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Combating Bacterial Colonization on Metals via Polymer Coatings: Relevance to Marine and Medical Applications

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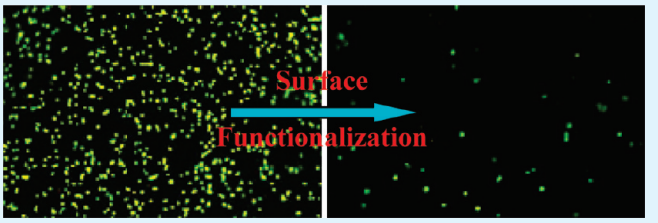
ABSTRACT: Metals are widely used in engineering as well as medical applications. However, their surfaces are easily colonized by bacteria that form biofilms. Among the numerous concerns with biofilm formation, biocorrosion is of particular importance in industry, because structural integrity may be compromised, leading to technical failures. In the food industry and medical field, biofilms also pose health risks. To inhibit bacterial colonization, the surfaces of metals can be coated with a polymeric layer which is antiadhesive and/or bactericidal. This article describes polymers that have these desired properties and the methodologies for immobilizing them on metal surfaces of relevance to the marine and medical fields. The focus is on polymer coatings that have a high degree of stability in aqueous medium and do not leach out. The efficacies of the different polymer coatings against bacteria commonly encountered in marine (*Desulfovibrio desulfuricans*) and medical applications (*Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli*) are demonstrated.



KEYWORDS: metals, bacterial adhesion, biofilms, biocorrosion, bactericidal polymers, implants

1. INTRODUCTION

Metals are widely used in daily engineering applications, and they are also an important class of biomaterial because of their superior mechanical strength and corrosion-resistant properties. However, bacteria are able to attach, grow, and form complex communities known as biofilms on metals as well as most other materials. Biofilms on metals present many industrial problems.[1] Accelerated deterioration of metals by processes directly or indirectly related to the activity of microbiological organisms, such as microorganism stimulated cathodic or anodic reaction or promoted establishment of electrolytic environments, is generally defined as microbiologically influenced corrosion (MIC) or biocorrosion.[2−4] Biocorrosion is extremely damaging to aquatic, maritime and process industries, as well as to the environment. It is estimated that at least 20% of corrosion can be attributed to biocorrosion and related activities at a direct cost of 30−50 billion annually.[5,6] Protection of structures against biocorrosion has therefore been the subject of extensive studies over the last few decades.[3] Biocorrosion is commonly caused by the actions of sulfate-reducing bacteria (SRB), a diverse group of bacteria that are active in an anaerobic environment.[7,8] SRB may coexist with other bacteria in the biofilm, and the biofilm-metal interactions at the molecular level are complex and have yet to be fully elucidated.[4] Besides the biocorrosion problem, biofilms also increase skin friction drag of ship hulls,[9] and support the attachment of the larvae of a broad range of sessile marine invertebrates[10] which adversely affect the ships' performance and increase fuel usage.

In addition to the economic losses that result from these industrial problems, biofilms pose hygiene risk in the food industry and increase the risk of infections in the medical field. The bacteria in a biofilm can be several orders of magnitude more resistant to antibacterial agents than their planktonic counterparts, and biofilms once formed are very difficult to eradicate.[11] It is apparent that the use of man-made materials and devices such as catheters, cardiac pacemakers, and prosthetic implants in the human body will continue to escalate, and as a result, a concomitant increase in biofilm-associated infection is also expected. It has been estimated that device-associated infections are responsible for about half of nosocomial infections.[12] The microorganisms that are most frequently associated with such infections are the staphylococci, particularly *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*), followed by *Pseudomonas aeruginosa* (*P. aeruginosa*) and other opportunistic bacteria in the environment.[13]

In view of the damaging consequences of biofilms, extensive efforts have been made to combat biofouling, bacterial adhesion, and biofilm formation on substrates. In aqueous media, fouling of hydrophobic surfaces occurs because of the adsorption of proteins and biomacromolecules, which results in the lowering of the high interfacial energy.[14] Surfaces that resist protein adsorption are usually hydrophilic, incorporate hydrogen-bond acceptors, but not donors, and exhibit overall charge neutrality.[15] Bacterial adhesion on surfaces is a complex process dependent on properties of the bacteria as well as the surfaces. The conventional model describing bacterial adhesion is based on the Derjaguin−Landau−Verwey−Overbeek (DLVO) theory but the molecular mechanism is mediated by cell appendages such as pili and flagella, which bind to abiotic surfaces as well as biomolecules on host cells, and are involved in biofilm formation.[16,17] Oxidizing substances such as chlorine and ozone

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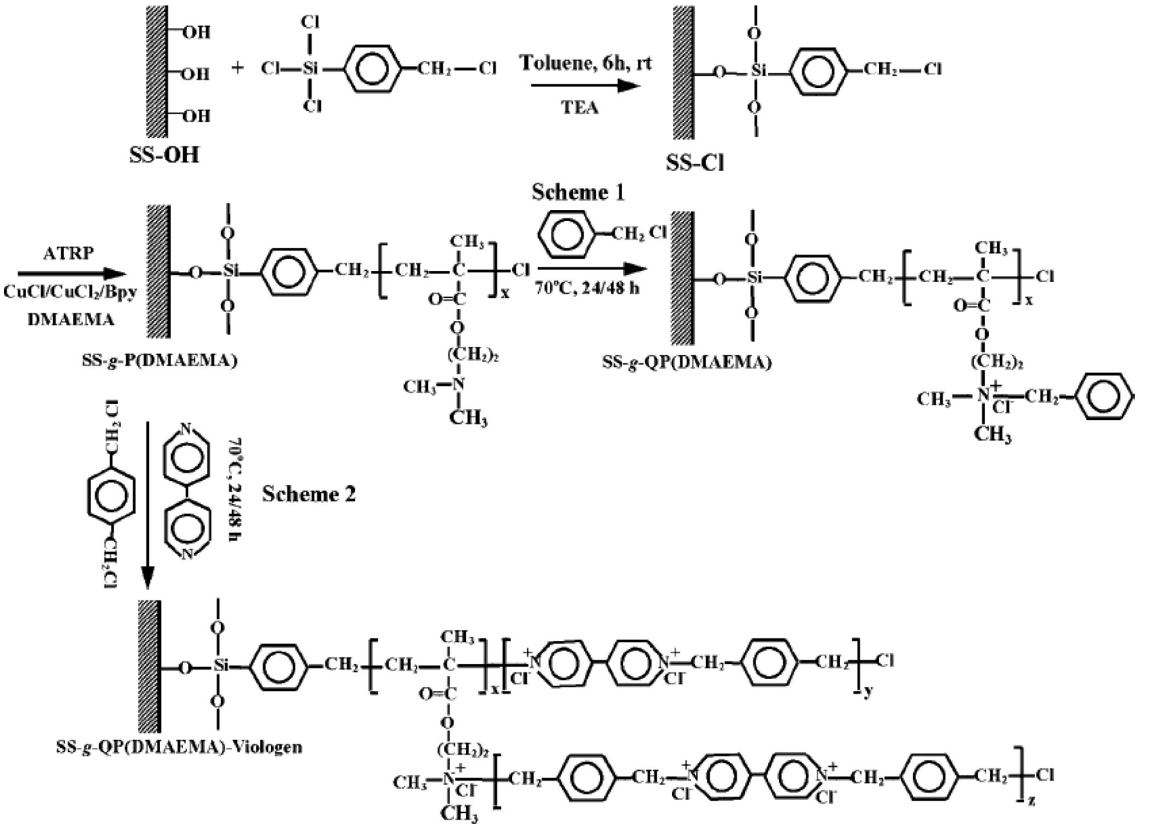

Figure 1. Schematic diagram illustrating the process of coupling of CTS to SS−OH surface (the SS-Cl surface), surface-initiated ATRP of DMAEMA from the SS-Cl surface (the SS-*g*-P(DMAEMA) surface), and subsequent functionalization, via quaternization, of the SS-*g*-P(DMAEMA) surfaces into antibacterial SS-*g*-QP(DMAEMA) and SS-*g*-QP(DMAEMA)-Viologen surfaces. Reprinted with permission from 68. Copyright 2010 Wiley Periodicals.

have been used to kill and/or remove biofilm organisms from water systems.[1,18] Biocides may also be combined with mechanical scrubbing or high pressure cleaning to remove the biofilm. A major disadvantage of applying biocides is that they subsequently end up in the environment, where they may cause adverse effects. For example, tributyltin-based paint had been widely applied to prevent biofouling of ship hulls, but since it is an endocrine disruptor and a persistent organic pollutant, it has been globally banned by the International Maritime Organization since January 2008.[19] In medical applications, devices such as catheters have been coated with silver[20−22] or antibiotics.[23−25] However, the use of medical devices containing silver must be undertaken with caution since silver has demonstrated toxicity to mammalian cells,[26,27] and the accumulation of silver, a heavy metal, in the environment may give rise to potential problems. Furthermore, the wide and uncontrolled use of silver products may result in more bacteria developing resistance,[28] and the clinical effectiveness of silver-coated catheters is controversial.[29] There is also some concern that widespread use of antibiotics may have contributed to the emergence of antimicrobial resistance.[30,31]

In both marine and biomedical applications, metals play a very important role, and similar problems of microbial adhesion and biofilm formation on the metal surfaces are encountered. These surface phenomena have detrimental consequences in both applications. Because removal of established biofilms cannot be readily accomplished, there is great motivation to develop preventative strategies. This review article describes methodologies developed in recent years for modifying the surfaces of metals to make them resistant to bacterial colonization in marine and medical applications. These techniques centered on the use of polymers that are antiadhesive and/or bactericidal. Because the requirements and constraints in marine and medical applications differ, the polymer coatings are correspondingly tailored to meet the specific challenges in each area as described in the sections below.

2. MICROBIOLOGICALLY INFLUENCED CORROSION (MIC) OF METALS AND ITS ENVIRONMENTALLY BENIGN REMEDIATION

2.1. Strategies for Combating Biocorrosion. Current approaches to combat biocorrosion include (i) the use of oxidizing (e.g., chlorine, bromine, ozone) or nonoxidizing (e.g., formaldehyde, glutaradehyde, quaternary ammonium compounds, isothiazolines) biocides to reduce the numbers and types of organisms in a bulk medium, (ii) cathodic protection, (iii) beneficial application of bacterial biofilms, and (iv) application of protective coatings.[3,32−35] Biocides are much less effective against sessile organisms within biofilms as compared to their effectiveness against a planktonic population,[36] because of dramatically enhanced resistance of biofilms to antimicrobial agents.[37,38] Because of their inherent toxicity,[39] biocides could pose negative impacts on the environment, as well as inhibit the growth of nontargeted organisms. Moreover, biocide treatments cannot be easily extended to open flow systems. Cathodic protection was reported to be effective in inhibiting biocorrosion of stainless steel by aerobic bacteria,[40] However, it was found to have little effect on the adhesion of anaerobic bacteria, and is thus unable to

prevent the initiation of localized corrosion by sulfate-reducing bacteria (SRB). Corrosion inhibition, arising from the presence and activities of bacteria within biofilms, has been reported for a number of metals and alloys, including carbon and stainless steels,[41−46] aluminum,[47,48] and copper.[49] However, the nature and formation of biofilms cannot be predicted with certainty and metal binding by extracellular materials has been reported as a mechanism for both biocorrosion[50,51] and corrosion inhibition.[33,50−52]

Close to 90% of total corrosion prevention costs are spent on protective coatings,[53] and the use of protective coatings has also been a major strategy against biocorrosion since the 1980s. Organometallic (e.g., tributyltin (TBT)-based paints), inorganic (e.g., titanium oxides and zinc), and organic (e.g., polymers) coatings have been widely used to protect metals and alloys against biocorrosion.[54−60] Despite their effectiveness in the marine environment, TBT-based protective antifouling coatings have been completely phased out since 2008 because of their detrimental effect on nontargeted marine organisms.[19] Stainless steel coated with multilayers of titanium oxide/butoxide has been prepared via a layer-by-layer (LbL) sol−gel deposition process. The titanium oxide/butoxide-coated coupons exhibited the desirable resistance in the biocorrosion environment of *Desulfovibrio desulfuricans* (*D. desulfuricans*) under anaerobic conditions. The passivity of the titanium oxide/butoxide multilayer coating not only remained stable under the harsh environment, it was also enhanced because of the deposition of calcium and phosphorus compounds associated with apatite.[61]

2.2. Antimicrobial Polymer Coatings. Polymers traditionally used in protective coatings against biocorrosion include polyurethane, silicone, epoxy resins, coal-tar epoxy, polyvinyl chloride, polyimides, and fluorinated compounds.[58,59,62−65] Defects and microbial degradation of polymer coatings, however, can give rise to localized attacks. In the past decade, robust techniques for fabricating environmentally friendly, dense, and tightly bonded antimicrobial polymer coatings and brushes on metallic substrates have been developed,[66−73] and studies have begun to ascertain the protective effects of well-defined polymer coatings and brushes against biocorrosion. The present review aims at summarizing the effectiveness of various antimicrobial polymer coatings and brushes containing quaternary ammonium compounds and natural biocides. Quaternary ammonium cations form a class of compounds which are used as biocides and corrosion inhibitor. As biocides, quaternary ammonium cations act on the plasmic membranes of cells, disrupt the lipid bilayers, and cause the release of intracellular materials.[74] Surfactant properties of these compounds provide additional protection against the formation of polysaccharidic materials released during the process of bacteria colonization.[62] Polymers from vinyl monomers containing tertiary amino groups, such as two-dimethylaminoethyl methacrylate (DMAEMA) and 4-vinyl pyridine (4VP), have been extensively used in the synthesis of antimicrobial surfaces via surface-initiated graft polymerization.[66−70,75,76] As a water-soluble polyamine, not only has polyethyleneimine (PEI) been widely used for the preparation of antimicrobial coatings,[77,78] it has also been employed as a corrosion inhibitor for the protection of steel.[79,80]

Conventional approaches for the immobilization of antibacterial polymer on metal surfaces are either by coupling reactions or by classical free-radical graft polymerization.[66,67] Yuan et al. designed a covalently coupled thin layer of quaternized viologens on silanized copper surface to combat biocorrosion.[66] Not only did the quaternized viologens exhibited good bacterial inhibition

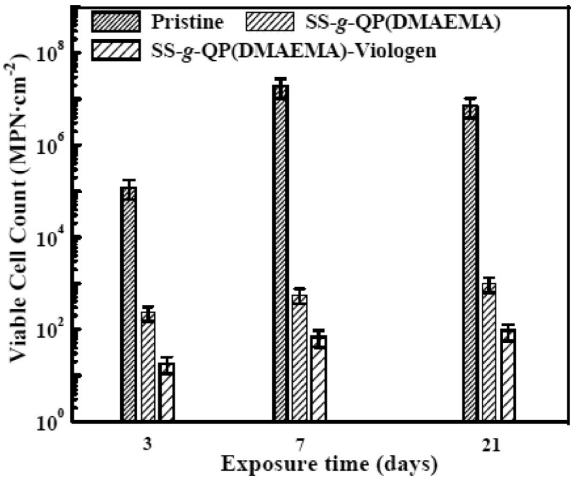

Figure 2. Number of viable *D. desulfuricans* cells adhered on the pristine, SS-*g*-QP(DMAEMA) and SS-*g*-QP(DMAEMA)-Viologen surfaces as a function of exposure time in the *D. desulfuricans* inoculated SSMB medium. Reprinted with permission from 68. Copyright 2010 Wiley Periodicals.

efficiency, they also markedly decreased the corrosion rate of metal specimens under the attack of microorganisms. However, both the antibacterial and corrosion inhibition abilities of the Cu surface deteriorated over long-term exposure because of the penetration of aggressive ions across the thin films. To enhance the antimicrobial ability and corrosion resistance of surface coatings, attempts were made to graft antibacterial poly(vinyl-*N*-hexylpyridinium) polymers on the metal (Cu) surfaces by a combination of surface-initiated free radical graft polymerization and coupling of alkyl halide.[67] The polymer brushes containing the pyridinium cations moieties exhibited high killing efficiency (>99%) of *D. desulfuricans*, and substantially enhanced the corrosion resistance of metal substrates with the corrosion inhibition efficiency remaining above 95% throughout the exposure period.

2.3. Antimicrobial Polymer Brushes from Controlled Radical Polymerization. Conventional free-radical graft polymerization from a substrate surface offers less control over the polydispersity, chain length, morphology, and density of functional polymer brushes. Surface-initiated controlled radical polymerization, such as atom transfer radical polymerization (ATRP), is an effective method to tether dense and narrowly dispersed polymer brushes of well-defined thickness, molecular architecture, and composition on solid substrates in a controlled manner.[81] Some vinyl monomers containing tertiary amino groups, such as DMAEMA and 4VP, can be polymerized or copolymerized via surface-initiated ATRP, followed by quaternization to induce the antibacterial activity. The original idea of grafting dense antibacterial polymer brushes of controlled molecular weight and low polydispersity on the stainless steel surface for combating biocorrosion involves surface-initiated ATRP of tertiary amine-containing DMAEMA directly the silane-functionalized stainless steel, as illustrated schematically in Figure 1.[68] Subsequent quaternization of the amino groups of DMAEMA polymer (P(DMAEMA)) brushes by benzyl halide produced the biocidal functionality on the polymer-modified stainless steel surface (SS-*g*-QP(DMAEMA) surface). Alternatively, the tertiary amino groups of PDMAEMA brushes were also quaternized by coupling with viologen to produce the SS-*g*-QP(DMAEMA)-Viologen surface (Figure 1). With the substantial increase in

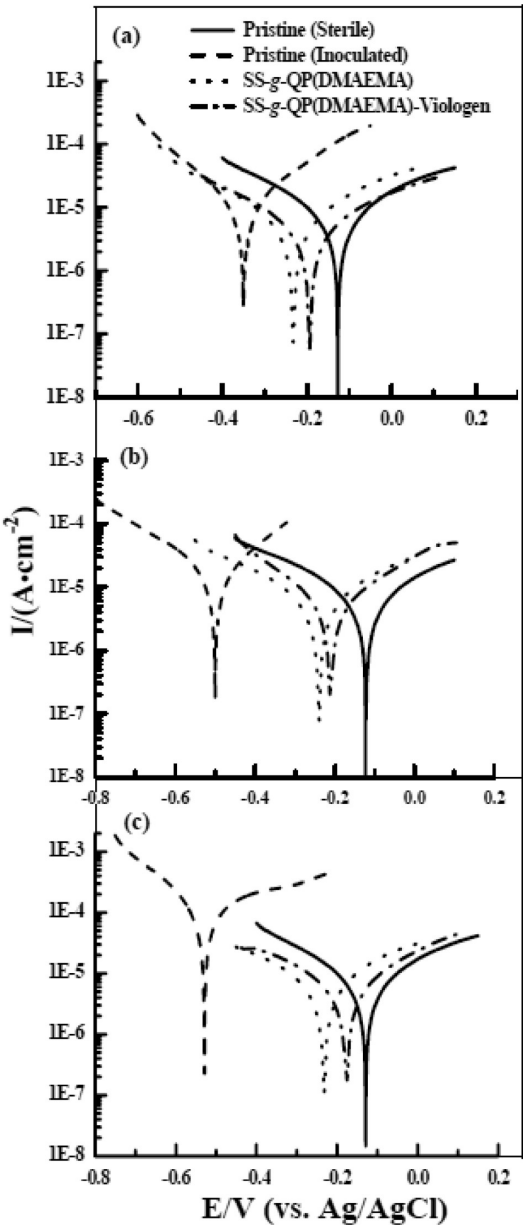

Figure 3. Tafel plots of the pristine, SS-*g*-QP(DMAEMA) and SS-*g*-QP (DMAEMA)-Viologen coupons in the *D. desulfuricans* inoculated SSMB medium, as well as the pristine coupons in the sterile SSMB medium for (a) 3, (b) 7, and (c) 21 days. Reprinted with permission from 68. Copyright 2010 Wiley Periodicals.

surface-bearing polycation concentration, the SS-*g*-QP(DMAEMA)-Viologen surface exhibited significantly enhanced antibacterial efficiency against *D. desulfuricans* in comparison to the SS-*g*-QP(DMAEMA) surface, although the latter also exhibited respectable bactericidal effect on *D. desulfuricans* (Figure 2).[68] At the same time, the electrochemical analysis revealed that the quaternized PDMAEMA brushes were effective in protecting the steel substrates from corrosion by *D. desulfuricans*, with the anticorrosion capability markedly enhanced by the coupled viologen layers. Figure 3a–c shows Tafel plots of the pristine (newly polished) and the surface-functionalized SS coupons after exposure in the sterile and *D. desulfuricans* inoculated simulated seawater-based modified Baar's (SSMB) medium for 3, 7, and 21 days, respectively.[68] The corrosion potential, E_{corr}, of the pristine coupons remains relatively constant with exposure time in sterile SSMB medium, whereas it undergoes an active shift with exposure time in the *D. desulfuricans* inoculated SSMB medium, and decreases rapidly to about −500 mV after 7 days of exposure. This phenomenon is usually attributed to the anodic dissolution process in terms of the mixed potential theory. E_{corr} of the SS-*g*-QP(DMAEMA) and the SS-*g*-QP(DMAEMA)-Viologen coupons undergoes a noble shift, relative to those of the pristine SS coupons, in the *D. desulfuricans* inoculated medium, indicating that the surface-grafted polymers possess the desired capability against the synergistic effects of aggressive Cl^-, biogenic S^{2-}, and bacterial cells of *D. desulfuricans*.

2.4. Inorganic–Organic Hybrid Coatings. To further improve the biocorrosion resistance of SS and to confer the bactericidal function on its surface for inhibiting bacterial adhesion and biofilm formation, well-defined inorganic–organic hybrid coatings, consisting of the inner compact titanium oxide multilayers and outer dense poly(vinyl-*N*-hexylpyridinium) brushes, have been successfully developed by a combined layer-by-layer (LbL) sol–gel deposition process and surface-initiated ATRP, as shown schematically in Figure 4.[69] Thus, nanostructured titanium oxide multilayers are first established on the SS surface via the LbL sol–gel deposition process. Subsequently, a trichlorosilane (CTS) coupling agent, containing the alkyl halide ATRP initiator, is immobilized on the titanium oxide-coated surface for surface-initiated ATRP of 4VP. The tertiary amino groups of the grafted poly (4-vinylpyridine), or P(4VP), brushes are finally quaternized with hexyl bromide to produce the biocidal quaternary ammonium compounds. Antibacterial assays and electrochemical studies revealed that in addition to its efficacy in inhibiting biofilm formation, the resulting inorganic–organic hybrid coatings significantly increased the resistance of the metal substrates to biocorrosion by *D. desulfuricans*.

Robust biocorrosion-resistant inorganic–organic coatings can also be introduced on metal surfaces via consecutive surface-initiated ATRP.[70] Well-defined inorganic–organic hybrid coatings, consisting of a polysilsesquioxane inner layer and quaternized P(DMAEMA) outer blocks, were prepared via successive surface-initiated ATRP of 3-(trimethoxysilyl) propyl methacrylate (TMSPMA) and DMAEMA from 2-(4-chlorosulfonylphenyl)-ethyl trichlorosilane) (CTCS)-coupled SS surface, as illustrated schematically in Figure 5.[70] The inner P(TMSPMA) block containing the reactive trimethoxysilyl groups can be readily hydrolyzed and condensed to form the cross-linked P(TMSPMA), or the polysilsesquioxane (CP(TMSPMA)) network, which provided a durable and resistant coating to electrolytes. Finally, the pendant tertiary amino groups of the P(DMAEMA) outer block were quaternized with alkyl halide to produce the SS-*g*-CP(TMSPMA)-*b*-QP(DMAEMA) surface with a high concentration of biocidal quaternary ammonium groups. The so-synthesized inorganic–organic hybrid coatings on the SS substrates exhibited good antibacterial and anticorrosion effects and inhibited biocorrosion induced by sulfate-reducing bacteria in seawater media, as revealed by antibacterial assay and electrochemical analyses. The bactericidal effect of the functionalized SS surface was investigated by comparing of the number of viable *D. desulfuricans* cells after being in contact with these substrate surfaces. Figure 6 shows the respective fluorescence microscopy images of the pristine SS and SS-*g*-CP(TMSPMA)-*b*-QP(DMAEMA) surfaces after exposure to the *D. desulfuricans* inoculated SSMB medium for 3 and 28 days.[70] Upon prolonging the exposure time

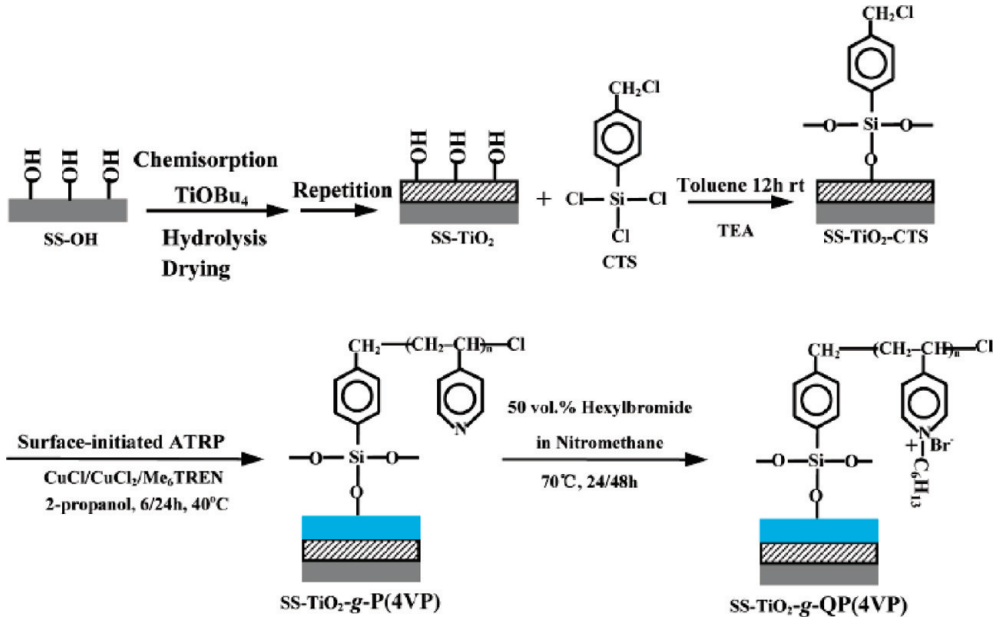

Figure 4. Schematic diagram illustrating the processes of formation of titanium oxide multilayers on the SS-OH surface via LbL sol−gel deposition (the SS-TiO_2 surface), coupling of CTS to the SS-TiO_2 surface (the SS-TiO_2-CTS surface), surface-initiated ATRP of 4VP to form the SS-TiO_2-*g*-P(4VP) surface, and subsequent quaternization of the SS-TiO_2-*g*-P(4VP) surface into the antibacterial SS-TiO_2-*g*-QP(4VP) surface. Reprinted with permission from 69. Copyright 2009 American Chemical Society.

to 28 days, the clusters of viable cells become dense and aggregate to form thick and heterogeneous biofilms on the pristine SS surface. In the case of the SS-*g*-CP(TMSPMA)-*b*-QP(DMAEMA) surface, there are only a few single viable cells and a high concentration of bacterial cells, large-sized colonies, and even patchy biofilms with red fluorescence bestrewed on the coupon surface, indicating that almost all bacterial cells are killed by the surface bearing a high concentration of quaternary ammonium (N^+) groups.

2.5. Electroactive Polymer Coatings. Arising from their intrinsic redox properties, electroactive polymers, such as polyaniline, polypyrrole, and polythiophene, have been proposed to serve as barriers and inhibitors, as well as to provide anodic protection and mediation of oxygen reduction, for aluminum, carbon steel, copper alloys and stainless steels.[82,83] A unique approach in inhibiting corrosion of mild steel (MS) and in rendering its surface biocidal for reducing bacterial adhesion, biofilm formation, and biocorrosion in seawater involved surface-initiated ATRP of glycidyl methacrylate from the MS surface-coupled trichlorosilane, containing the benzyl sulfonic chloride ATRP initiator, followed by thermal curing with electroactive polyaniline (PANI) and *N*-alkylation of PANI.[72] The MS surface functionalized by quaternized electroactive bilayer exhibits high inhibition efficiency (up to 95% reduction in corrosion current density from that of the pristine MS surface) against the attack of corrosive environment and significantly reduces bacteria adhesion and biocorrosion. Another approach to impart metal surfaces with enhanced resistance to corrosion, bacterial adhesion and biocorrosion involved oxidative graft polymerization of 2,2′-bithiophene from the copper surface with self-assembled 2,2′-bithiophene monolayer. Subsequent reduction of silver ions to silver nanoparticles (Ag NPs) on the surface gives rise to a homogeneous bithiophene polymer (PBT) film with densely coupled Ag NPs on the copper surface (Cu-*g*-PBT-Ag NP surface).[73] Arising from the chemical affinity of thiols for the noble and coinage metals, the copper surface functionalized with both PBT brushes and Ag NPs exhibits good stability. The immobilized Ag NPs significantly inhibit bacterial adhesion and enhance the bactericidal properties of the functionalized copper surface.

2.6. Bioinspired Coatings. Development of novel surfaces bioinspired by nature is expected to further enhance the environmental friendliness of the present nonreleasing antimicrobial surfaces and self-release surfaces.[84] Surface molecular architecture consisting of biomimetic anchors for biocompatible polymer brushes with covalently attached natural biocides has been explored. Surface-initiated ATRP of poly(ethylene glycol) monomethacrylate (PEGMA) from the SS surface-coupled catecholic L-3,4-dihydroxyphenylalanine (dopamine or DOPA) with terminal alkyl halide initiator was first carried out, followed by the immobilization of lysozyme at the chain ends of poly(ethylene glycol) branches of the grafted PEGMA polymer brushes.[85] The resulting lysozyme-coupled SS-*g*-P(PEGMA)-*c*-Lysozyme surface displayed high antifouling activity as compared to the pristine SS surface, as well as exhibited a high bactericidal efficiency toward both gram-positive *S. aureus* and gram-negative *Escherichia coli* (*E. coli*) bacteria in contact with the surface, as revealed by antibacterial assays. The environmentally benign approach to impart SS surfaces with antifouling and antibacterial functionalities is shown schematically in Figure 7.[85] More recently, in perhaps what is an ironic twist, the undesirable adhesive properties of barnacle cement in its native state have been employed beneficially on SS to serve as the initiator anchor for surface-initiated ATRP of 2-hydroxyethyl methacrylate (HEMA).[86] The hydroxyl groups of the HEMA polymer brushes were subsequently converted into carboxyl groups for coupling of chitosan to impart the SS surface with both antifouling and antibacterial properties. The bactericidal effects of chitosan are discussed below in conjunction with the inhibition of bacterial adhesion on metal implants.

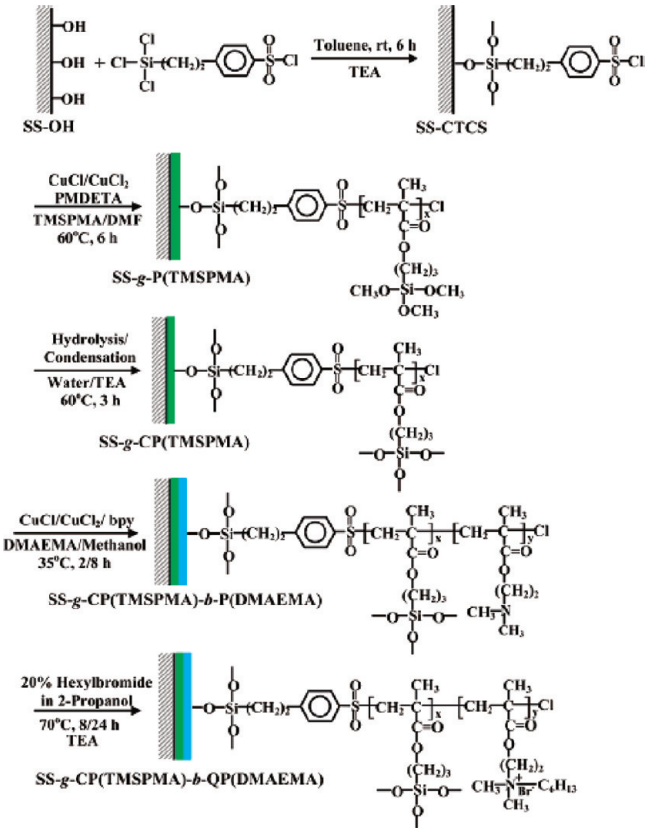

Figure 5. Schematic diagram illustrating the process of coupling CTCS to the SS-OH surface (the SS-CTCS surface), surface-initiated ATRP of TMSPMA from the SS-CTCS surface (the SS-*g*-P(TMSPMA) surface), interchain cross-linking of the trimethoxysilyl groups (-Si-$(OMe)_3$) after hydrolysis and subsequent condensation of the silanol groups (-Si-OH) to form the cross-linked polysilsesquioxane layer (the SS-*g*-CP(TMSPMA) surface), surface-initiated ATRP of DMAEMA from the SS-*g*-CP-(TMSPMA) surface to form the SS-*g*-CP(TMSPMA)-*b*-P(DMAEMA) surface, and subsequent N-alkylation to produce the quaternized and bactericidal SS-*g*-CP(TMSPMA)-*b*-QP(DMAEMA) surface. Reprinted with permission from 70. Copyright 2010 American Chemical Society.

3. INHIBITION OF BACTERIA ADHESION ON IMPLANTS WITHOUT COMPROMISING OSTEOBLAST FUNCTIONS

3.1. Inhibition of Bacterial Colonization on Implant Surfaces.

In the previous section, a number of techniques for conferring antibacterial properties on metals to inhibit MIC have been described. While similar techniques may be applied for coating medical implants with a polymeric layer, the critical constraint faced in such applications is that the antibacterial coating must not pose significant cytotoxic effects to mammalian cells or compromise the tissue integration process. One of the most commonly used metals for orthopedic implants is titanium and its alloys. When an implant is inserted into the body, there is competition between bacterial adhesion and tissue integration, which was described by Gristina as the "race for the surface".[87] The adherence of bacteria to the implant surface is recognized as the initial step toward possible implant infection. For an implant to be successful, tissue integration should occur before appreciable bacterial colonization because the tissue cells cannot displace these bacterial colonizers. A number of studies have shown that bacterial adhesion on surfaces can be inhibited by means of

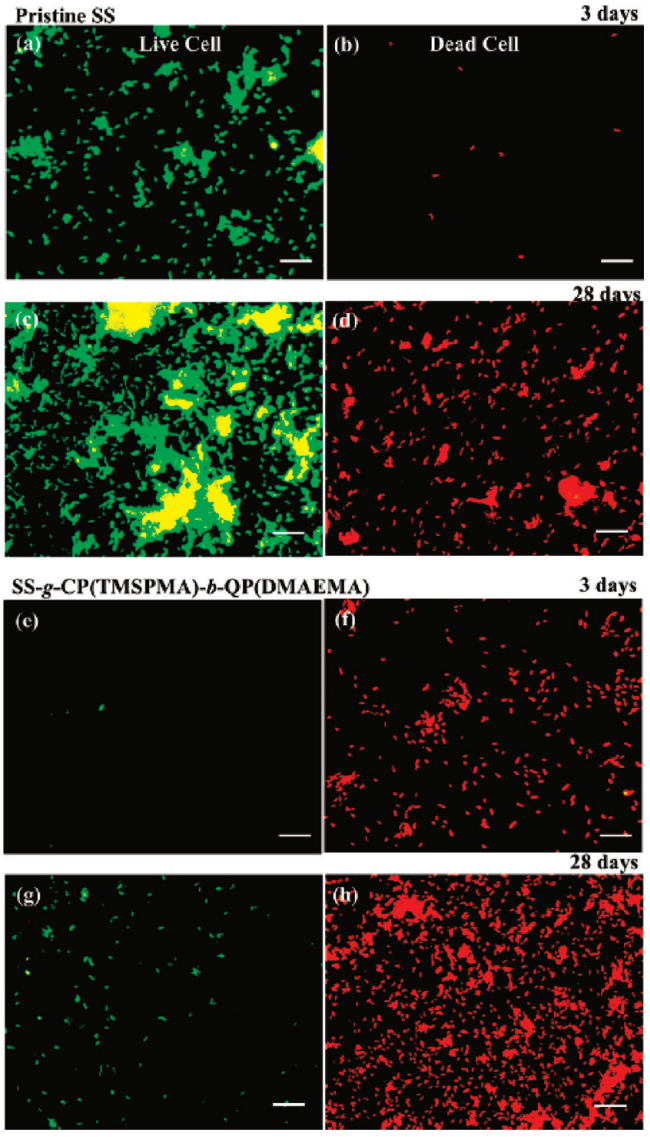

Figure 6. Representative fluorescence microscopy images of (a−d) the pristine SS and (e−h) the SS-*g*-CP(TMSPMA)-*b*-QP(DMAEMA) surfaces under the green filter (a,c,e,g) and the red filter (b,d,f,h) after exposure to the *D. desulfuricans* inoculated SSMB medium for 3 and 28 days. Scale bars = 50 μm. Reprinted with permission from 70. Copyright 2010 American Chemical Society.

hydrophilic polymeric brushes.[88−93] The most commonly employed polymers are based on poly(ethylene glycol) (PEG) or poly(ethylene oxide) (PEO). A coating of these highly hydrated polymer chains on a surface presents a large exclusion volume effect which inhibits protein and bacterial adhesion.[91] To coat titanium with PEG brushes, peptide sequences with specific affinity for titanium[94,95] were recently identified using a combinatorial phage display technique and applied as anchors for PEG chains. The peptide-conjugated PEG was deposited onto the selected surface through adsorptive mechanisms from dilute solution. The coated surface efficiently blocked the adsorption of fibronectin and significantly reduced the extent of *S. aureus* attachment and biofilm formation in vitro. In another method, a graft copolymer comprising a polycationic poly(L-lysine) (PLL) backbone and PEG side chains (PLL-*g*-PEG) is adsorbed

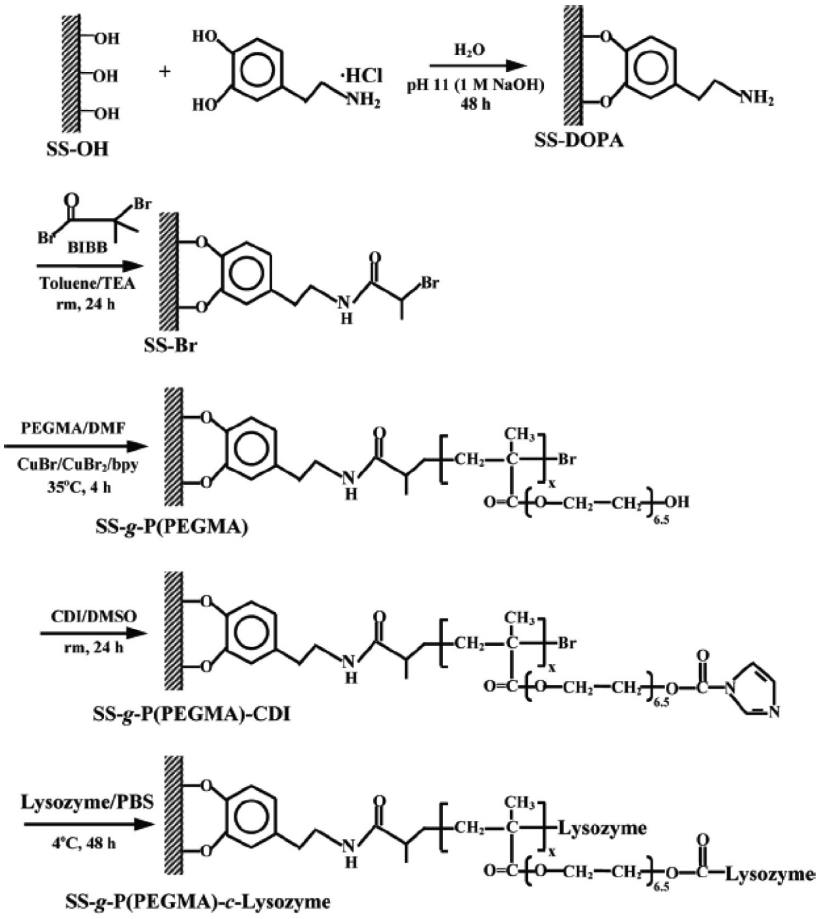

Figure 7. Schematic diagram illustrating the process of coupling dopamine (DOPA) to SS-OH surface to give the SS-DOPA surface, immobilization of alkyl bromide initiator via condensation reaction (the SS-Br surface), surface-initiated ATRP of PEGMA from the SS-Br surface (the SS-*g*-P(PEGMA) surface), activation of the hydroxyl side chain by 1,1′-carbonyldiimidazole (CDI) (the SS-g-P(PEGMA)-CDI surface) and subsequent immobilization of lysozyme to produce the SS-*g*-P(PEGMA)-*c*-Lysozyme surface. Reprinted with permission from 85. Copyright 2011 American Chemical Society.

through electrostatic interactions on negatively charged surfaces such as TiO_2 at physiological pH, rendering them highly protein and bacterial resistant.[96−98] Another class of nonbiofouling polymer brushes are the long-chain zwitterionic polymers. Jiang et al. have modified gold and glass surfaces with zwitterionic poly(sulfobetaine methacrylate) and poly(carboxybetaine methacrylate) to inhibit biofilm formation.[99,100]

A highly hydrophilic polymer coating on titanium can also be obtained via surface-initiated ATRP of methacrylic acid sodium salt (MAAS) using surface-immobilized trichlorosilane coupling agent as the ATRP initiator.[101] The surface modified with the poly(methacrylic acid) (P(MAA)) layer has a contact angle of 8° which is significantly lower than that of the pristine titanium surface (53°). The P(MAA) brushes behave as an antiadhesive polymer and the number of adherent *S. epidermidis* and *S. aureus* on the P(MAA)-modified titanium is about three to four times lower than on the pristine titanium. Surface-initiated ATRP of HEMA on titanium can be carried out in a similar manner to achieve an antibacterial surface.[102] Although the coating of hydrophilic polymers on the titanium surface is effective in inhibiting bacteria adhesion, it will also inhibit the attachment of osteoblasts on the titanium and subsequent tissue integration.[103−105] Thus, strategies, which confer selective biointeractivity by inhibiting bacterial attachment but promoting osteoblast functions, will be more desirable, and will be discussed in the next section.

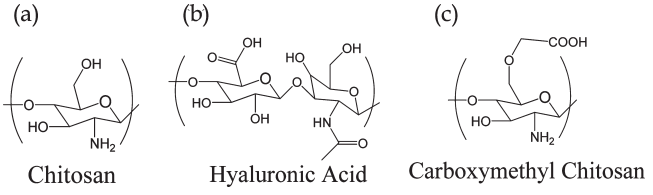

Figure 8. Structures of (a) chitosan, (b) hyaluronic acid, and (c) carboxymethyl chitosan.

Bactericidal coatings that are not cytotoxic to mammalian cells can also be used to inhibit bacterial colonization on the metal implant. In this respect, chitosan (Figure 8a) is a good candidate. Because of its cationic nature, chitosan interacts with the negatively charged bacterial cell wall and disrupts the cell membrane function leading to cell death[106,107] Furthermore, chitosan is also deemed one of the most promising biopolymers for tissue engineering and possible orthopedic applications since it enhances osteoblast functions.[106,108,109] Because chitosan is cationic, LbL electrostatic deposition has been employed to form stable multilayers of chitosan with the polyanionic hyaluronic acid (Figure 8b) on titanium.[105,110] LbL electrostatic deposition is a versatile and yet facile technique applicable for the functionalization of a variety of surfaces and geometries,[111,112] and thus

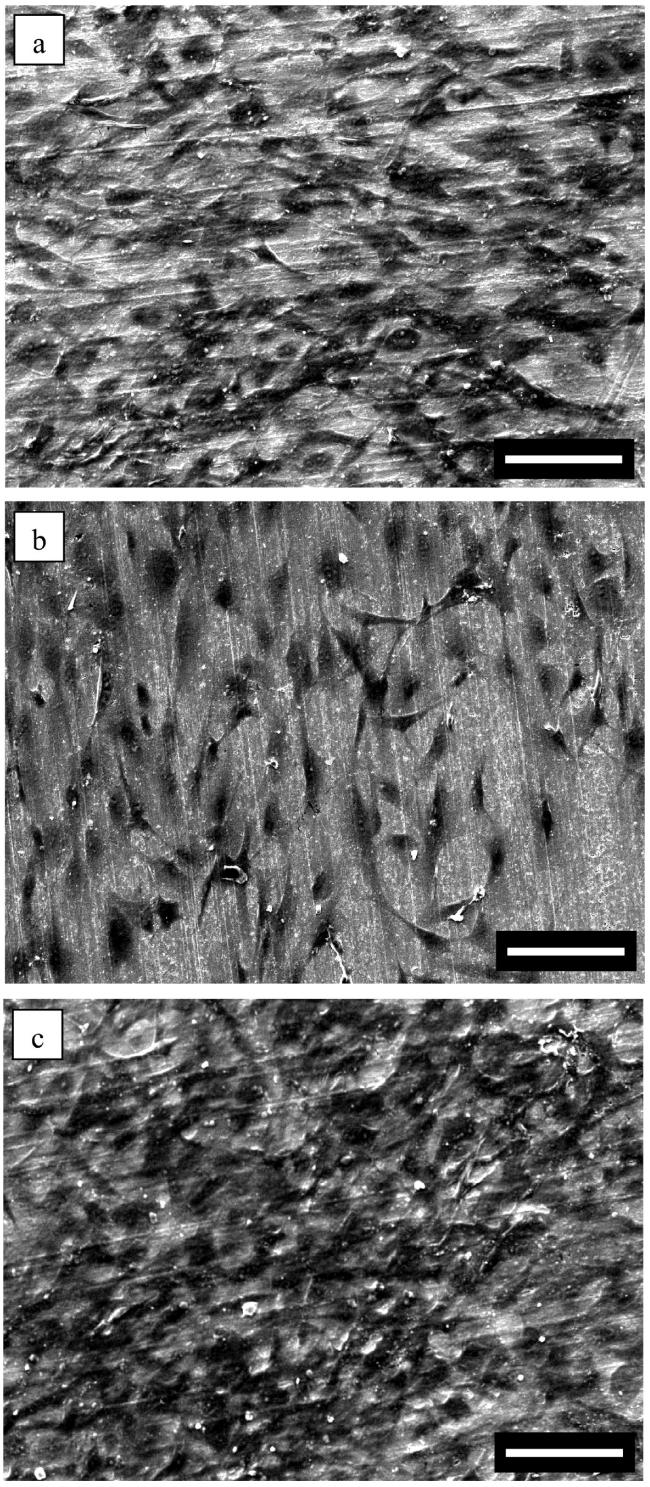

Figure 9. Osteoblast proliferation after 7 days on (a) pristine titanium, (b) titanium coated with five bilayers of hyaluronic acid and chitosan, and (c) titanium coated with five bilayers of hyaluronic acid and chitosan with conjugated RGD at a surface density of 14.5 pmol/cm^2. Scale bar = 100 μm. Reprinted with permission from 105. Copyright 2008 Elsevier.

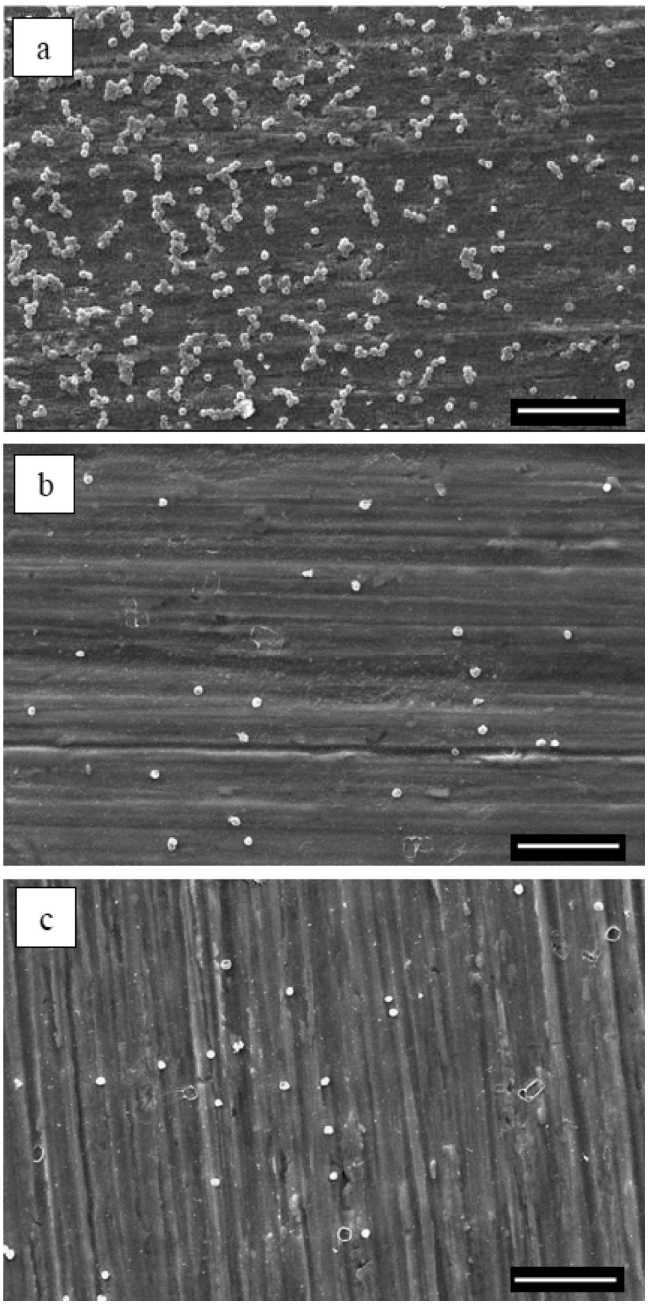

Figure 10. Scanning electron microscopy images of titanium surfaces after exposure to *S. aureus* suspension (1×10^6 cells/mL) in PBS for 4 h: (a) pristine titanium, (b) titanium coated with five bilayers of hyaluronic acid and chitosan, and (c) titanium coated with five bilayers of hyaluronic acid and chitosan with conjugated RGD at a surface density of 14.5 pmol/cm^2. Scale bar = 10 μm. Reprinted with permission from 105. Copyright 2008 Elsevier.

would be useful for implants. With 9−10 layers of chitosan and hyaluronic acid deposited on the titanium substrate, the number of adherent gram-negative *E. coli* and gram-positive *S. aureus* can be decreased by up to an order of magnitude. By cross-linking the chitosan and hyaluronic acid chains, the polyelectrolyte multilayers are highly stable, and the bacteria-resistant properties are preserved even after the functionalized substrates were aged for 21 days in phosphate buffered saline (PBS). Chitosan can also be grafted on metal surfaces which have been treated with dopamine[113] or 3-aminopropyltriethoxysilane.[114] These two linker molecules provide an effective way of functionalizing metal surfaces with amino groups. The interaction of titanium with dopamine is likely to take the form of a bidentate coordination

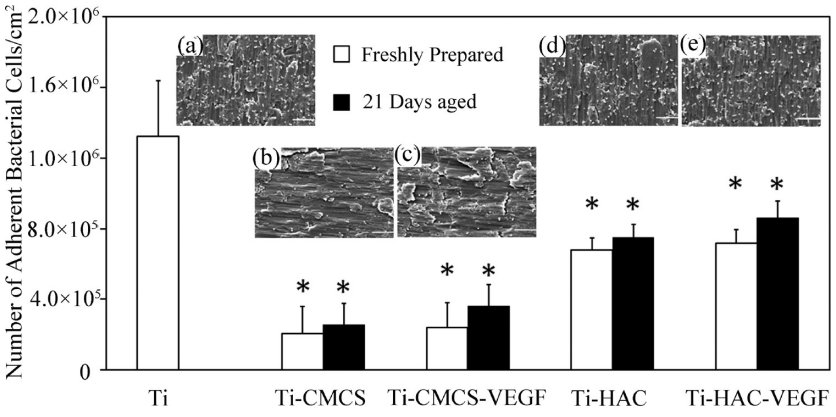

Figure 11. Number of adherent *S. aureus*/cm^2 on the pristine titanium (Ti), titanium modified with carboxymethyl chitosan (Ti-CMCS), carboxymethyl chitosan with conjugated VEGF (Ti-CMCS-VEGF), hyaluronic acid (Ti-HAC), and hyaluronic acid with conjugated VEGF (Ti-HAC-VEGF) substrates after exposure to bacterial suspension (5×10^7 cells/mL) in PBS for 4 h. * denotes significant differences ($P < 0.05$) compared with pristine Ti. Insets a−e show the corresponding scanning electron microscopy images of the surface of Ti, Ti-CMCS, Ti-CMCS-VEGF, Ti-HAC, and Ti-HAC-VEGF substrates, respectively, after the 4 h incubation period. Reprinted with permission from 123. Copyright 2010 Elsevier.

complex between the catechol oxygens of dopamine and a titanium atom at the native oxide surface[115] while 3-aminopropyltrieth-oxysilane reacts with the hydroxyl groups on the metal surface.[116] Glutaraldehyde was then employed to provide the reactive aldehyde groups for covalent bonding with either dopamine or silane and the chitosan molecule. Gentamicin can be loaded into the chitosan to provide additional antibacterial effect.[114]

3.2. Inhibition of Bacterial Colonization and Promotion of Osteoblast Functions. Although chitosan supports osteoblast functions, further enhancement is possible when combined with the RGD (Arg-Gly-Asp) motif which is present in a number of proteins including fibronectin and fibrinogen. RGD is known to interact specifically with cell surface integrin receptors, and it has been found that bacteria such as *P. aeruginosa*, *S. aureus* and *S. epidermidis* do not recognize the RGD motif.[117] PLL-g-PEG functionalized with RGD at the PEG side chain (PLL-*g*-PEG/PEG-RGD) was used to coat Ti which decreased bacterial adhesion by 69%. On the other hand, the PLL-*g*-PEG/PEG-RGD-coated surfaces are adhesive to cells such as fibroblasts, osteoblasts and endothelial cells.[98] RGD can be conjugated via its carboxylic acid groups to the amine groups of chitosan grafted on titanium using *N*-hydroxysuccinimide and 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide. A significant increase in osteoblast cell attachment, proliferation, and alkaline phosphatase activity was observed compared to the pristine titanium surface as well as the chitosan-grafted surface. The presence of RGD did not compromise the antibacterial efficacy of the chitosan graft layer to a significant extent.[113] RGD can be similarly conjugated to chitosan/hyaluronic acid multilayers deposited on titanium using carbodiimide chemistry.[105] The osteoblast proliferation (Figure 9) and alkaline phosphatase activity were increased by 100−200% over that of pristine Ti substrates and high antibacterial efficacy was retained (Figure 10). Silk sericin, which is made up of 18 amino acids, with aspartic acid and serine being the dominant components, has also been used to confer osteoblast adhesion, proliferation, and alkaline phosphatase activity on P(MAA)-modified titanium.[101] The P(MAA) chains retained their antibacterial property while acting as linkers for the coupling of sericin to their pendant carboxyl end groups via carbodiimide chemistry.

The strategy of using immobilized biomolecules to enhance osteoblast functions of antiadhesive surfaces can also be applied to signaling proteins such as bone morphogenetic proteins (BMPs). Direct introduction of BMP into the body is not desirable because of its relatively short half-life and diffusion from the site of its intended action which may lead to adverse effects on other tissues.[118,119] Among the members of the BMP family, BMP-2 has been demonstrated to be one of the most potentially effective osteoinductive substances for enhancing bone formation in vivo.[120] BMP-2 has been grafted onto the surfaces of titanium alloy substrates via an intermediate polymer layer of either oxidized dextran[121] or carboxymethyl chitosan (Figure 8c).[122] The intermediate layer was first immobilized on titanium using a dopamine linker. The oxidized dextran serves as an antiadhesive layer and conjugation with BMP-2 was carried out via a reductive amination reaction. Carboxymethyl chitosan has bactericidal properties, and BMP-2 was conjugated using carbodiimide chemistry. In the presence of the dextran layer on titanium, the adhesion of both bacteria as well as osteoblast was inhibited. However, when a sufficiently high density of BMP-2 is conjugated to the dextran layer, the osteoblast attachment can be enhanced but no such effect was observed with bacteria. Osteoblast spreading, alkaline phosphatase activity, and calcium mineral deposition were also promoted on substrates with a surface BMP-2 density of >50 ng/cm^2 or higher. The carboxymethyl chitosan graft layer reduced *S. aureus* and *S. epidermidis* adhesion by a factor of 4−5 compared to that on the pristine surface without affecting osteoblast adhesion.[112] After conjugation of BMP-2 to the carboxymethyl chitosan graft layer, osteoblast attachment, alkaline phosphatase activity, and calcium mineral deposition of both osteoblast and human bone marrow-derived mesenchymal stem cells were promoted. The achievement of the dual functions of bacterial adhesion reduction and cell function promotion by the combination of BMP-2 and dextran or carboxymethyl chitosan grafted on titanium substrates illustrates the good potential of such surfaces for enhancement of tissue integration and implant longevity. Recently, it was shown that when vascular endothelial growth factor (VEGF) was conjugated to either carboxymethyl chitosan or hyaluronic acid grafted on titanium, similar results were obtained, i.e., bacterial colonization was inhibited (Figure 11) while concomitantly osteoblast functions were enhanced (Figure 12).[123] Figure 11 also illustrates the stability of the antibacterial properties of the coating since there is

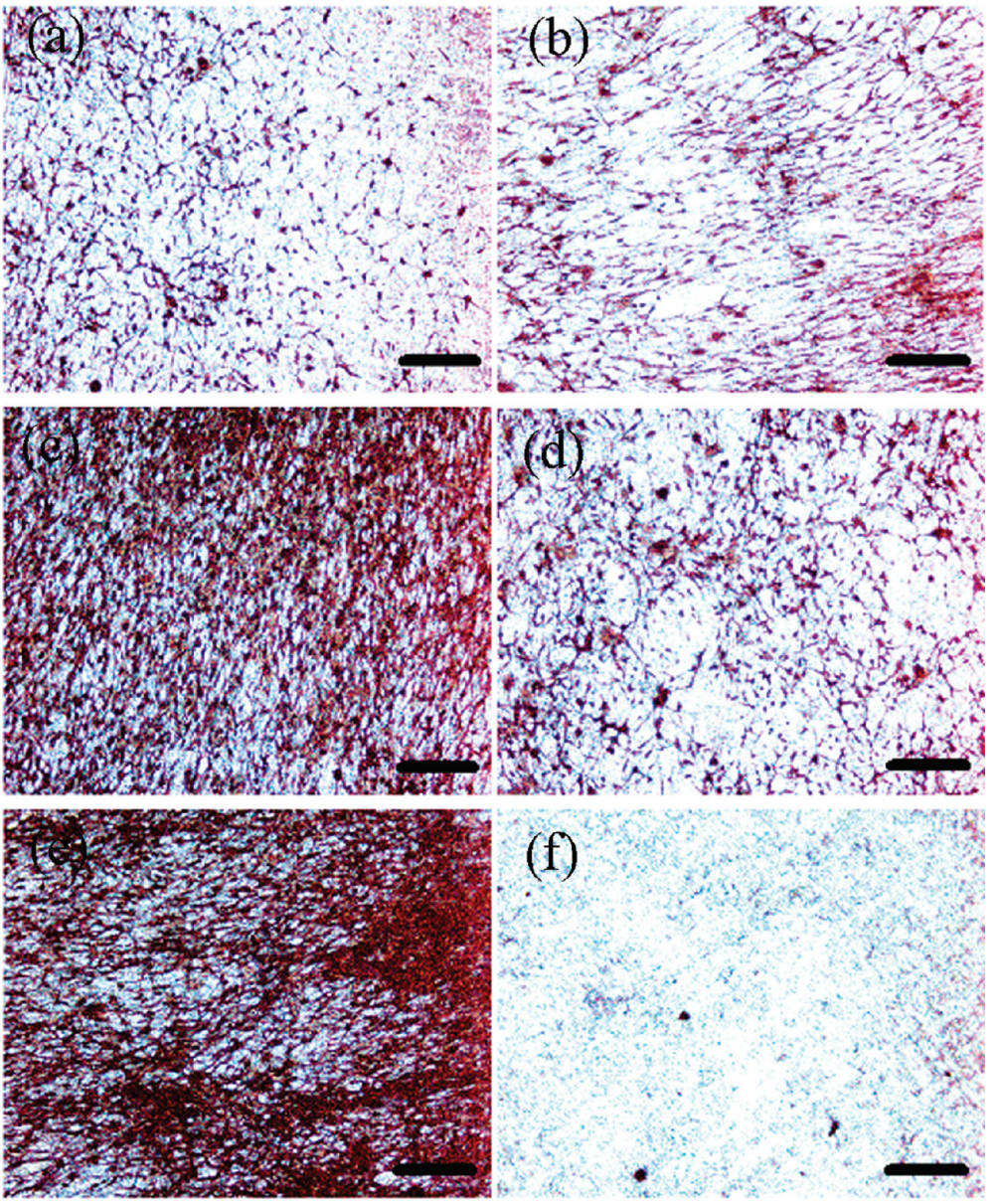

Figure 12. Optical microscopy images of Alizarin Red stained osteoblasts after culturing for 14 days on (a) pristine titanium (Ti), titanium modified with (b) carboxymethyl chitosan (Ti-CMCS), (c) carboxymethyl chitosan with conjugated VEGF (Ti-CMCS-VEGF), (d) hyaluronic acid (Ti-HAC), and (e) hyaluronic acid with conjugated VEGF (Ti-HAC-VEGF). Initial seeding was carried out with 3×10^4 cells/cm^2. (f) Ti-CMCS substrate that had been placed in cell culture medium for 14 days without cell seeding after Alizarin Red staining. Scale bar = 200 μm. Reprinted with permission from 123. Copyright 2010 Elsevier.

no significant difference in bacterial cell counts on the functionalized Ti substrates when they are freshly prepared and after aging in PBS for 21 days at room temperature. The stability of the growth factor (BMP-2 and VEGF) immobilized on the polymeric coating was also confirmed from experiments, which showed that no leaching of the growth factor from the surface was detected after 14 days in PBS. Furthermore, assays of osteoblasts cultured for 14 days on a permeable support in transwells with or without the growth factor-functionalized substrates placed at the bottom of the wells showed no significant differences in cell attachment and proliferation or calcium mineral deposition. Thus, the enhancement of osteoblast functions observed on growth factor-functionalized substrates is due to the immobilized growth factor and not due to leaching into the growth medium.[122,123]

CONCLUSION

The prevention of bacterial or microbial adhesion and biofilm formation on metal surfaces in engineering and biomedical applications remains a scientific and technological challenge. The conventional practice of incorporating biocides in surface coatings to combat microbiologically influenced corrosion (MIC) presents environmental problems. Recent advances in surface-initiated controlled radical polymerization offers an environmentally benign approach for tethering robust and non-releasing antimicrobial polymer brushes on metal surfaces. Further enhancement of the environmentally friendly nature of the coating can be achieved via the use of biomimetic anchors for antimicrobial polymer brushes and covalently bonded natural

biocides. While similar approaches of tethering polymer brushes can be applied to inhibit bacterial colonization of medical implants, the polymer coatings must also not pose significant cytotoxic effects to mammalian cells or compromise the tissue integration process. Thus, only biocompatible polymers can be used, and if these polymers are antiadhesive to osteoblasts in addition to the bacteria, then biomolecules such as peptides or growth factors have to be incorporated to enhance the osteoblast functions.

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