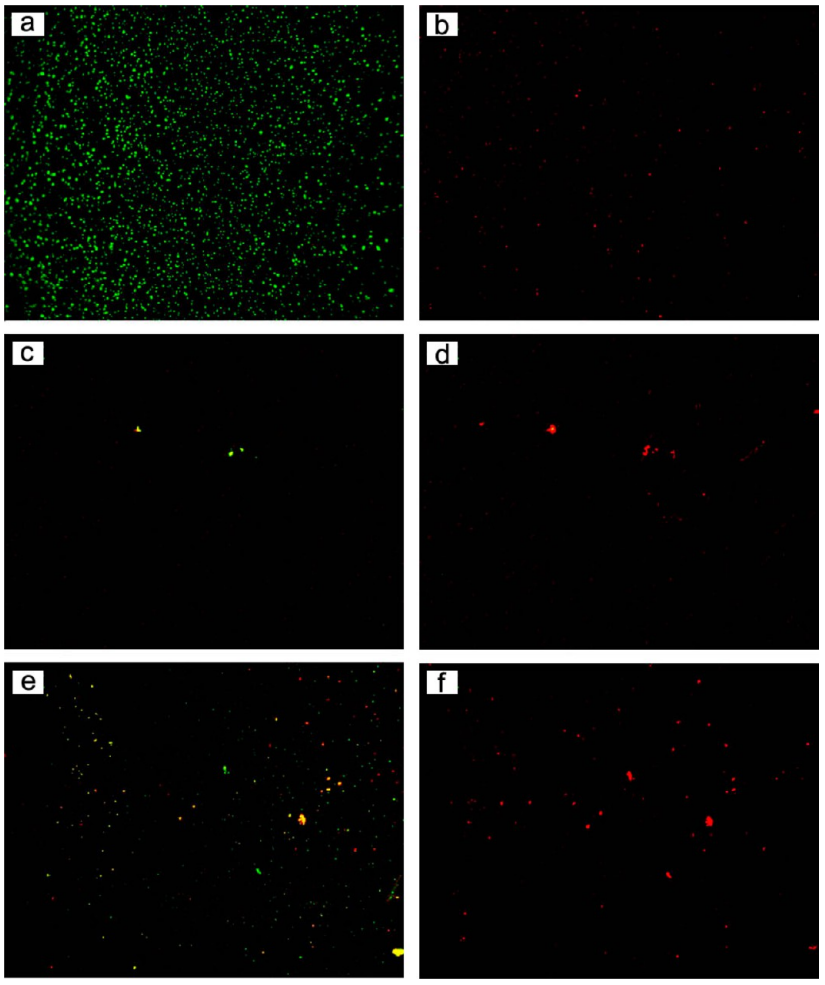

Figure 12. Fluorescence microscopy images of (a, b) Pristine Ti, (c, d) Ti-*g*-P(MAA), and (e) and (f) Ti-*g*-P(MAA-Silk) surface after exposure to a suspension of *S. aureus* (1×10^7 cells/mL) for 5 h. Reprinted with permission from ref 17. Copyright 2008 Elsevier.

cells and platelet adhesion and activation is insignificant thus boding well for applications in vivo.[68]

The antimicrobial activity of AMPs can also be influenced by the properties of the intermediate polymeric complex (also called spacer), including the length, flexibility and kind of spacer between the active sequences and solid matrices according to the precise mechanism of the bactericidal action of AMPs.[144,150−153] In addition, the immobilization location influences the effect. Gabriel et al. have observed that the length of the spacer must be rather long and flexible so that there is lateral diffusion of the peptide molecules in the lipid double layer of the microbial membrane.[144] This theory has been confirmed circuitously by the loss of bactericidal functions on randomly immobilized LL37 and that only the peptide properly bound to the PEGylated Ti surface is capable of killing *E. coli* upon contact.[144] Another conclusion that N-terminal conjugation permits appropriate parallel orientation of the peptide helices has been obtained, but subsequent results indicate that C-terminal immobilization of the peptide has similar effects. For example, Bagheri and co-workers have observed that C-terminal immobilization of the KLAL peptide at a concentration between 0.1 and 0.2 mM and 0.6 and 0.8 mM is sufficient to impose bactericidal effects toward *B. subtilis* and *E. coli*.[152] In addition, by estimating the influence of the parameters of fixation on the activity spectrum, a conclusion can be obtained. The length of the spacer and amount of surface peptide are critical parameters whereas the chain position of the linkage is another factor.[152] This conclusion seems to be inconsistent with that of Gabriel that random immobilization of LL37 reduces the antibacterial activity and it may be also attributed to the difference in the experimental model and methods. These results indicate that it is not easy to control the activity of AMP tethering because of the various interlinked factors and complex interplay mechanism.

All in all, covalent immobilization of AMPs on metallic implants is a promising way to prevent bacterial infection but more studies on the antibacterial mechanism and efficiency are needed to broaden the application in this field.

4. ADHESION RESISTANT POLYMERIC NANOARCHITECTURES

Besides direct incorporation of biocides into the polymeric architectures, an important route is to build an adhesion resistant polymer layer on the metallic implant because initial adhesion of microorganisms plays a vital role in biofilm formation on biomaterials.[35] Other important factors include surface characteristics such as topography, hydrophilicity, charge, surface energy, and functional groups.[154]

Some hydrophilic polymers like poly(methacrylic acid) (PMAA), poly(ethylene glycol) (PEG) and their derivatives have intrinsic antiadhesive properties.[17,70,71] Modification of Ti-based implants by these polymers is a good means, but these

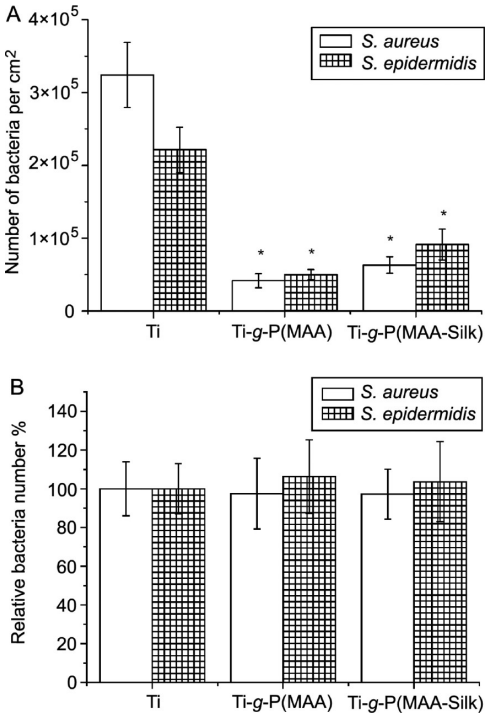

Figure 13. (A) Number of adherent of *S. aureus* and *S. epidermidis* cells per cm^2 on the pristine and functionalized Ti substrates. (*) Denotes significant differences ($p < 0.05$) compared with the pristine Ti. (B) Number of *S. aureus* and *S. epidermidis* viable cells in the suspension after contacting the pristine and functionalized Ti substrates for 5 h. The number of cells is expressed relative to that after contacting the pristine Ti. No significant statistical difference can be observed among the different surfaces. Reprinted with permission from ref 17. Copyright 2008 Elsevier.

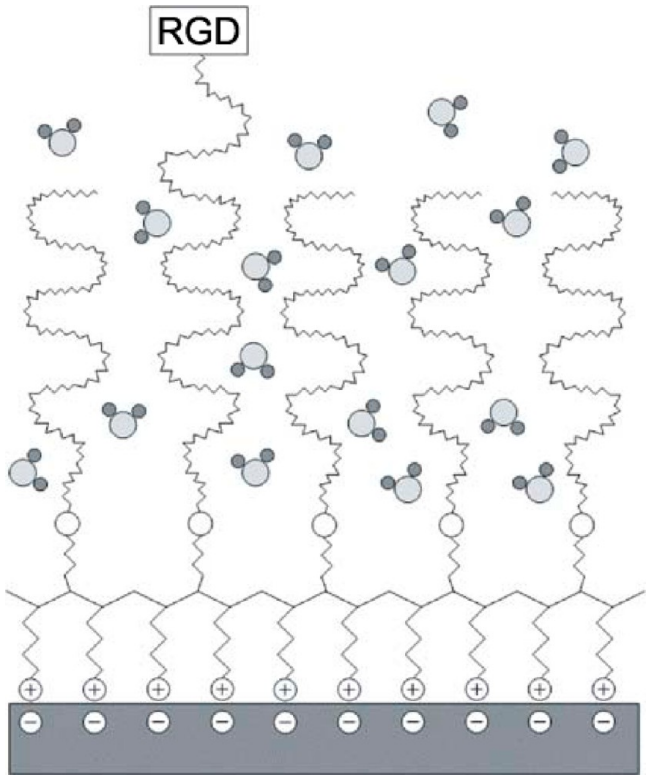

Figure 14. Schematic view of a PLL-*g*-PEG/PEG-peptide adlayer on the titanium oxide surface. The positively charged PLL backbone is attached to the negatively charged titanium oxide (TiO_2) layer on the titanium metallic substrate via electrostatic interaction. The graft PEG chains are hydrated (represented by the H_2O molecules between the PEG chains) and extend into the aqueous environment. Reprinted with permission from ref 159. Copyright 2003 Elsevier.

hydrophilic surfaces also inhibit mammalian cell adhesion.[37,155−157] In order to utilize the surfaces to balance between bacteria killing and osteoblasts compatibility, further modification is required. It can be realized by anchoring the bioactive molecules such as growth factors, RGD peptide, and silk sericin.[17,71,158,159] Normally immobilization of bioactive molecules cannot affect the antibacterial efficacy but it can retain the ability of promoting osteoblast functions on the polymeric architectures.

Generally, the adhesion resistant polymeric architecture is fabricated by two means, namely covalent bond interaction and noncovalent interaction. In the first way, the antiadhesion polymer is immobilized on the substrate by covalent bond interaction. For example, PMAA brushes with or without silk sericin have been fabricated on titanium using ATRP by Zhang et al. as schematically illustrated in Figure 11.[17] As shown in Figures 12 and 13A, the grafted Ti plates by ATRP, designated as Ti-*g*-P(MAA) and Ti-*g*-P(MAA-Silk), possess obvious antibacterial capability. Furthermore, the quantity of attached *S. aureus* and *S. epidermidis* on the ATRP modified samples decrease substantially compared to the untreated Ti. Meanwhile, there is no statistically significant difference in the viable cell numbers for both *S. aureus* and *S. epidermidis* in the suspensions after exposure ($p > 0.05$) (Figure 13). Therefore, the bacteria reduction may be ascribed to the antiadhesive properties of the PMAA modified surfaces.

Another way is to anchor the antiadhesion polymer onto the substrate via a noncovalent connection. For example, Ungureanu et al. have produced a hybrid coating of polypyrrole/Poly(ethylene glycol) (PPy/PEG) on Ti with various PEG concentrations by electrodeposition.[70] The antibacterial efficiency increases from 67 to 88% when the PEG concentration is increased from 0 to 2%. The hybrid coating with 2% PEG concentration possesses hydrophilic properties and smaller roughness compared to those with PEG concentrations of 0, 0.5, and 4%. It can be explained according to the interaction mechanism between biomaterials and bacteria.[70,160]

Because of the surface charges and reduction in the Lifshitz -van der Waals attraction, long PEG chains are more effective in resisting bacteria adhesion as well as protein adsorption. It is attributed to the steric barrier, excluded volume effects, and osmotic repulsion exerted by the highly hydrated and flexible PEG chains.[71,161] As shown in Figure 14, poly(L-lysine)-grafted-poly(ethylene glycol) (PLL-*g*-PEG) brushes with/without RGD peptide can be fixed on negatively charged titanium dioxide by a simple dipping process in an aqueous polymeric solution.[71,159] The copolymers assemble spontaneously from the aqueous solution and attach to different surfaces through electrostatic interaction, for instance, smooth and rough surfaces as well as flat and 3D objects with a complex shape.[71] In comparison with the untreated titanium, the PLL-*g*-PEG and PLL-*g*-PEG/PEG-RGD modified titanium samples have 89−93% and 69% less adherent viable *S. aureus*, respectively.

However, it is difficult to produce a zero-defect polymeric architecture to completely inhibit bacterial adhesion on the biopassive surface. Consequently, initial adhesion of microorganisms

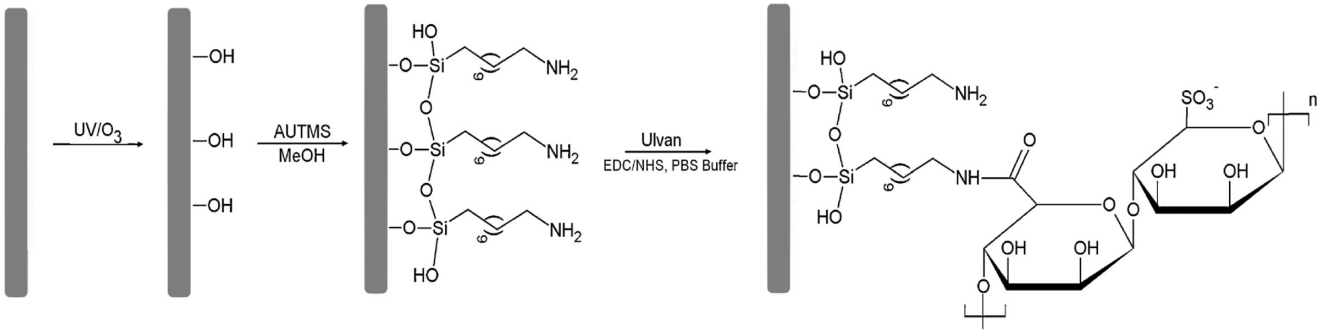

Figure 15. Schematic showing ulvan immobilization on titanium. Reprinted with permission from ref 72. Copyright 2013 Elsevier.

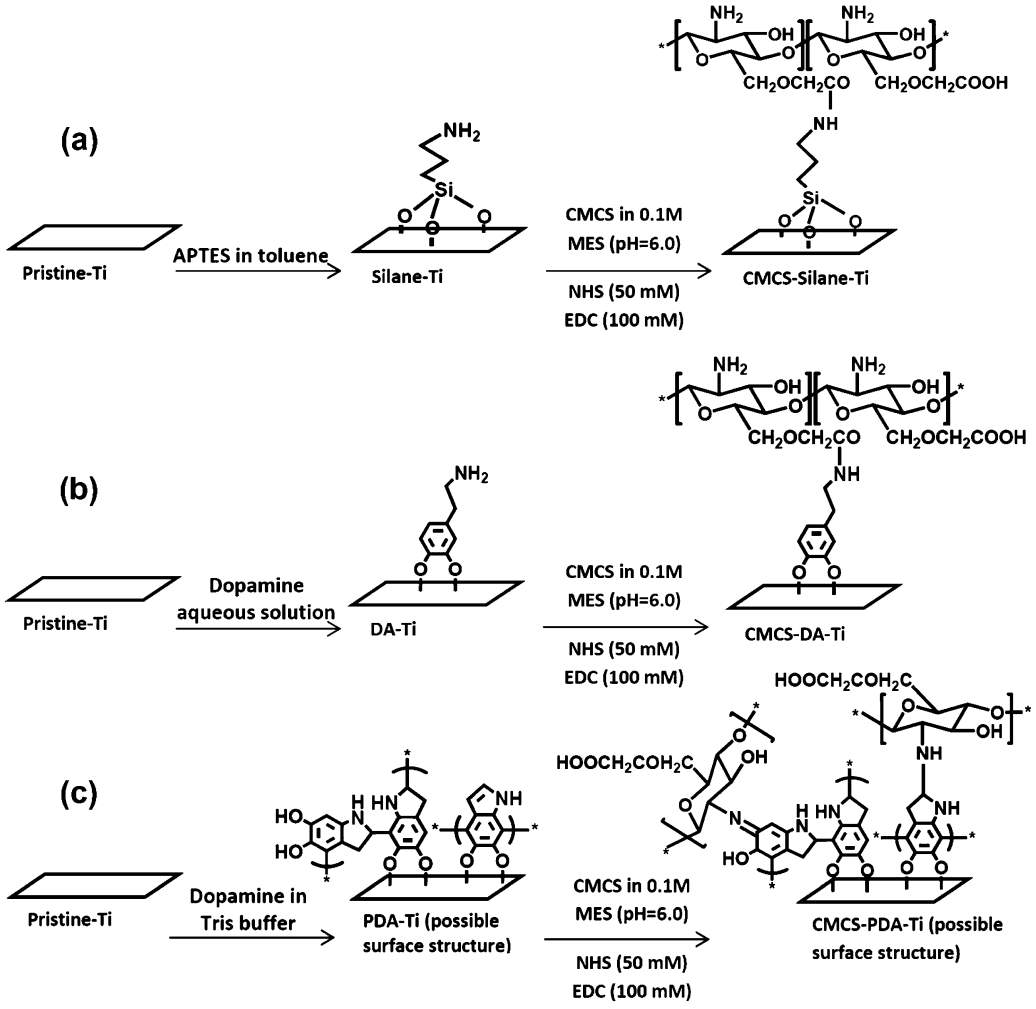

Figure 16. Schematic diagram illustrating grafting of CMCS on Ti using (a) silane, (b) DA, and (c) PDA anchors. Reprinted with permission from ref 77. Copyright 2013 Elsevier.

may occur causing implant failure. Moreover, it is difficult to assess the efficacy of the antiadhesive polymeric architectures accurately under clinical conditions in vivo.

5. ANTIBACTERIAL POLYSACCHARIDE NANOARCHITECTURES

Polysaccharides, a sort of bioactive macromolecules, are found in plants,[162] microorganisms,[163] and animals.[164] Some polysaccharides such as chitosan (CH), hyaluronic acid (HA), and their derivatives have good biological characteristics such as biocompatibility,[76] biodegradability to nontoxic products,[165] osteoblast function-enhancing nature, and antimicrobial properties.[37,72,166,167] With regard to antibacterial properties, ulvan, a non biocidal algal polysaccharide, has been shown to restrict bacterial adhesion,[72] whereas chitosan (CH) can suppress the growth of bacteria and even induce inactivation. The effectiveness is related to the molecular weight, concentration, pH, and so on.[37,73,166,168]

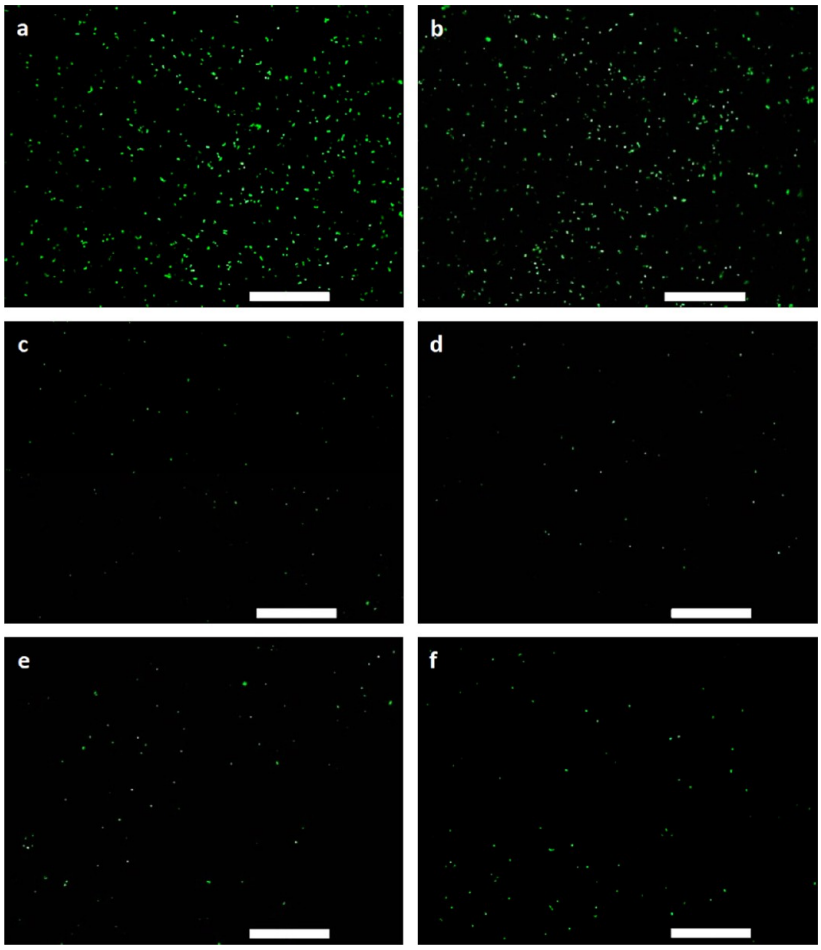

Figure 17. Fluorescence microscopy images of: (a) Pristine-Ti, (b) DA-Ti, (c) CMCS-DA-Ti, (d) CMCS-DA-Ti-ET, (e) CMCS-DA-Ti-AC, and (f) CMCS-DA-Ti-PBS after exposure to a PBS suspension of S. epidermidis of 1×10^7 cells/mL for 4 h. (a–f) are acquired using a green filter. Scale bar = 50 lm. Reprinted with permission from ref 77. Copyright 2013 Elsevier.

5.1. Antiadhesive Polysaccharides. Bacterial infection and inflammation can be effectively prevented by fixing polysaccharides on Ti-based implants.[72,169−172] The antibacterial action on the modified surface can be categorized in two modes, antiadhesion and growth inhibition of bacteria, as determined by the properties of the polysaccharides. For example, Gadenne et al. have prepared an adhesion-resistant surface by covalent immobilization of ulvans polysaccharides on titanium using a self-assembled aminoundecyltrimethoxysilane (AUTMS) monolayer as an intermediate layer (shown in Figure 15).[72] The efficiency of the modified surface against colonization of *P. aeruginosa* and *S. epidermidis* can reach 96% with reduction of initial adhesion, and it can be ascribed to the antiadhesive effect of the grafted ulvans.[72]

5.2. Antibacterial Polysaccharides. Positively charged surfaces benefit bacterial adhesion compared to uncharged and negatively charged surfaces.[169] This is the reason why hyaluronic acid (HA) has good antibacterial properties. The large number of carboxyl groups generates a large concentration of acidic and negative charges.[170] The combination of HA and CH is considered a good way to construct a polymeric architecture on Ti-based implants to achieve synergistic antibacterial effects. Recently, Chua et al. have functionalized Ti implants with RGD grafted HA/CH by layer-by-layer (LbL) electrostatic deposition.[37,171] The number of bacteria attached to the HA/CH functionalized Ti substrate is less than that on the pristine Ti by about 80% due to the synergistic effects of the bactericidal nature of CH and antiadhesion of HA.[37,172] At the same time, the inherent antibacterial performance of the HA/CH modified substrate is not altered by the RGD moieties that can enhance mammalian cell attachment, proliferation, and ALP activity.[15,37,173] It has been shown that incorporation of CH nanospheres with drug molecules into the mineralized collagen coatings enhances the bioactivity and antibacterial properties of the Ti implants.[12] Other coatings such as hydroxyapatite/chitosan composite coatings, silica xerogel/chitosan hybrid coatings, and BMP2-encapsulated chitosan coatings also have these effects in principle, but the antibacterial activity of these coatings is still unclear.[174−176]

Compared to CH, its derivatives have better antibacterial performance by introducing various types of side chains, for example, alkynyl chitosan,[177] chitosan-lauric acid (CH-LA),[76,178] chitosan-atorvastatin,[179] hydroxypropyltrimethylammonium chloride chitosan (HACC),[180] and *O*-carboxymethylated chitosan (*O*-CMCS),[181] whereas the primary biological properties are maintained or even enhanced. The effects of these side chains on the biocompatibility of CH are few or positive. For example, O-CMCS promotes proliferation of normal skin fibroblasts and osteogenesis and does not show cytotoxicity, while HACC is noncytoxic to L-929 cells exhibiting comparable biocompatibility as CH.[180−182] These derivatives can be fixed on titanium through a coupler such as

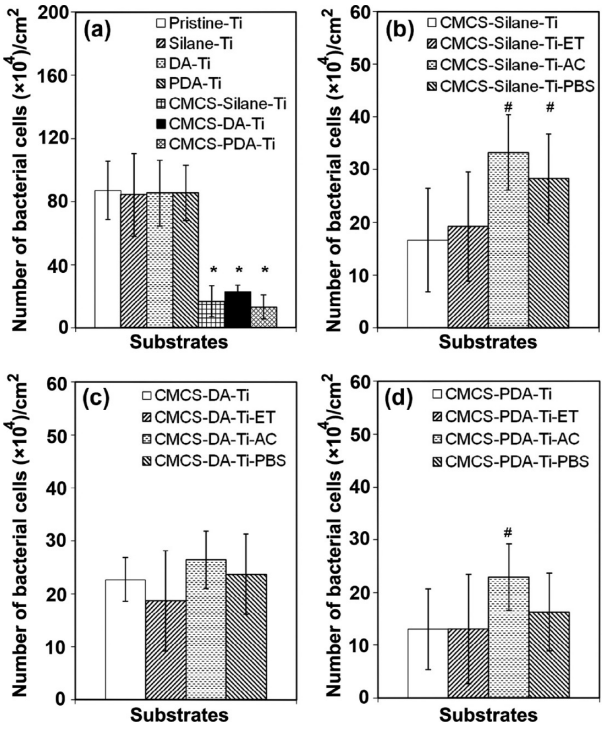

Figure 18. Quantities of adherent S. epidermidis per cm^2 on different substrates after exposure to the bacterial suspension in PBS (1×10^7 cells/mL) for 4 h. (*) denotes significant difference ($P < 0.05$) compared to Pristine-Ti in (a), ($^{\#}$) denotes significant difference ($P < 0.05$) compared toh CMCS-Silane-Ti or CMCS-PDA-Ti in (b) and (d), respectively. Reprinted with permission from ref 77. Copyright 2013 Elsevier.

dopamine and silane coupling agent.[76,179,183−186] For example, titanium grafted with hydroxypropyltrimethylammonium chloride chitosan (HACC) by APTES has enhanced antibacterial property.[183,184] The CH-LA conjugated with different content of LA is fixed on the titanium surface by the polydopamine film as the intermediate layer.[76] The Ti-PDA-CH-2.5%LA still has the highest antibacterial effects of 93.3% against P. eruginosa and 95.6% against S. aureus after culturing for 7 days. In addition, the functionalized polymeric architecture improves proliferation of osteoblasts and simultaneously inhibits bacterial adhesion because of the excellent properties of PDA and CH-LA.[76,178,187] *O*-carboxymethylated chitosan (*O*-CMCS) has been found to have higher antibacterial activity and the ability of conjugation with bioactive molecules due to the COOH groups.[185,186] Immobilization of *O*-CMCS on Ti through the SI-ATRP of dopamine can reduce the number of adherent *S. aureus* and *S. epidermidis* by more than 75% compared to the pristine Ti.[155,188]

5.3. Stability of Antibacterial Coatings. In the antibacterial architecture, the stability of the polymeric coating is particularly important in order to maintain long-term antibacterial effects on the implants. Zheng et al. have assessed the stability of antibacterial coatings on titanium anchored with different agents such as (3-aminopropyl) triethoxysilane (Silane), dopamine (DA), and polydopamine (PDA), and the grafting process is schematically shown in Figure 16.[77] The stability can be evaluated by estimating the change of the coatings after treatment with 70% ethanol, autoclaving, phosphate buffered saline (PBS). The results are shown in Figures 17 and 18.[77] The antibacterial property of CMCS-Silane-Ti is influenced slightly after autoclaving and prolonged immersion in PBS but not affected after treatment in 70% ethanol.[77] Among the three modes, only autoclaving can slightly decrease the antibacterial property of CMCS-PDA-Ti,[69,77] but the antibacterial ability of CMCS-DA-Ti is not altered.[77] It can be concluded that DA anchoring is a good way to construct functionalized polymeric architectures on Ti-based implants.

6. CONCLUSION AND OUTLOOK

Bacterial infection often leads to failure of Ti-based implants because of the formation of biofilms on the surface in the initial stage. Recent research and development pertaining to polymeric nanoarchitectures on Ti-based implants are reviewed with the focus on antibacterial applications. Grafting of polymeric nanoarchitectures endows the metallic implants with enhanced self-antibacterial capability. The materials have excellent adaptability and can deliver different kinds of antibacterial agents to ensure stable release in the biological environment. The polymeric nanoarchitectures on Ti-based implants can be categorized into three types according to the antibacterial modes, namely bactericidal group, adhesion-resistant group, and synergistic copolymers. Almost all antibacterial agents from organic antibiotics to inorganic silver nanoparticles can be incorporated into these polymeric nanoarchitectures to introduce antibacterial effects. Similar to other fields, in order to improve the antibacterial effectiveness, further investigations are needed, for instance, comprehensive and mechanistic studies of the effects of the size and shape on the antimicrobial activity of silver nanoparticles and relevant cytotoxicity. Furthermore, considering the ability of bacteria to adapt in a living environment, the use of multiple approaches with more than one type of antibacterial agents and anti-adhesive compounds may be key to producing effective antibacterial polymeric nanoarchitectures. Besides the antibacterial activity, the stability and lifetime of the nanoarchitectures under physiological conditions are important for long-term antibacterial efficiency. Future studies should focus on the construction of polymeric nanoarchitectures to obtain the desired properties of the coupling layers. Although in vitro and in vivo experiments disclose that these polymeric nanoarchitectures have little effects on osseointegration, some problems such as excessive proliferation of osteoblasts, drug-resistant bacteria, and other uncertain effects on tissue and cells should be investigated systematically in order to expedite and broaden the application of functionalized polymers to metallic implants.

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Notes

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