Antifouling Coatings: Recent Developments in the Design of Surfaces That Prevent Fouling by Proteins, Bacteria, and Marine Organisms

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REVIEW

Antifouling Coatings: Recent Developments in the Design of Surfaces That Prevent Fouling by Proteins, Bacteria, and Marine Organisms

*Indrani Banerjee, Ravindra C. Pangule, and Ravi S. Kane**

The major strategies for designing surfaces that prevent fouling due to proteins, bacteria, and marine organisms are reviewed. Biofouling is of great concern in numerous applications ranging from biosensors to biomedical implants and devices, and from food packaging to industrial and marine equipment. The two major approaches to combat surface fouling are based on either preventing biofoulants from attaching or degrading them. One of the key strategies for imparting adhesion resistance involves the functionalization of surfaces with poly(ethylene glycol) (PEG) or oligo(ethylene glycol). Several alternatives to PEG-based coatings have also been designed over the past decade. While protein-resistant coatings may also resist bacterial attachment and subsequent biofilm formation, in order to overcome the fouling-mediated risk of bacterial infection it is highly desirable to design coatings that are bactericidal. Traditional techniques involve the design of coatings that release biocidal agents, including antibiotics, quaternary ammonium salts (QAS), and silver, into the surrounding aqueous environment. However, the emergence of antibiotic- and silver-resistant pathogenic strains has necessitated the development of alternative strategies. Therefore, other techniques based on the use of polycations, enzymes, nanomaterials, and photoactive agents are being investigated. With regard to marine antifouling coatings, restrictions on the use of biocide-releasing coatings have made the generation of nontoxic antifouling surfaces more important. While considerable progress has been made in the design of antifouling coatings, ongoing research in this area should result in the development of even better antifouling materials in the future.

1. Introduction

Biofouling or biocontamination is relevant in a wide range of applications, including but not limited to surgical equipment and protective apparel in hospitals,[1–3] medical implants,[1–6] biosensors,[7] textiles,[8–11] food packaging[12–14] and food storage,[15] water purification systems,[16] and marine and industrial equipments.[16–20] Surfaces that resist the nonspecific adsorption of protein and microbes are also vital in catheters, prosthetic devices, and contact lenses, in immunological assays like enzyme-linked immunosorbent assay (ELISA), in devices for drug delivery, and in materials for patterned cell culture.[21–24]

Adsorption of proteins reduces sensitivity in the case of in vitro diagnostics such as immunological assays.[21] Protein fouling on biological implants reduces the efficacy of the devices and may also result in harmful side effects, such as thrombosis.[25] Moreover, protein adsorption and the subsequent formation of a layer of protein on the surfaces of biological implants provides a conditioning layer for microbial colonization and subsequent biofilm formation.

Microbial contamination and the associated risk of infection is one of the most serious complications in food industries, hospitals, and community settings.[23,26–28] The attachment of bacteria to a surface leads to subsequent colonization resulting in the formation of a biofilm.[23,29,30] This adhesion of bacteria to a surface is mediated by different types of interactions which can be specific, for example through a protein film that might have formed on the surface, or through nonspecific interactions.[30–32] Biofilm formation on biological implants like catheters, prosthetic devices, and contact lenses leads to infection.[24] Typical treatment methods for biofilm-mediated infection of medical devices involves surgical replacement of the contaminated devices, along with prolonged antibiotic therapy, which incurs additional health care costs.[26] Such treatments are often associated with long periods of hospitalization, morbidity, severe functional impairment, and increased mortality.[33] Biocontamination is of greatest concern when it comes to life-threatening infectious diseases caused by attached bacteria, especially when caused by antibiotic-resistant strains.[26,28,29] Among various cases of infections caused by pathogenic bacteria, the infections mediated by methicillin-resistant *Staphylococcus aureus* (MRSA) have been of great concern in hospitals and community settings.[1,34,35]

Fouling is also a concern in the case of surfaces subjected to aquatic environments where marine microorganisms can bind

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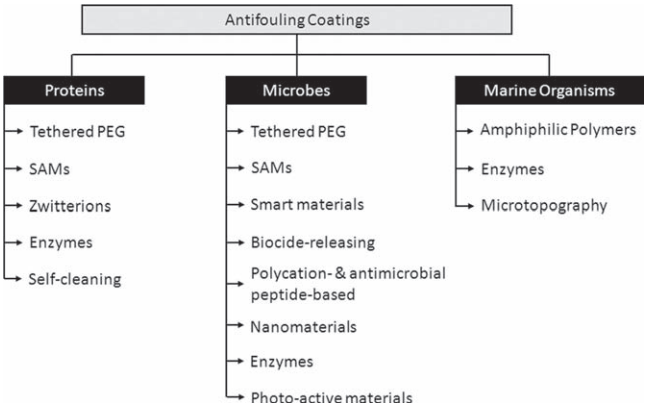

Figure 1. Schematic representation of the various approaches to fabricate antifouling surfaces.

to a surface and form a conditioning layer, which then provides an easily accessible platform for other aquatic species, such as diatoms and algae, to attach and proliferate.[17,36,37] The critical issues associated with biofouling include increased operational and maintenance cost due to fouling of water conduits and ship hulls and the degradation of abiotic materials, among others.[18,20]

Herein, we review the key strategies for preparing antifouling coatings[10,21,38–56] that resist the adhesion of biocontaminants or degrade or kill them. We discuss various approaches including the immobilization of polymers, such as poly(ethylene glycol) (PEG) on surfaces, photoactivated self-cleaning coatings, incorporation of biocidal agents (silver, antibiotics, nanoparticles, polycations, enzymes, and antimicrobial peptides), and the use of structured surfaces (**Figure 1**). This review specifically focuses on recent developments in strategies relevant to protein antifouling, antimicrobial, and marine antifouling applications.

2. Coatings That Prevent Protein Fouling

2.1. Coatings That Resist Protein Adsorption

2.1.1. PEG-Based Coatings

Immobilizing PEG is one of the most commonly used approaches to impart protein resistance to a surface. The antifouling properties of PEG-based coatings have been widely reported in the literature.[25,38,57–63] Physical adsorption, chemical adsorption, direct covalent attachment, and block or graft copolymerization are some of the techniques that have been used to attach PEG to surfaces.[63] The physical adsorption or covalent attachment of PEG chains usually cannot reduce protein adsorption below a certain limit because of steric issues that limit the density of the attached polymer chains.[64–66]

Several studies sought to theoretically understand the protein resistance conferred to a surface by attaching PEG to it.[67–74] Jeon et al. studied the basis for the exceptional protein resistance of PEG-functionalized surfaces.[67] They considered the PEG chains to be terminally attached to a hydrophobic substrate placed in water. The protein was modeled as a block of infinite length placed parallel to the substrate, separated by the polymer chains and water in between (**Figure 2**). According to their studies, the approach of the protein towards the substrate results in compression of the PEG chains leading to repulsive elastic forces. Additionally, the removal of water molecules from hydrated polymer chains during compression creates a thermodynamically unfavorable osmotic penalty. These unfavorable elastic and osmotic stresses generate a repulsive force, the magnitude of which depends on the surface density and chain

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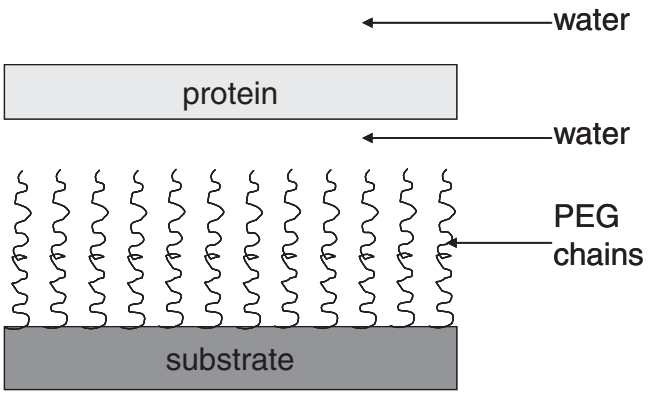

Figure 2. Model picture for the theoretical study by Jeon et al.[67] showing a protein of infinite size in water with a solid substrate having terminally attached PEG chains.[67]

length of the PEG. The paper concluded that the protein resistance of the surface should increase monotonically with the surface density of the grafted PEG and its chain length. Jeon and Andrade later extended these findings by modeling proteins as finite spherical particles and also concluded that for a given surface density of polymer, an increase in chain length would lead to greater protein resistance.[68]

Prime and Whitesides provided a breakthrough in the development of protein-resistant surfaces based on self-assembled monolayers (SAMs) presenting oligo(ethylene oxide) groups.[38,61] Highly protein-resistant surfaces were generated by forming mixed SAMs from thiols such as $HS(CH_3)_{11}(OCH_2CH_2)_nOH$ and $HS(CH_3)_{10}CH_3$. They measured the extent of adsorption of four different proteins, fibrinogen, pyruvate kinase, lysozyme, and ribonuclease (RNase), which differed in both structure and molecular weight. While previous studies had suggested that only surfaces grafted with very long PEG chains can resist protein adsorption,[75–78] Prime et al.[38,61] reported remarkable protein resistance with SAMs of alkanethiolates with as few as two ethylene oxide groups. The observations reported by Prime et al.[38,61] do not contradict, as it might seem, the former studies with covalently grafted PEG. The benefit of using longer chains is that they can cover the surface more effectively. In the case of SAMs, it is possible to incorporate a larger number of chains per unit surface area than with most other grafting techniques, thus making it possible to obtain a more effective surface coverage even with shorter chain lengths. In addition, Prime et al.[61] observed that replacing the terminal –OH of oligo(ethylene oxide) by $–OCH_3$ did not decrease the ability of the surface to resist protein adsorption.

Szleifer and co-workers[69,70] used single-chain mean field (SCMF) theory to explain the protein resistance of SAMs presenting shorter oligo(ethylene oxide) chains as reported by Prime et al.[38,61] They took into account the forces of interaction between the protein and the polymer-modified substrate averaged over all the possible conformations of polymer chains. According to their results, an important parameter in determining protein resistance of the PEG layer is the density of polymer molecules in the region close to the substrate.[69] However, the kinetics of protein adsorption depend on the thickness of polymer layer. Thus, in the case of grafting techniques, in which only a limited number of the polymer chains can be attached per unit surface area, it is advantageous to increase the thickness of the polymer layer by increasing the chain length, thereby creating a kinetic barrier that prevents protein adsorption on the surface.

Grunze and co-workers[71–74] reported that the protein resistance of methoxy-terminated oligo(ethylene glycol) SAMs significantly depends on the conformation of the polymer chains on the surface. The authors reported that oligo(ethylene oxide) groups have a helical conformation in the SAMs formed on gold which are inert to protein adsorption.[72] In the case of SAMs formed on silver, however, the chains have a trans conformation and cannot thwart protein adsorption.[72] Monte Carlo simulations indicated that the helical SAMs interact more with water compared to trans SAMs, suggesting that this interaction with water plays a vital role in determining protein resistance.[73,74]

Even though SAMs are by far the best known systems in terms of surface coverage and ease of fabrication, they are prone to defects and a lack of robustness.[21,79] PEG or polymers tethered to substrates by direct covalent attachment are robust, but they cannot reduce protein adsorption as effectively as SAMs.[21] Chilkoti and co-workers reported a strategy to combine the advantages of SAMs, high surface density and ease of formation, with those of grafted polymers, which form thicker and more robust films.[79] The schematic of the reaction is shown in **Figure 3a**. They first formed SAMs of the initiator ω-mercaptoundecyl bromoisobutyrate (compound 1) on gold. The terminal bromoisobutyrate groups enabled surface-initiated atom transfer radical polymerization (SI-ATRP). The polymerization was carried out in the absence of oxygen by immersing the SAM in a solution of oligo(ethylene glycol) methyl methacrylate (OEGMA) monomer prepared in a mixture of water and methanol in the presence of CuBr/bipyridine as catalyst.[79] These surfaces were tested and successfully resisted the nonspecific adsorption of proteins when exposed to the solutions of fibronectin and 10% fetal bovine serum (FBS) as monitored by surface plasmon resonance (SPR) spectroscopy (see Figure 3b).[79]

Researchers have also developed biomimetic strategies to attach PEG to various substrates.[80–89] Messersmith and co-workers[80,81] used 3,4-dihydroxyphenylalanine (DOPA), which is present in large amounts in mussel adhesive proteins (MAPs), to tether PEG to titania (TiO_2) substrates. DOPA is a catecholic amino acid that is formed by the post-translational modification of tyrosine. Peptides containing up to three residues of DOPA were attached to monomethoxy-terminated PEG (m-PEG) polymers. The resultant m-PEG-DOPA was adsorbed on TiO_2 substrates. The formation of a charge transfer complex between the DOPA and the Ti-OH groups present at the surface[80] anchors the polymer to the TiO_2 surface. These m-PEG-DOPA modified surfaces were found to resist protein adsorption, monitored using spectroscopic ellipsometry and optical waveguide lightmode spectroscopy (OWLS). Using DOPA, the mass of PEG adsorbed per unit surface area exceeded the previously reported values for the same molecular weight PEG.[80]

Gademann and co-workers[82] used an iron chelator called anachelin to tether m-PEG to TiO_2 surfaces. Anachelin has a strong binding affinity for metal oxide surfaces and is used by cyanobacteria for biofilm formation. The authors synthesized

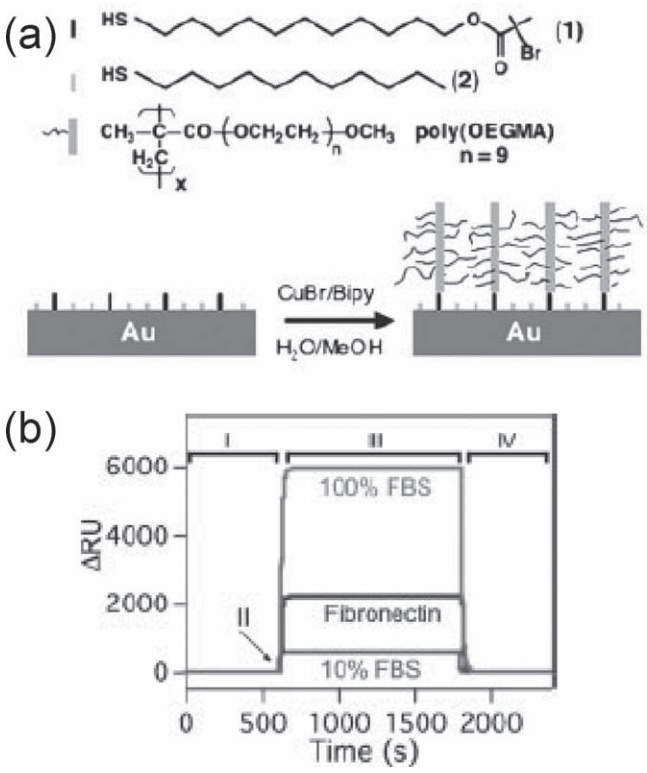

Figure 3. a) Schematic showing the SI-ATRP of OEGMA from a mixed SAM of an initiator-functionalized alkanethiol (1) and a diluent alkanethiol (2) on gold. b) Protein adsorption measurements conducted on SPR chips coated with poly(OEGMA) brushes. The chips were primed with phosphate buffer saline (PBS) for 10 min (region I), followed by injection of 10% FBS/100% FBS/fibronectin (region II) for 20 min (region III), which was followed by a PBS rinse for another 10 min (region IV). Reproduced with permission.[79] Copyright 2004, Wiley-VCH.

a compound which contained m-PEG conjugated with a fragment of anachelin (**Figure 4**) and the resultant conjugate was tethered to a TiO_2 surface. The surface, thus formed, showed excellent protein resistance upon exposure to human serum. Spencer and co-workers[83] have reported other ways to tether PEG onto metal oxide surfaces like TiO_2, $Si_{0.4}Ti_{0.6}O_2$, and Nb_2O_5 by using a class of copolymers based on poly(L-lysine)-*graft*-PEG

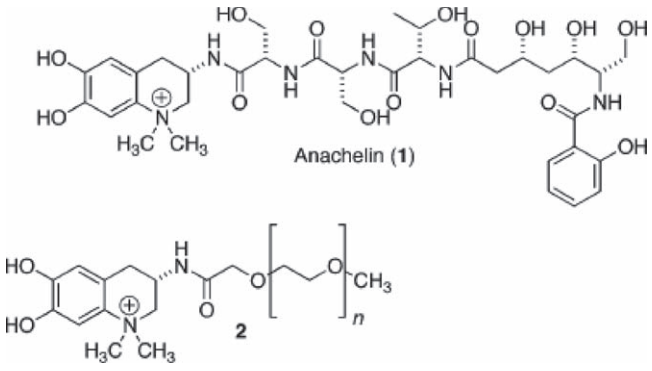

Figure 4. Structure of the cyanobacterial iron chelator anachelin (1) and m-PEG conjugate derivative (2). Reproduced with permission.[82] Copyright 2006, American Chemical Society.

(PLL-*graft*-PEG). PLL has positively-charged amine groups, which bind to the negatively-charged metal oxide substrate, while the hydrophilic, uncharged PEG chains remain free and exposed to the aqueous phase and form comb-like structures. The resulting surfaces were effective in reducing protein adsorption. Thus, a variety of techniques for generating antifouling surfaces have been developed in recent years, by using different strategies for tethering PEG on various types of substrates.

2.1.2. Other Protein-Resistant Surfaces

Even though PEG is the most commonly used substance to impart protein resistance to a surface, it has a tendency to auto-oxidize and form aldehydes and ethers in the presence of oxygen,[21] which causes the surfaces to lose their protein resistance ability. Thus, it is important to design alternative molecules that resist protein adsorption. Whitesides and co-workers[39,90,91] have investigated the properties that confer such exceptional protein resistance ability to SAMs presenting oligo(ethylene glycol) groups and used these properties to design other protein-resistant surfaces. Deng et al.[90] designed SAMs presenting oligomers of propylene sulfoxide instead of ethylene glycol. In designing these surfaces, three main properties of ethylene glycol were kept in mind, namely hydrophilicity, the ability to form hydrogen bonds with water, and conformational flexibility. Although the biocompatibility of propylene sulfoxide is not known, the parent compound, dimethyl sulfoxide, is more biocompatible than ethylene glycol. It was shown that both pure and mixed SAMs presenting oligomers of propylene sulfoxide resisted adsorption of RNase A and fibrinogen completely. The pure SAMs of this new compound were indistinguishable from SAMs presenting hexaethylene glycol in their protein resistance ability.

Chapman et al.[91] formed SAMs of compounds containing different functional groups and tested their protein resistance using SPR. The compounds that gave positive results in the screen had four common parameters that were likely to be important for protein resistance: the presence of polar functional groups, the absence of any net charge, the presence of hydrogen bond acceptor groups, and the absence of hydrogen bond donor groups. Ostuni et al.[39] extended the work by Chapman et al.[91] and designed several compounds that could be conveniently prepared using commercially available agents. SAMs of these compounds were formed on gold-coated glass surfaces using the anhydride method (**Figure 5**) and were tested for their ability to avoid protein adsorption, as monitored by SPR spectroscopy. The authors examined SAMs of derivatives of ethylene glycol and ethers, amines and ammonium salts, amides and amide-based amino acids, crown ethers, nitriles, carbohydrates, and several other groups.[39] The effect of hydrogen bond donors on the inertness of the substrate was tested by using functional groups with multiple amino acids, such as glycine. One of the hydrogen atoms on the amino groups was replaced by a methyl group, which reduced the number of hydrogen bond donors. This substitution improved the inertness of the substrate, consistent with the hypothesis that the decrease in the number of hydrogen bond donors increases the protein resistance ability of the SAMs. For derivatives of ethylene glycol, the protein resistance increased with the increase in the number of oligomers of

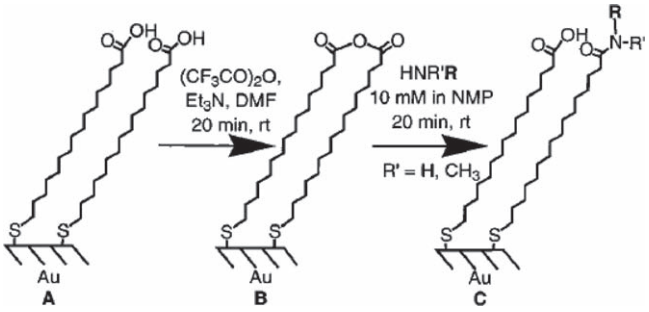

Figure 5. Schematic showing the synthesis of SAMs containing a 1:1 mixture of –CONRR' and –COOH/–COO^- using the anhydride method. NMP is anhydrous *N*-methyl-2-pyrrolidinone, Et_3N is triethyl amine, DMF is dimethyl formamide, and rt indicates room temperature. This method was used by Chapman et al.[91] and Ostuni et al.[39] to find molecules, other than PEG, that can form protein-resistant SAMs. Reproduced with permission.[39] Copyright 2001, American Chemical Society.

ethylene glycol in the functional group. It was observed that disaccharides resisted the adsorption of proteins to a greater extent than monosaccharides and replacement of the hydrogen in the hydroxyl group (hydrogen bond donor) with a methyl group increased the protein resistance of the substrates. Although Ostuni et al.[39] observed that a decrease in the hydrogen bond donors increases the protein resistance ability, which is consistent with the hypothesis presented by Chapman et al.,[91] it should be noted that Mrksich and co-workers[92] have observed that SAMs of mannitol resist protein adsorption even though they contain a large number of hydrogen bond donors. These results[39,91,92] brought forth several alternative compounds that resist protein adsorption and confirmed that resistance to nonspecific protein adsorption was not unique to surfaces presenting oligomers or polymers of ethylene glycol.

Zwitterionic SAMs and Polymers: Phosphorylcholine (PC)-based zwitterionic surfaces have also been widely studied for their protein resistance properties.[93–101] The most prominent hypothesis behind such activity is that the PC zwitterions bind strongly to water, creating a hydration layer that does not allow proteins to adhere to the surface.[95] In one of the earliest reports on PC-based SAMs by Tegoulia et al.,[95] the authors prepared single- and two-component monolayers of pure PC and mixed monolayers of PC and methyl- or hydroxyl-terminated thiol. Interestingly, all of these SAMs showed significant and comparable reduction in the amount of protein adhesion, due to the presence of PC moieties. A similar extent of protein resistance was observed in the case of PC-containing polymeric surfaces and brushes.[98–101] Furthermore, the protein resistance property of oligo(ethylene glycol) and zwitterionic PC groups was found to be synergistic; a hybrid alkanethiol had a greater suppressive effect on protein adsorption than the corresponding oligo(ethylene glycol)-alkane thiol.[97]

Holmlin et al.[102] prepared mixed SAMs of thiols carrying opposite terminal charges. SAMs obtained from a 1:1 mixture of oppositely charged thiols significantly prevented the nonspecific adsorption of two representative proteins, lysozyme and fibrinogen. On the other hand, single-component SAMs presenting a net charge (positive or negative) adsorb nearly complete monolayers of proteins. Furthermore, some single-component SAMs that had zwitterionic groups also resisted protein adsorption. While the results were not universally applicable to all the zwitterionic SAMs tested, several were comparable to the best known systems for resisting protein adsorption.[102]

Kosmotrope-Based Protein-Resistant Surfaces: Kane et al.[22] presented a hypothesis that relates the ability of a given molecule to render surfaces protein-resistant to its ability to be excluded from the surface of a protein in a ternary system comprising of that molecule, water, and the protein. A compound is said to be preferentially excluded from the surface of a protein if the concentration of the compound in the local environment around the protein is less than the net bulk concentration (**Figure 6**) as

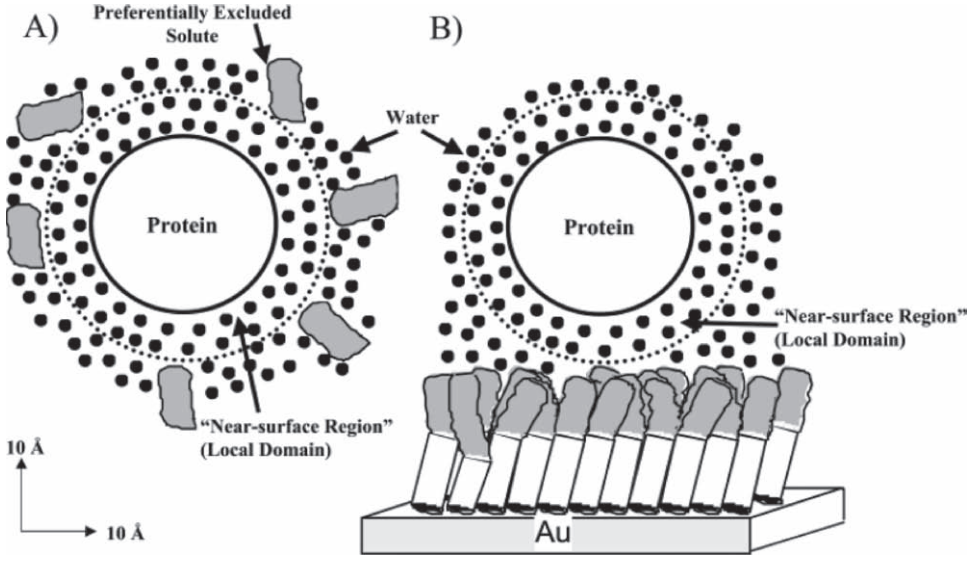

Figure 6. Schematic illustration of a) a solute that is completely excluded from the surface of the protein (the local domain) and b) a protein that does not adsorb onto a surface. Reproduced with permission.[22] Copyright 2003, American Chemical Society.

is the case with osmolytes,[103] which are synthesized by cells to relieve osmotic pressure. Kosmotropes stabilize the structures of proteins by being preferentially excluded from the protein surface.[104] The authors examined data from the literature and found that most of the known protein-resistant surfaces were based on displays of kosmotropes.[22] SAMs based on the osmolytes, betaine, and taurine were formed and observed to be protein-resistant, consistent with their hypothesis. The connection between protein resistance, kosmotropicity, and the ability of molecules to function as osmolytes may help shed light on all three properties.[22]

Trimethylamine *N*-oxide (TMAO) is a kosmotrope that has the ability to counteract the protein denaturant urea by imparting stability to the folded state of the protein. The unfolded state is unstable in the presence of TMAO due to the osmophobic protein backbone being exposed in the unfolded state as opposed to the folded state.[105] Dilly et al.[106] reported that this molecule conferred protein resistance when grafted to a polymeric support. This result is consistent with the hypothesis of Kane et al.[22] that kosmotropicity has a strong correlation with the protein resistance property of a substrate. Although the detailed mechanism by which TMAO imparts protein resistance is not well understood and requires further study, the authors commented that this moiety forms strong hydrogen bonds with water leading to the ordering of water molecules near the substrate, which preferentially excludes proteins, thereby conferring protein resistance to the substrate.

Peptide-Based and Peptoid-Based Protein-Resistant Surfaces: Chelmowski et al.[107] reported the ability of peptide-based SAMs to confer protein resistance to gold-coated surfaces. Although the use of peptides in preparing protein-resistant surfaces may seem somewhat counterintuitive, the authors rationally designed the peptides using amino acids that were hydrophilic, had uncharged side-chains, provided hydrogen bond acceptor groups, and formed α-helical structures.[107] Several of these design rules are consistent with the ones described in other studies[39,91,108] for designing protein-resistant surfaces. Gold surfaces, when functionalized with peptide-SAMs designed according to these rules, showed significant resistance to the adsorption of several proteins including streptavidin, bovine serum albumin, and fibronectin. The peptide-based surfaces showed resistance to streptavidin adsorption comparable to that of oligo(ethylene glycol)-based SAMs.

Statz et al.[41] designed a glycine peptoid that was *N*-substituted by a methoxyethyl side chain, following the guidelines provided by Whitesides and co-workers.[39,91] Peptoids are non-natural mimics of peptides that have a protein-like backbone with side chains on the nitrogen atom instead of the α-carbon. The peptoid designed in this particular study was tethered to TiO_2 using a 5-mer peptide containing alternate DOPA and lysine residues (**Figure 7**). MAPs have the highest content of DOPA and 75% of the DOPA residues lie adjacent to lysine residues. The same design was conserved in the anchoring 5-mer peptide. The amount of protein adsorbed on the peptoid-modified surfaces was significantly lower than on the controls. In fact, the protein resistance of these surfaces was comparable to that of SAMs presenting oligo(ethylene glycol) groups.

Glycerol and Carbohydrate Derivatives: Haag and co-workers reasoned that dendritic polyglycerols (PGs) exhibit structural

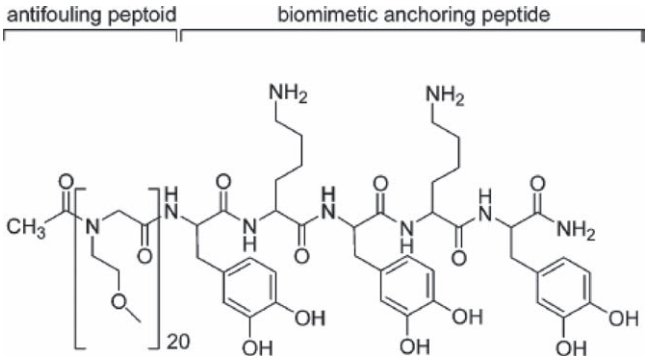

Figure 7. Structure of the glycine peptoid that was conjugated with a 5-mer anchoring peptide containing alternate DOPA and lysine residues designed by Statz et al.[41] The antifouling peptoid is tethered to TiO_2 substrates using the anchoring peptide. Reproduced with permission.[41] Copyright 2005, American Chemical Society.

features similar to those of several highly protein-resistant surfaces.[109] These features include the presence of highly flexible, aliphatic polyether or hydrophilic groups and a highly branched architecture. Based on these observations, Haag and co-workers[109] prepared gold surfaces coated with thiol-functionalized dendritic PG derivatives of varying molecular weights. SPR spectroscopy studies indicated that these surfaces displayed resistance to protein adsorption comparable to that of PEG SAMs. Additionally, bulk PG showed higher thermal and oxidative stability than PEG, based on thermogravimetric analysis. It would be interesting to determine whether the same holds for surfaces presenting PG derivatives.

In their recent work, Wyszogrodzka and Haag[110] report the preparation of SAMs on gold that present methylated or hydroxyl-terminated PGs. Specifically, mixed SAMs were prepared by reacting amine-functionalized PG structures with a SAM that presented interchain anhydrides.[110,111] For the linear PGs, the resistance to protein adsorption increased with increasing numbers of glycerol repeat units. However, a similar effect was not seen with dendritic structures because an increase in the number of PG units resulted in increased steric hindrance and a decrease in the efficiency of the reaction with the SAMs. Methylation of all-terminal hydroxyl groups resulted in a significant improvement in protein resistance compared to hydroxyl-terminated PGs.[110] The authors attribute this effect to the increase in coupling yields as a result of the elimination of hydrogen bond-donor (hydroxyl) groups, which otherwise react with anhydride groups and may compete with amine coupling, especially for hindered amines.[110] Furthermore, they suggested that the elimination of hydrogen bond donors by methylation increased the mobility and flexibility of individual molecules on the surface.[110]

Following the same set of rules outlined by Whitesides and co-workers,[39,91] Guan and co-workers[112] designed a carbohydrate-derived side-chain polyether, the structure of which is analogous to the polyether structure present in PEG; however, it is a side-chain polyether rather than a main-chain polyether. The authors also prepared SAMs using a thiol-capped polyether. Protein resistance of the polyether SAMs was confirmed against fibrinogen and lysozyme by SPR analysis. Furthermore, Guan

and co-workers used living ring-opening polymerization to synthesize degradable protein-resistant polymers from permethoxylated ε-caprolactone (P(OMe)CL).[113] Block copolymers of permethoxylated lactone with ε-caprolactone (P(OMe)CL-*b*-PCL) were also synthesized and characterized for their protein resistance. Interestingly, both forms (P(OMe)CL and P(OMe)CL-*b*-PCL) showed resistance to fibrinogen and lysozyme, which was evaluated using SPR spectroscopy. Next, Guan and co-workers synthesized side-chain ether polyesters and polyamides using condensation polymerization of monomers derived from simple carbohydrates.[114] While permethoxylated polyesters showed resistance to protein adsorption, polyamides did not.

Another carbohydrate-derived protein-resistant formulation includes the use of PLL-*graft*-dextran.[115] Based on the previous work involving the use of PLL-*graft*-PEG polymer brushes, which prevented non-specific adsorption of protein onto oxide surfaces,[83,116] Perrino et al. rationalized the use of dextran which, like PEG, is neutral and therefore does not interact with the polycationic backbone.[115] As expected, copolymer-coated silica-TiO_2 waveguides were protected from protein adsorption.

2.2. Protein-Degrading Films

2.2.1. Protease-Based Antifouling Films

Incorporation of proteases into coatings is one of the approaches taken for imparting protein-degrading properties to a surface. In one of the earliest reports on using proteases for preparation of antiprotein fouling coatings, Kim et al.[42] immobilized hydrolytic enzymes, namely pronase and α-chymotrypsin (α-CT), into a poly(dimethylsiloxane) (PDMS) matrix by either sol-gel entrapment or using covalent attachment methods (**Figure 8a**). Kim et al. showed that these enzyme-PDMS formulations could be cast directly into thin biocatalytic films or incorporated into oil-based paints.[42] While all enzyme-containing formulations showed biocatalytic activity, for α-CT the activity of the film was higher with sol-gel entrapment than when prepared by covalent attachment. The decrease in activity upon covalent attachment has been attributed to the partial denaturation of α-CT because of the formation of covalent bonds between the enzyme and PDMS during polymerization. On the other hand, in the case of pronase, higher activity was observed for covalent attachment than sol-gel entrapment. The proteolytic activity of α-CT–containing films was improved by using a lyoprotectant, poly(vinylpyrrolidone) (PVP), during the freeze-drying step. Both pronase- and α-CT-containing PDMS films prepared by covalent attachment significantly inhibited protein adsorption (Figure 8b). The reduced protein adsorption on protease-containing films was attributed to the increase in conformational entropy due to degradation of protein molecules, thereby decreasing the Gibbs free energy of adsorption for the adsorbing peptide fragments.[42] In another study by Dordick and co-workers, α-CT-containing silicates were prepared by both of the methods discussed earlier.[117] Consistent with previous results,[42] silicates prepared by covalent attachment were more stable than sol-gel entrapped α-CT.[117] The enhanced stabilization was attributed to the insignificant leaching and the protection of the attached enzyme from

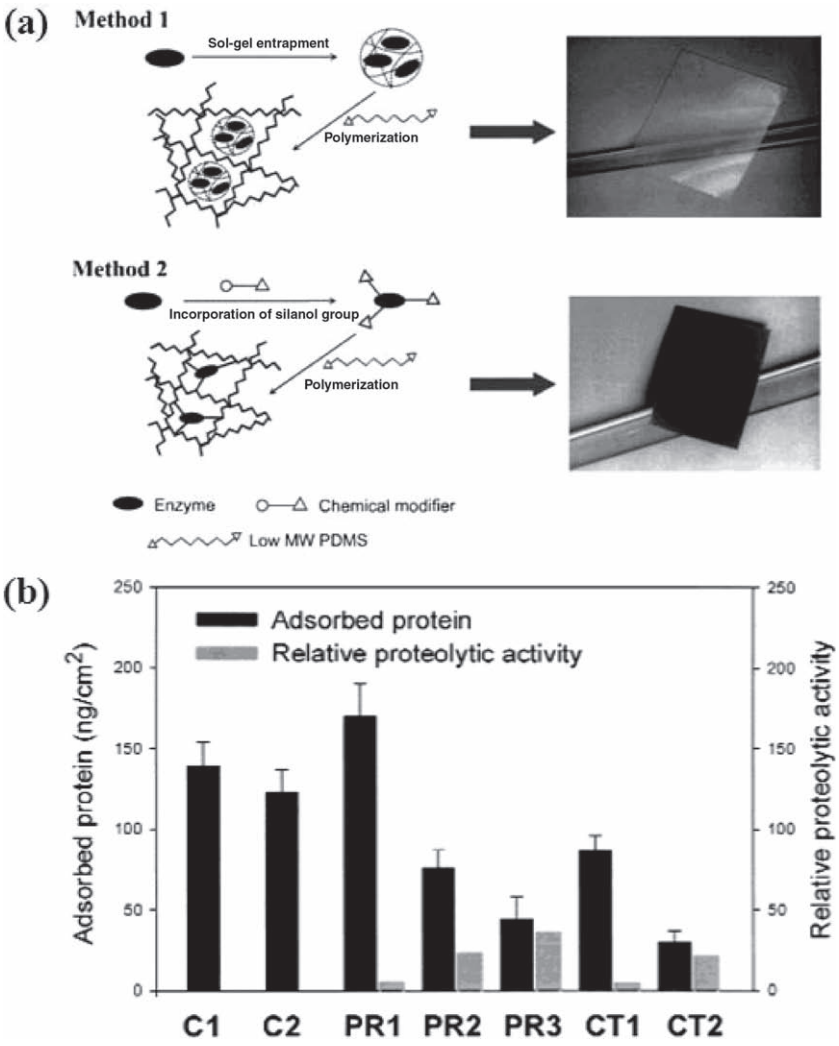

Figure 8. Protease-based antifouling films: a) Incorporation of enzyme into PDMS polymers via sol-gel entrapment (method 1) and covalent attachment (method 2). b) Proteolytic activity and adsorption of BSA onto PDMS films containing pronase and α-chymotrypsin (α-CT). C1: PDMS control; C2: PDMS control containing sol-gel particles without enzymes; PR1: film prepared by method 1 (pronase = 0.7 wt%); PR2: film prepared by method 2 (pronase = 0.9 wt%); PR3: film prepared by method 2 (pronase = 1.1 wt%, solution cast in tetrahydrofuran (THF)); CT1: film prepared by method 2 (α-CT = 0.5 wt%, solution cast in THF); CT2: film prepared by method 2 (polyvinylpyrrolidone-treated α-CT = 0.3 wt%, solution cast in THF). Reproduced with permission.[42] Copyright 2001, John Wiley & Sons, Inc.

autolysis. Interestingly, α-CT-silicates were able to significantly decrease the extent of protein binding. Specifically, there was ca. 80% reduction in the adsorption of human serum albumin (HSA) on silicate films containing 10% w/w α-CT compared to silicates that did not contain any α-CT or that contained pre-inactivated α-CT.

Recently, Asuri et al.[16] used nanotubes as a support for enzyme immobilization. The authors exploited the fact that the nanoscale supports provide larger functional enzyme loading and better stability than their macro- and microscopic counterparts.[16] Moreover, due to their cylindrical structure, nanotubes may be entrapped in the polymer matrix, thereby decreasing the leaching of the immobilized enzyme from the matrix. Taking advantage of these properties, Asuri et al. prepared polymer composites containing conjugates of nanotubes with the protease subtilisin Carlsberg (SC). Poly(methyl methacrylate) (PMMA) films containing nanotube-SC conjugates reduced the adsorption of HSA and fibrinogen by ca. 85% and 70%, respectively, compared to control PMMA films. The antifouling performance against HSA adsorption was improved upon incorporation of conjugates of nanotubes with another protease, trypsin (TRY), into the film to provide a broader range of proteolytic activity.[16] In this case, as much as 95% lower binding of HSA was obtained relative to control films. To broaden the range of biopolymers that enzyme-containing films can degrade, nanotube conjugates of polysaccharide-degrading enzymes, such as dextranase (that can degrade dextran) were incorporated into PMMA matrix.[16] Since several bacteria and aquatic organisms use proteins and polysaccharides to establish contact with synthetic substrates, such biocatalytic nanocomposites[16] may help inhibit biofilm formation and hence prevent biofouling.

2.2.2. Photoactivated Self-Cleaning Films

Self-cleaning coatings have become a major focus in recent years.[4,9–11,118–127] These coatings can be characterized into two main types: superhydrophobic films[119,128–139] or photoactive films.[24,120,140] Nature uses superhydrophobic substrates to form self-cleaning surfaces by an effect called the "lotus leaf" effect.[24,141–145] Water droplets roll down these surfaces and pick up dirt, bacteria, or other fine debris during the rolling movement, leaving behind a cleaner surface.[24] Superhydrophobic surfaces can be fabricated by employing a combination of low surface energy materials and high surface roughness. There are several types of superhydrophobic films based on silica nanoparticles,[128–133] carbon nanotubes,[119,134] polymers,[138,139] etc. Superhydrophobic films prevent contaminants from sticking to their surface but do not degrade the contaminants. Photoactive self-cleaning films, on the other hand, break down the foulants in the presence of ultraviolet (UV) or visible radiation due to the production of reactive oxygen species (ROS). The mechanism of ROS generation has been well-studied[24,146] and is schematically illustrated in **Figure 9**. When UV or visible light is incident on the photosensitizer, the electrons in the ground singlet state are promoted to the excited singlet state. These electrons can relax back to the ground state by emitting light or can undergo intersystem crossing to their excited triplet state, which can lead to the production of ROS via two reaction pathways, type I and II.

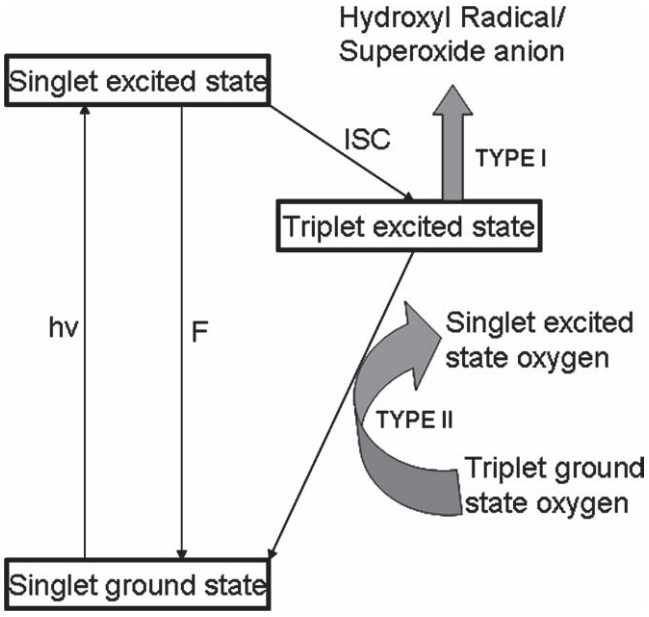

Figure 9. Jablonski diagram showing energetic transitions from the photosensitiser molecule to molecular oxygen. (hv is the incident visible light energy, F = fluorescence, ISC = intersystem crossing).[24]

In the type I pathway, superoxide ion or hydroxyl radicals may be produced, whereas in the type II pathway, energy is transferred to oxygen, which is promoted to the excited singlet state. These highly reactive species (superoxide ion, hydroxyl radicals, and singlet oxygen) that are produced can degrade various types of contaminants.

The most commonly known photoactive material is TiO_2. TiO_2 can decompose a wide range of contaminants, such as stearic acid[121,122,146] and gaseous formaldehyde,[147] and can keep itself clean under UV as well as visible light. Due to its self-cleaning action, TiO_2 has been coated onto different types of surfaces, including glass,[121,122,147,148] textiles,[9,11] and catheters,[4] and has been used extensively for environmental clean-up.[123]

Recently, Joshi et al.[125] reported the fabrication of transparent photoactive self-cleaning films based on carbon nanotubes. The nanotube films inhibited fouling by bovine serum albumin (BSA) in presence of visible and near-infrared (NIR) irradiation. Thus, these nanotube films provide an alternative transparent, self-cleaning system.

3. Antimicrobial Coatings

3.1. Coatings That Prevent Bacterial Adhesion

3.1.1. PEG and Other Coatings

Bacterial attachment to a surface occurs through several mechanisms, including hydrophobic and electrostatic interactions.[23,30,40,149,150] Microbial attachment to biological implants is dangerous and may lead to biofilm formation and

infection.[30] The type of interaction varies from one kind of bacteria to another and even changes within a particular type of bacteria due to mutations, thus adding to the complexity of the problem. Researchers have found that control over hydrophobicity, surface roughness, electrostatic interactions, and surface compliance can dramatically reduce bacterial attachment.[49,151–156] It is also postulated that bacterial attachment on a substrate can occur through a layer of adsorbed protein, and thus surfaces that resist adsorption of protein should also resist adsorption of bacteria.[40] Such surfaces are critical since they help combat the serious issues of biofilm formation and microbial contamination. Since PEG has been known to confer protein resistance to a substrate, many researchers have attempted to fabricate substrates that resist bacterial adhesion using PEG.[23,27,30,40,149,150,157–159] PEG chains are flexible and exhibit large steric repulsion forces, which may impede the approach of bacteria towards the surface.

Park et al.[23] reported the preparation of PEG-modified polyurethane substrates. PEG molecules carrying terminal hydroxyl, amino, and sulfonate groups were tested against *Escherichia coli* (*E. coli*) and *Staphylococcus epidermidis* (*S. epidermidis*). They tested the adhesion of bacteria in different media, such as tryptic soy broth (TSB) and human plasma-containing media. The bacterial attachment was found to be dependent on the media, the functionalization, and the molecular weight of PEG. In general, higher molecular weight PEGs exhibited greater resistance to bacterial attachment than the lower molecular weight ones. Surfaces with terminal sulfonate groups were most effective in reducing bacterial attachment.

Norde and co-workers[30] reported the influence of chain length of PEG brushes on the adhesion of different types of bacteria and yeast. The interaction of proteins with polymer brushes has been studied extensively, but bacteria and yeasts are larger in size and present a more complicated system. Two bacteria, *S. epidermidis* and *Pseudomonas aeruginosa* (*P. aeruginosa*), and two different types of yeasts, *Candida tropicalis* (*C. tropicalis*) and *Candida albicans* (*C. albicans*), were used. It was seen that the higher molecular weight PEG and longer brushes resisted the bacterial adhesion more strongly. It was also observed that relatively hydrophobic microbes (*P. aeruginosa* and *C. tropicalis*) adhered more strongly than the hydrophilic ones (*S. epidermidis* and *C. albicans*), which suggests that hydrophobic interactions favor the attachment of the microbes to the surface. The microbes that adhered to the PEG brushes could be more readily removed by passage of an air bubble than the microbes that adhered to the bare substrate, indicating that the attachment force is weaker on the PEG-modified surface.

As discussed in a previous section, Ostuni et al.[39] designed SAMs presenting a number of functional groups that were comparable to SAMs presenting oligo(ethylene glycol) groups in their ability to resist the nonspecific adsorption of proteins. The authors further investigated the relationship between the protein resistance of SAMs and their ability to resist bacterial adhesion.[40] They fabricated SAMs of alkanethiolates presenting different groups and compared the adhesion of protein to that of bacteria. It was observed that, for a given surface, the extent of bacterial resistance did not correlate linearly with the protein resistance. Thus, it is clear that the parameters required to design a surface that resists the adsorption of proteins are not sufficient to render a surface bacteria-resistant. Though attachment of PEG to a surface is one of the most widely explored approaches in fabricating protein-resistant surfaces, it is not as effective in reducing bacterial colonization. This ineffectiveness may be due to the complexity of the mechanisms through which bacteria attach to a surface, many of which are still not well understood.[40] Additionally, PEG undergoes oxidation in complex media and is not suitable for long term use.[22,160]

Other materials that reduce bacterial adhesion are based on surfactants such as hydroxyapatite and chitosan or biological molecules such as albumin and heparin.[161] Friedman and co-workers[162] coated TiO_2 surfaces with BSA that was crosslinked using carbodiimide chemistry. The coatings were found to be effective at resisting bacterial adhesion and did not show significant degradation of the albumin in the coating when tested in vitro at 37 °C for 20 days. An in vivo evaluation of albumin coatings in rabbits was reported by An et al.[163] The authors found that TiO_2 implants coated with albumin had a much lower infection rate of 27% as compared to controls, which had a 62% rate of infection. Thus, albumin coatings were shown to be effective both in vitro and in vivo.

Lichter et al.[49] used polyelectrolyte multilayers of poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA) and studied bacterial attachment as a function of the mechanical stiffness of the multilayers. The stiffness of the multilayers could be easily tuned by changing the pH at which the multilayers were assembled. The authors observed that the extent of bacterial attachment on a substrate depends on the mechanical stiffness of the substrate; an increase in the stiffness of the substrate resulted in an increase in the number of bacteria attached.

3.1.2. Smart or Stimuli-Responsive Materials

Smart or stimuli-responsive materials show rapid and reversible changes in their physicochemical properties in response to small changes in environmental conditions (e.g., pH, temperature, salt concentration, electrical potential, light, and the surrounding media).[164–166] These materials play an important role in a diverse range of applications, which include drug delivery, biosensors, tissue engineering, coatings, and textiles.[164–171] In the context of antifouling coatings, thermo- and pH-responsive polymers have been used to impart a biofouling-release property to coated surfaces.[43,172–175] Thus, by changing the temperature of the underlying material that is coated with such polymers, biofoulants attached to the material surface may be released, rendering the surface clean.

Poly(*N*-isopropylacrylamide) (PNIPAAM) is one of the most studied and widely-used environmentally sensitive (smart) polymers for controlling the wettability of surfaces.[43,172–177] PNIPAAM is a thermoresponsive polymer that exhibits a lower critical solubility temperature (LCST) in water of 32 °C. At temperatures below the LCST, PNIPAAM is soluble in water and is hydrophilic with an extended coil conformation. On the other hand, at temperatures above the LCST, PNIPAAM undergoes a phase transition to a water-insoluble and collapsed hydrophobic structure.[168,173] In the initial reports based on using PNIPAAM as a biofouling-release agent, Lopez and co-workers exploited the stimuli-responsive wettability of PNIPAAM in preparing

fouling-release surfaces.[43,172] Ista and Lopez demonstrated the fouling-release potential of PNIPAAM-coated glass surfaces prepared using a dip coating method.[172] While initial 2 h and 18 h incubations of PNIPAAM-coated and control glass surfaces with *Halomonas marina* (*H. marina*) cells were carried out at 37 °C, the samples were rinsed with 4 °C artificial seawater to obtain the fouling-release properties. The authors observed a greater than 95% reduction in the number of *H. marina* cells that remained attached to PNIPAAM-coated surfaces, compared to ca. 40% reduction obtained in case of control glass surfaces.[172]

Further, Lopez and co-workers prepared PNIPAAM-tethered polystyrene (PS) surfaces using radical polymerization in order to study the fouling-release properties of covalently functionalized PNIPAAM.[43] The temperatures at which the attachment of *S. epidermidis* and *H. marina* onto PNIPAAM-tethered surfaces was favorable were 25 °C and 37 °C, respectively. On the other hand, fouling-release properties were optimal at 37 °C and 4 °C for *S. epidermidis* and *H. marina*, respectively. Detachment studies for *S. epidermidis* were performed at 37 °C instead of 4 °C, because *S. epidermidis* cells were found to attach preferentially to hydrophilic PNIPAAM surface rather than hydrophobic surface.[43] These species-specific temperature conditions were maintained during attachment and detachment studies, depending on the microorganism studied.[43] The authors showed that greater than 90% of cultured microorganisms (*S. epidermidis*, *H. marina*) that attached to the PNIPAAM-grafted surfaces during 2, 18, 36, and 72 h incubations were removed when the hydration state of the polymer was changed from a wettability that favors bacterial attachment to a condition that was less favorable.[43] In addition to the surfaces prepared by dip coating and covalent functionalization, Lopez and co-workers prepared films on gold surfaces by in situ polymerization of NIPAAM on initiator-modified SAMs (**Figure 10**).[173] The attachment and detachment studies showed that the percentage of cells released from PNIPAAM-functionalized SAM surface (ca. 90%) was far greater than that for the controls (carboxylic acid and methyl-terminated SAMs) and was comparable to the results obtained for PNIPAAM-tethered PS surfaces.[173]

In other studies by Alexander and co-workers, PNIPAAM and a copolymer of NIPAAM and *N-tert*-butylacrylamide (N^tBuAAM) (PNIPAAM-*co*-N^tBuAAM) were tethered onto amine-functionalized glass surfaces by free-radical polymerization.[176,177] At temperatures above the LCST, a larger number of bacteria (*Salmonella typhimurium* and *Bacillus cereus*) attached to the surface functionalized with PNIPAAM-*co*-N^tBuAAM compared to controls (PNIPAAM- and amine-functionalized surfaces). However, after reducing the temperature below the LCST, a greater number of cells detached from surfaces functionalized with PNIPAAM-*co*-N^tBuAAM compared to controls.[176,177]

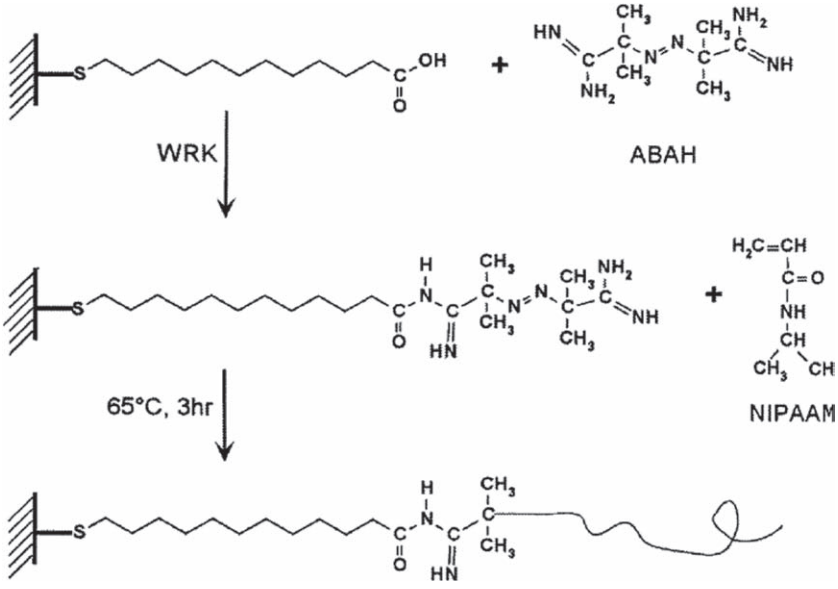

Figure 10. Schematic representation of the process of in situ polymerization of NIPAAM on initiator-derivatized SAMs. 2,2′-azobis(2-amidopropane) hydrochloride (ABAH) is used as a free-radical initiator; NIPAAM = *N*-isopropylacrylamide. Reproduced with permission.[173] Copyright 2001, American Chemical Society.

3.2. Microbicidal Coatings

3.2.1. Release-Based Approach

Silver-Releasing Coatings: Silver has been widely used for its bactericidal activity.[178] Silver and its compounds have widespread uses in a number of applications including wound dressings,[179] the treatment of burns,[180,181] medical devices and catheters,[182,183] and textiles.[184] Silver nanoparticles have also been shown to be strongly bactericidal.[185,186] Antimicrobial silver ions or nanoparticles have been grown or embedded in polyamides,[187] polyelectrolyte multilayers,[53,188–190] fiberglass,[191] and other polymers,[192,193] as well as in hydrogels,[194] to form antimicrobial films. In fact, due to the extensive use of silver, bacterial strains with silver resistance are emerging.[178]

Dai and Bruening[188] described the design of polyelectrolyte multilayers containing silver nanoparticles that exhibited antimicrobial activity. Lee et al.[189] reported the antibacterial activity of silver nanoparticle-loaded multilayers of PAA and poly(acrylamide) formed on magnetic microspheres as well as on plain glass.[195,196] The coatings were found to be effective against *E. coli* (Gram-negative) and *S. epidermidis* (Gram-positive).

Recently, Kumar et al.[44] reported a simple, inexpensive method for producing silver nanoparticles embedded in household paints. The paint used in this work was composed of vegetable oil, which contains a variety of unsaturated fatty acids. These fatty acids undergo auto-oxidation resulting in the production of free radicals during the curing and drying processes. The authors took advantage of the free radicals to reduce silver benzoate and form silver nanoparticles. The advantages of using household paint are that it forms a uniform crack-free coating and is non-toxic. The choice of the metal salt was based

on the solubility of nanoparticle precursors in the oil medium. The oil, along with the metal ion, was coated on surfaces like wood, glass, PMMA, polystyrene, and polypropylene. It was seen that after about 6 h the color changed to brownish-yellow due to the formation of silver nanoparticles, which were characterized by UV-vis spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and X-ray photoelectron spectroscopy (XPS). The nanoparticles formed were extremely stable and did not show aggregation even when heated to 200 °C for 1 h. The surfaces coated with silver-containing paint showed strong antimicrobial activity when tested against *E. coli* and *Staphylococcus aureus (S. aureus)*.

Eby et al.[197] reported the production of lysozyme-reduced silver nanoparticles, followed by the electrophoretic deposition of the enzyme-nanoparticle hybrids on the surfaces of stainless steel and surgical blades. The rationale for using both lysozyme and silver was to increase the range of antimicrobial activity, since lysozyme is active against Gram-positive bacteria and silver is active against both Gram-positive and Gram-negative bacteria. Indeed, the coated surgical blades showed biocidal activity against a variety of Gram-positive and Gram-negative strains, such as *Bacillus anthracis* (*B. anthracis*) Sterne strain, *Bacillus subtilis* (*B. subtilis*), and *S. aureus*, but were not very effective against *E. coli* or *C. albicans*. It was observed that lysozyme, which was tested and found to be active in the coatings, not only helped in the strong adsorption of the coating on stainless steel surfaces, but also facilitated the diffusion of the active components from the surface into the aqueous media. The major disadvantage associated with release coatings based on silver or other antimicrobial components is the depletion of the active species, which leads to a loss in the antimicrobial activity of the coatings with time. This problem may be addressed by using reloadable coatings such as polyelectrolye multilayers and zeolites. Another option is to use slow releasing coatings that release the biocide over longer periods of time.[198]

Recently, it was suggested that silver nanoparticles may be toxic to mammalian cells.[199–202] Silver nanoparticles can easily penetrate through the pores and glands of the human skin and may cause damage. Aymonier et al.[203] reported the design of a silver-based coating which deactivates pathogens without releasing the silver. The authors synthesized silver nanoparticles, which were 1 to 2 nm in diameter and modified with highly branched amphiphilic poly(ethyleneimines) (PEI), and coated them on glass slides. The glass slides showed excellent antimicrobial activity against *E. coli* without any significant release into the environment. The authors found no antimicrobial activity with glass slides coated with just the modified PEI or a mixture of PEI and silver nitrate (the starting material) or a mixture of PEI and lithium triethylborohydride (reducing agent), suggesting that the antimicrobial activity was due to the silver nanoparticles. The amphiphilic nature of the PEI particles imparts effective binding to the substrate. The polar core solubilizes the precursor for particle synthesis and stabilizes the nanoparticles while the hydrophobic alkyl chains prevent the particles from leaching into water. Recently Niskanen et al.[204] reported the synthesis of polymeric coatings with thiol groups that effectively bind silver nanoparticles. Such polymeric coatings, may also be used as an alternative, longer lasting, and more environmental friendly antimicrobial coatings.

Antibiotic or Antiseptic-Releasing Coatings: Prevention of post-surgical bacterial infections remains a challenging problem in orthopedic surgery that may be resolved by using implants treated to kill or prohibit the attachment of bacteria in their proximity.[205–207] One of the approaches to avoid bacterial infection on implants, and hence prevent biofouling, is to cover them with the coatings that can release antibiotics in the local niche. Such antibiotic-containing coatings can be prepared either by soaking the carrier material in a solution containing antibiotics or by directly impregnating antibiotics into the coating material.[45,46,205–209]

While several antibiotic carriers, such as biodegradable polymers (e.g., poly(lactic-co-glycolic acid) (PLGA), PMMA) and bioactive ceramics, have been developed, hydroxyapatite (HyA) is frequently used due to its osteoconductivity and biocompatibility.[205] However, HyA scaffolds possess poor mechanical properties (low strength and brittleness). To overcome this problem, Kim et al.[205] recently fabricated HyA-based scaffolds with controlled porosity and better mechanical properties. These scaffolds were then coated with an antibiotic-based composite containing HyA and poly(ε-caprolactone) (PCL) at varying concentrations using a dipping-and-drying method.[205] The PCL polymer was used as a coating component to overcome the brittleness and low strength of the HyA scaffold and to effectively entrap the antibiotic (tetracycline hydrochloride). With the increasing amount of HyA-PCL on HyA, there was an increase in the drug amount and elastic modulus, irrespective of the composition of the individual components in the HyA-PCL layer.[205] However, the composition did have an effect on the dissolution behavior. Composites containing higher amounts of HyA dissolved faster. With regard to the antibiotic release, after the initial burst of drug, the drug release pattern corresponded with the coating dissolution. Thus, release of the antibiotic could be controlled by manipulating the composition and concentration of the coating formulation.[205] Indeed, entrapment of antibiotics into coating material is a desired option to achieve slow and local release of antibiotics at the required concentration.[207]

Stigter et al.[206] reported the use of carbonated hydroxyapatite (CHyA) coatings as a host material for incorporating antibiotics that differ from each other in terms of their chemical structure. The incorporation of antibiotics was carried out through co-precipitation of antibiotics with calcium phosphate on titanium surfaces. The authors showed that the incorporation efficiency varied for different antibiotics and was governed by the compatibility of the antibiotics and the coating material.[206] Specifically, antibiotics containing carboxylic acid groups (e.g., amoxicillin, cephalothin, carbenicillin, and cefamandol) showed better incorporation and slower release compared to those lacking these groups; the slow release rate was attributed to a higher affinity of the antibiotics towards the coating material. Bacterial inhibition tests were performed for each of these coatings with *S. aureus* cells; the CHyA coatings containing CHyA-compatible antibiotics showed higher inhibition compared to the coatings containing antibiotics that lacked favorable interactions with CHyA.[206] These results suggest the suitability of CHyA-based coatings for incorporating antibiotics containing carboxylic acid groups, such as cephalothin, which is a broad spectrum antibiotic. It is, therefore,

critical to consider the nature of antibiotic when designing the antibiotic-based implant coatings.

Recently, Hammond and co-workers have used layer-by-layer deposition technique to fabricate polyelectrolyte multilayer films that incorporate gentamicin (GS), an antibiotic that is effective against MRSA, as well as against biofilms of several staphylococci.[45] One of the salient features of this approach is the incorporation of charged small molecules without physical or chemical modification, rendering the construction of the film simple and efficient. The layer-by-layer heterostructure consisted of a hydrolytically degradable poly(β-amino ester) (Poly X), a biocompatible polyanionic hyaluronic acid (HA), and GS. For the purpose of this study, the films assembled in a tetralayer ($(((\text{Poly X/HA})_1(\text{GS/HA})_1)_n$) architecture were used, where n is the number of tetralayers (**Figure 11a**). Hydrophilicity and electrostatic interactions between the film components were modulated using Poly Xs of varying molecular weights (Figure 11b). The GS loading density increased with increasing hydrophilicity and charge density of Poly X, suggesting that stronger electrostatic interactions within the films led to higher GS loadings.[45] Moreover, these interactions also governed the release rate of the antibiotic from the film, with stronger binding affinity resulting in a slower GS release rate. For instance, for Poly X with a higher charge density and hence stronger electrostatic interactions, 95% of the GS release occurred over prolonged time periods (10 to 15 h) compared to the burst release of the drug observed in case of Poly X with a lower charge density (95% drug release within 4 h, Figure 11c). In the first case, the release of GS involved two phases, with the first phase, occurring in the initial 8 h, accounting for 80% of the drug release. The authors suggest that this phase was driven by destabilization and degradation of the film components (specifically, hydrolytically degradable Poly X).[45] On the other hand, release of the remaining GS was driven by its diffusion out of uneroded HA residues. Additionally, the release rate was shown to be tunable by varying the film architecture (the number and composition of multilayers). Thus, control over encapsulation and release dosage could be easily obtained by modifying the number of deposited layers, the polymer chemistry, and the film architecture.[45]

While most of the discussion in this section so far has dealt with coatings that release antibiotics, another approach that has recently emerged is to bind or polymerize antibiotic derivatives

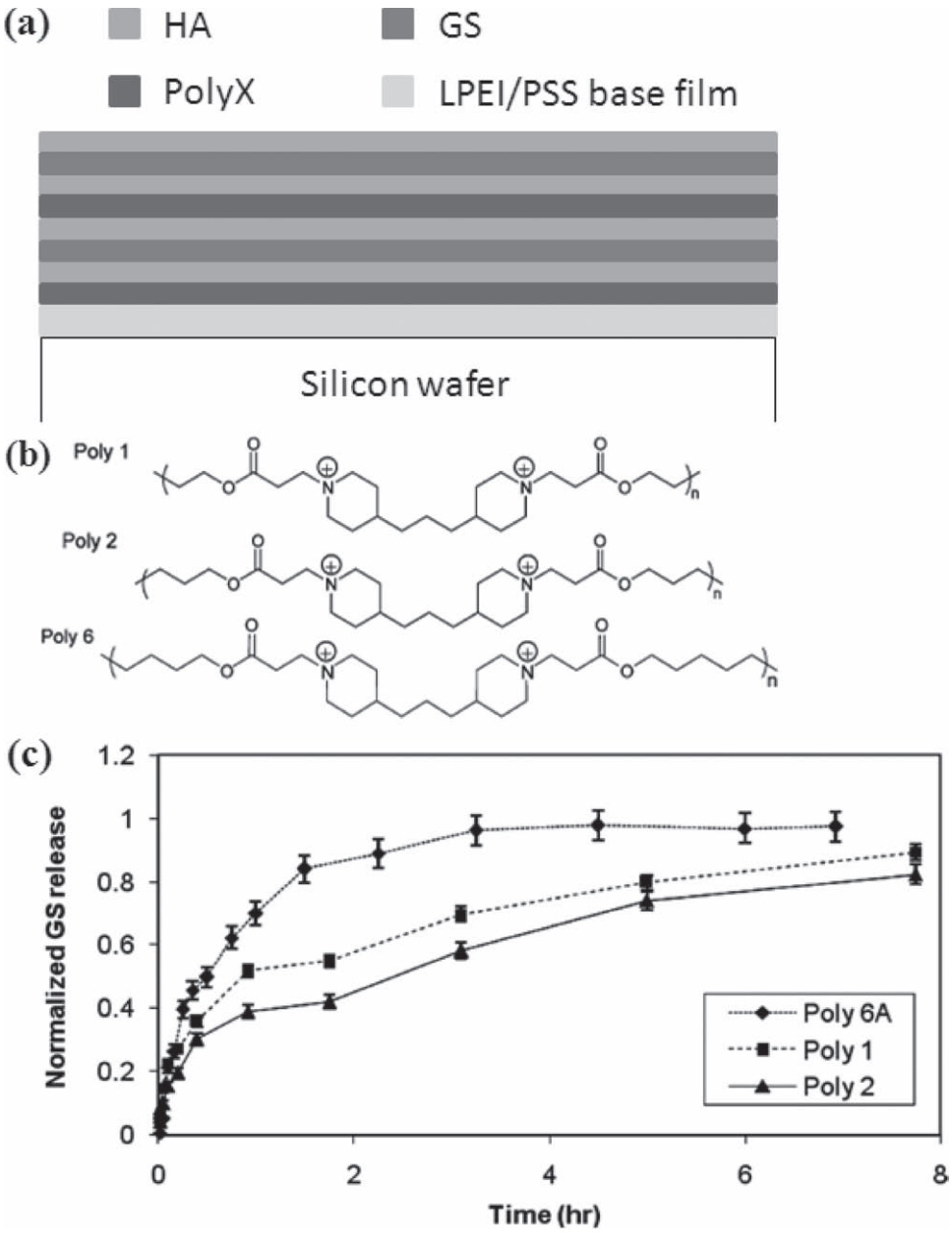

Figure 11. Polyelectrolyte multilayers for incorporation and release of gentamicin sulfate. a) $((\text{Poly X})/\text{HA})_1(\text{GS/HA})_1)_n$ architecture formed by layer-by-layer assembly. The base layer consists of $(\text{LPEI/PSS})_{10}$, where LPEI and PSS stand for linear PEI and poly(styrene-4-styrenesulfonate), respectively. Poly X: poly(β-amino ester), HA: hyaluronic acid, GS: gentamicin sulfate.[365] b) Structures of different Poly X materials. c) Normalized cumulative GS release from 50-tetralayer films made from different Poly Xs. Panels (b,c) reproduced with permission.[45] Copyright 2008, American Chemical Society.

on implantable materials such as metal oxides.[46,208,209] Anseth and co-workers[46,208] reported the synthesis of PEG-acrylate derivatives of vancomycin that can be polymerized on an orthopedic titanium alloy to form a bactericidal surface (**Figure 12**). In general, a decrease in solution-based and surface-based antimicrobial activity has been reported upon conjugation of vancomycin with PEG-acrylate. The authors attributed the decrease in activity to the intramolecular interference with the hydrogen bonds, which are essential for the binding of vancomycin to its bacterial target and to the destabilization because of an unfavorable interaction with glucose. However, modification of vancomycin at different sites and optimization of the length of PEG chains led to an improvement in the antimicrobial activity.[46] While solution-based minimum inhibitory concentration (MIC) assays showed decreasing antimicrobial activity with increasing PEG spacer length, experiments with polymerized vancomycin derivatives on a substrate suggest that PEG tethers may be useful for avoiding the interaction of the antibiotic moiety with the polymer backbone and for facilitating biochemical interactions required for obtaining an antimicrobial effect. In a study reported by Wach et al.,[209] anachelin was hybridized with vancomycin because of its strong binding ability to metal-oxide surfaces. In addition, a PEG linker was used between anachelin and vancomycin to ensure optimal positioning of the antibiotic on the surface and to prohibit the attachment of proteins and cells. The functionalization of these natural product hybrids was stable and showed antimicrobial and antifouling activity against *B. subtilis*.

Octenidine dihydrochloride (OCT) is another potent cationic antiseptic[210] and is used as a skin disinfectant for treating patients with MRSA[211,212] and as a mouth-rinse[213] because of its activity against oral plaque forming organisms. OCT is active against a wide range of Gram-positive and Gram-negative bacteria and fungi. Recently, Schneider and co-workers[214] developed OCT-coated uncuffed polymer tracheotomy tubes and tested their inhibitory effect on biofilm formation by *S. aureus* and *P. aeruginosa*. The coatings were found to be effective against *S. aureus* but were less effective against *P. aeruginosa*. The authors comment that a long-term benefit would not be achieved by using the modified uncuffed tubes due to reduced antimicrobial effect after daily reprocessing. These coatings may, however, be useful in single use trachetomy tubes. Matl et al.[215] studied the use of OCT-releasing coatings in surgical sutures and compared their effectiveness to the well-established surgical Vicryl Plus. Synthetic poly(glycolic acid) (PGA) surgical sutures were coated with two different concentrations of OCT. Palmitic acid was used as a mediator agent to bind the hydrophilic antiseptic (OCT) to the lipophillic biomaterial. The mediator agent also functioned as a drug carrier and retarded the drug release, making the effect last longer. In addition to antimicrobial tests, cytotoxicity tests were carried out with the coated and uncoated sutures on L929 fibroblasts from mouse to check biocompatibility. The coatings were found to have superior anti-infective properties but were less biocompatible than Vicryl Plus.

The fast exhaustion of the antibiotics and antiseptics and the necessity of frequent loading of the antimicrobial agent are some of the disadvantages of the releasing coatings. Nonetheless, recent advances in the material properties of the carrier matrix, increasing the compatibility of antibiotics with the carrier matrix, control over the drug release by manipulation of the coating architecture, and the

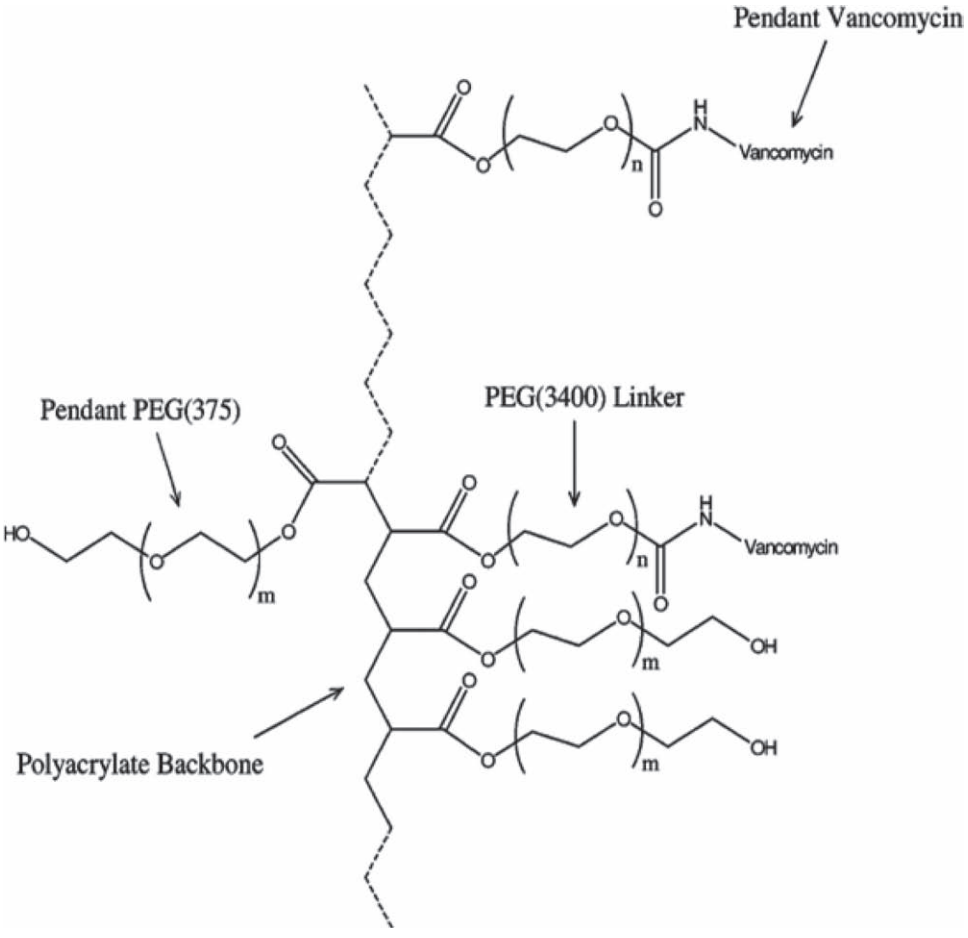

Figure 12. Polymer structure formed on copolymerizing PEG(375) acrylate with vancomycin-PEG(*X*)-acrylate monomer, where *X* is the average molecular weight of the PEG chain, either 3400 or 5000. A polyacrylate backbone is formed by chain polymerization. Pendants to the backbone are both PEG(375) functionalities ($m \approx 9$) and vancomycin-PEG(*X*) groups ($n \approx X/44$, where 44 g mol^{-1} is the molecular weight of an ethylene glycol repeat). Reproduced with permission.[46] Copyright 2009, American Chemical Society.

design of polymerizable and chemically functionalized antibiotics for nonreleasing antibiotic-based coatings offer considerable promise.

Nitric Oxide–Releasing Coatings: Nitric oxide (NO) is naturally produced in the body and is important for bioregulatory functions in the cardiovascular, respiratory, and nervous systems.[216] In fact, NO is produced by the immune system of the body as an antimicrobial agent in response to bacterial infection.[216] NO is a strong oxidizing agent and causes damage to the bacterial cell membrane, then diffuses into the cell causing further damage to DNA and proteins inside.[216]

Since NO is released naturally by several sources in the body, including endothelial cells, and has a very short half life within the body, NO-releasing coatings have been considered for improved biocompatibility of biological implants.[216] NO-releasing materials have been used to decrease thromboresistivity of vascular grafts[217] and intravascular oxygen sensors,[218,219] as well as in the formation of antibacterial coatings.[220–222] To address toxicity issues due to the leaching of the carcinogenic NO donor molecules, Nablo et al.[220,222] demonstrated that diazeniumdiolates (NO donor molecules) can be covalently linked to a siloxane polymer using sol-gel chemistry and used to make antibacterial coatings. Due to the mild synthesis conditions and tremendous chemical flexibility, sol-gel chemistry provides a versatile platform for the synthesis of NO-based coatings. Schoenfisch and co-workers[221] demonstrated the effectiveness of sol-gel coatings for orthopedic implants using medical grade stainless steel. Nablo et al.[223] further studied the effectiveness of NO-releasing sol-gel coatings in vivo in rats and obtained an 82% reduction in the number of infected implants by using the NO-releasing coating. However, in another study, Nablo and Schoenfisch[224] reported that the sol-gel coatings were mildly cytotoxic in an in vitro assay with L929 mouse fibroblasts.

Transition-metal-exchanged zeolites are highly porous and extensively used as a gas storage material. The stored gas (e.g., NO) is liberated under aqueous conditions making the zeolite-based materials apt for biological settings. Recently, Fox et al.[225] reported a new strategy to use Zn^{2+} exchanged zeolite materials to store NO. The materials were found to have a strong bactericidal effect. However, for such materials to be of practical use, the extent of leaching and toxicity need to be characterized. Thus, an optimized design of zeolites is required to develop a material with strong antimicrobial effect and minimal toxicity.

3.2.2. Non-Release-Based Antimicrobial Systems

Polycation-Based Coatings: The antimicrobial activity of solution-based quaternary ammonium compounds has been exploited for some time.[226] However, coating common surfaces with polymers containing cationic and quaternized functional moieties is a novel and is an excellent approach to render the surface antiseptic.[47,53,227–239] Coatings that physically entrap quaternary ammonium compounds also show significant bactericidal effect, as a result of the release of the biocidal quaternary species.[53,240] However, such coatings lose their antibacterial activity as the biocidal substance gets released into the surrounding environment or solution.[228] On the other hand, surfaces with covalently attached and stable quaternized polymers can be bactericidal through a contact mechanism. Since the functional groups are covalently attached, the surface can retain its antimicrobial property even after multiple uses.[236]

In one of the initial reports, Tiller et al.[47] covalently functionalized various common materials, including metals, polymers, and ceramics, with cationic poly(4-vinyl-*N*-alkylpyridinium bromide). The authors suggested that the quaternized polycationic moieties had the same mechanism of action as antimicrobial peptides, i.e., cell penetration through interaction with cell wall components (**Figure 13a**). Tiller et al. reasoned that a certain level of amphipathic nature in the immobilized polymer assists in stabilizing these groups on the glass surface.[47] Specifically, the authors speculated that these tethered groups interact with each other through attractive hydrophobic interactions between alkyl moieties, as well as through electrostatic repulsion between cationic groups. A balance between these two interactions, created by optimizing the chain length of alkyl groups, was shown to be useful in obtaining a stable formulation and greater bactericidal activity (Figure 13b). Further optimization of the surface concentration to achieve efficient bacterial killing and the effectiveness of these optimized coatings against both airborne and waterborne bacteria was demonstrated by Tiller et al.[228] It has been speculated that these polycations disrupt the outer membrane of Gram-negative bacteria by interacting with the anionic lipopolysaccharide network and making their way into the inner membrane to cause further cell damage.[47] It has also been suggested that these polycationic groups penetrate through the outer peptidoglycan layer of Gram-positive bacteria to reach the cytoplasmic membrane.[47] While it is unclear how such initial damage to the outer or cytoplasmic membrane caused by tethered polycations leads to bacterial killing, the authors suggest that polycations may "actively participate" by inducing autolysis.[47] Poly(vinyl-*N*-hexylpyridinium)-coated surfaces were also able to kill multidrug-resistant strains that operate multidrug resistance pumps, the known mechanism of resistance to cationic antiseptics.[231]

Klibanov and co-workers further investigated whether the same approach can be extended to a coating formulation consisting of a different polycationic functionality.[229] They functionalized surfaces with PEI instead of poly(4-vinylpyridine) (PVP). A bactericidal effect was obtained after *N*-alkylation of the PEI groups; *N*-alkylation not only raised the positive charge on the tethered polymer groups but also stabilized them by increasing hydrophobic interactions. The effectiveness of PEI-derivatization against airborne and waterborne Gram-negative and Gram–positive bacteria was tested on macroscopic and nanoscale materials to broaden the scope of applications. For instance, nanoparticles coated with N-alkylated PEI could be used as an antimicrobial component in paints. Additionally, bactericidal fabrics were prepared by covalently attaching these functional moieties to commonly woven textiles.[230] Cotton cloths derivatized with higher molecular weight *N*-alkylated PEIs were shown to exhibit strong bactericidal effects (>90% killing) even after multiple washes.[230] Furthermore, surfaces coated with PEI were found to be fungicidal and virucidal,[227,230] yet benign to mammalian cells.[236] Milović et al.[236] addressed issues related to the mechanism and kinetics of the bactericidal effect shown by PEI-functionalized surfaces using a fluorescence-based membrane integrity assay and plate-count viability

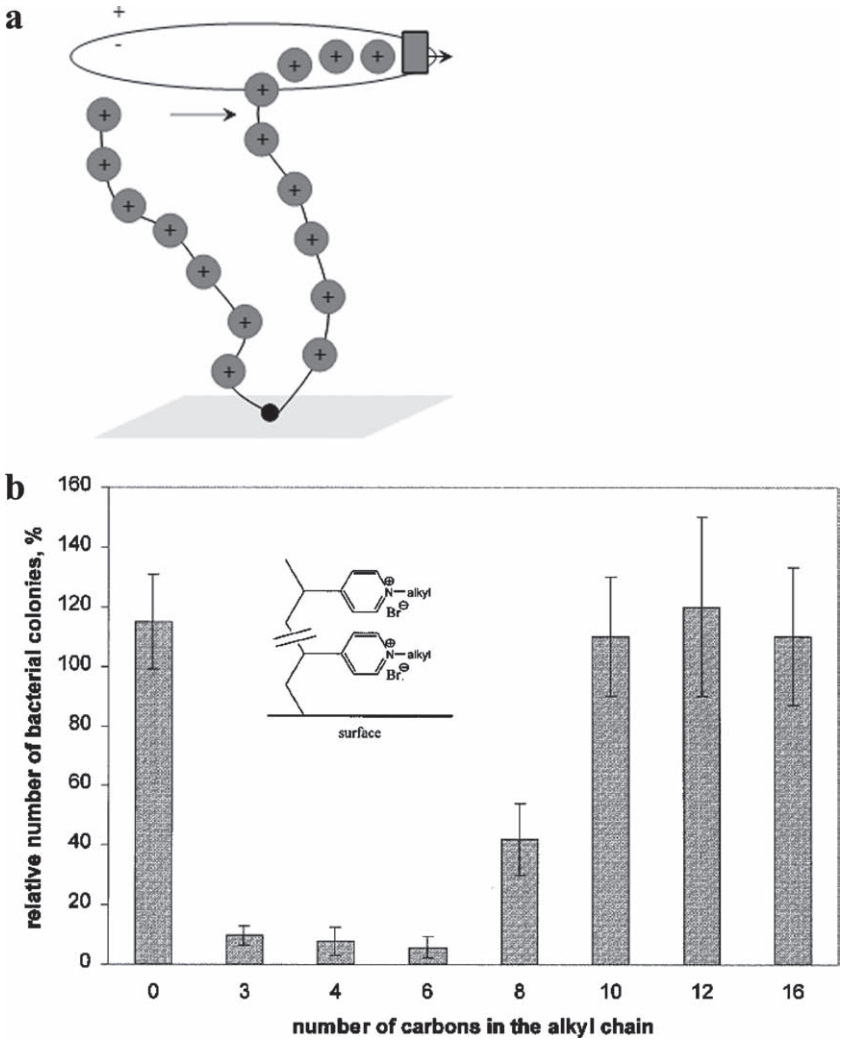

Figure 13. Polycation-based antimicrobial coating. a) Cell penetration of polycationic polymer through the interaction with cell wall components.[235] b) Percentage of *S. aureus* colonies grown on the infected surface of glass slides modified with PVP that was *N*-alkylated with different linear alkyl bromides relative to the number of colonies grown on a commercial NH_2-glass slide. Panel (b) reproduced with permission.[47] Copyright 2001, National Academy of Sciences, USA.

tests. The results suggest that *N*-hexyl, methyl-PEI coatings rapidly and severely undermine the bacterial cell wall integrity, thereby inactivating or killing the cells on contact. One of the challenges outlined by Klibanov is the sustainability of the antimicrobial effect of polycationic functionalities as the surface becomes covered by layers of nonviable cells during the course of exposure to microbes.[241] Fortunately, the surfaces that suffered this type of fouling have been shown to have their antimicrobial activity restored by washing with cationic detergents.[231]

Building on the studies discussed above, Fuchs and Tiller[234] synthesized an antimicrobial emulsifier composed of hydrophobic polystyrene (PS) and hydrophilic poly(4-vinyl-*N*-methylpyridinium iodide) (P4VMP). In order to make the polymer coatings more elastic and less brittle, PS was copolymerized with butyl acrylate (BA) in different weight ratios, resulting in the formation of a PS-*co*-PBA copolymer, which has a lower glass transition temperature than PS. Coatings containing PS-*co*-PBA and PS-*b*-P4VMP were prepared and characterized to check for the presence of P4VMP group on the outer surface of the polymer particles. A glass slide with part of its surface coated with the copolymer showed bactericidal activity without any leaching of the biocidal component. However, a film containing 2.5% PS-b-P4VMP lost all of its activity after one day of washing. The stability of the coating was enhanced by increasing the PS content in PS-*b*-P4VMP which, in turn, increased the hydrophobicity.

In a recent report, Tiller and co-workers reported synthesis of macromers with one biocidal, one polymerizable end group, and a polymeric spacer between the two domains.[242] Specifically, they used *N, N*-dimethyldodecylammonium (DDA) as the antimicrobial end group, poly(2-methyl-1,3-oxazoline) (PMOX) as the polymeric spacer, and a methacrylamide function as the polymerizable group at the other end. As a control, they used a spacer-free co-monomer of DDA and methacrylamide. The macromer (and control co-monomer) were copolymerized with 2-hydroxyethylmethacrylate (HEMA) and 1,3-glyceroldimethacrylate to obtain a biocidal polymer network. Antimicrobial and zone inhibition assays suggested that the comonomer-containing polymeric films acted as a releasing system, while the macromer–containing films rendered the surface contact-active.[242] In addition, the minimal required active weight fraction of macromers was up to 20 times lower than that of the co-monomer. By performing FTIR and Raman spectroscopy studies, the authors found that the effect was caused by surface enrichment of the biocidal groups in the case of macromer-containing films.[242] A fluorescence-based assay also suggested preferential concentration of the additive at the surface. Thus, the authors demonstrated the synthesis of a contact-active polymeric network that showed a strong antimicrobial effect as a result of surface-enrichment of the biocidal groups and their immobilization by a polymeric spacer.[242] Waschinski and Tiller further studied the effect of two polymeric backbones, poly(2-ethyl-1,3-oxazoline (PEOX) and PMOX, on the antimicrobial activity of terminal quaternary ammonium groups.[243] Interestingly, the authors found that antimicrobial activity was independent of the polymer chain lengths of PMOX or PEOX. However, the MIC for PMOX derivatives in free solution was approximately twice as high as that for PEOX derivatives.[243] Therefore, acrylate films containing macromers of PEOX derivatives may perform better than those containing PMOX-based macromers.

Kurt et al. exploited the thermodynamically-driven surface enrichment of bactericidal groups to obtain contact-active coatings based on polyurethane polymer surface modifiers

(PSMs).[244] The structure and composition of the antimicrobial polymer ingredients (e.g., the ratio of charged to uncharged groups) was based on that for naturally occurring antimicrobial peptides, such as magainin and defensin. An optimized antimicrobial polymer formulation was obtained through rational design, synthesis, and biocidal tests of polyurethanes with alkylammonium-functionalized soft blocks.[244] The alkylammonium polyurethane coatings containing polyurethane backbone, chaperone-like (PEG-like methoxyethoxyethoxy and trifluoroethoxy side chain) soft blocks, and antimicrobial surface-concentrating (alkylammonium co-telechelics with C_6 and C_{12} side chains) soft blocks, were synthesized. The results suggest that longer alkyl chains (C_{12}) result in better biocidal activity than shorter chains (C_6). In terms of the chaperoning effect, trifluoroethoxy side chains were found to be better than the PEG-like methoxyethoxyethoxy side chains, with higher surface-enrichment effect in the case of alkylammonium C_6 chain. However, in the case of the PSMs with PEG-like methoxyethoxyethoxy side chains, alkylammonium C_{12} chains showed a self-chaperoning and self-concentrating effect along with higher biocidal activity.[244] By controlling the weight fraction of PSMs and charged groups, length of side chains, chaperoning, and surface enrichment effect, the authors were able to prepare contact-active polyurethane coatings that killed *P. aeruginosa, E. coli,* and *S. aureus* within 30 minutes of contact.[244]

Russell and co-workers used atom transfer radical polymerization (ATRP) to synthesize antimicrobial polycationic polymers of controlled molecular weights and low polydispersity on the surfaces of glass and paper.[233] Following the polymerization of tertiary amine 2-(dimethylamino) ethyl methacrylate (DMAEMA) directly onto filter paper or glass slides via ATRP, DMAEMA was quaternized using ethyl bromide. The quaternary ammonium-covered glass and paper showed bactericidal activity, as well as moderate sporicidal activity when tested against 10^4–10^6 *B. subtilis* spores.[233] In a recent study, Yuan et al. used ATRP to grow well-defined polymer brushes of DMAEMA grafted onto stainless steel to avoid biofilm formation. Instead of an alkyl bromide, Yuan et al. used an equimolar mixture of dichloro-para-xylene and 4,4′-dipyridine to quaternize the surface-grafted DMAEMA polymers, which not only resulted in a higher number of positive charges but also increased the hydrophilicity of the surface.[245] As a result of the polycationic nature of the surface, antibacterial and antifouling effects were obtained when the modified stainless steel substrates were exposed to *Desulfovibrio desulfuricans* in anaerobic seawater.[245] In other studies, bifunctional copolymers containing a reactive silane-based group and positively-charged antimicrobial quaternary ammonium group have also been used to confer antimicrobial properties to silicone rubber and hydroxylated surfaces such as glass.[232,246] Thus, these and previously discussed studies demonstrated the feasibility of functionalizing various common surfaces (e.g., metals, fabrics, polymers, ceramics, silicon, and glass) with polycationic polymers to obtain antimicrobial or antifouling surfaces.

Polyelectrolyte multilayers have also been used extensively to embed silver ions/nanoparticles or other biocides and form antimicrobial coatings, some of which are discussed in this review. Rubner and co-workers developed various types of microbicidal coatings based on polyelectrolyte multilayers.[49,247,248] Lichter et al. suggested that highly swelled multilayer films can efficiently reduce bacterial attachment.[49] Lichter et al.[247] also reported that by tuning the pH of assembly of the multilayer and post-assembly conditions, polycationic sites can be generated in the multilayer, giving rise to biocidal activity even without the incorporation of external biocidal agents. They performed tests with sulfonated polystyrene (SPS) and PAH as well as PAA and PAH multilayers. These multilayers generally do not show any antimicrobial activity even when the polycationic species are in the top layer because the positive charges are not free. However, when SPS/PAH multilayers assembled at pH 9.3 were acid-treated at pH 4.0, free ammonium groups formed on the surface. The advantage of this system is that the films retain charge even when the pH is increased up to 10. These multilayers showed biocidal activity at neutral pH conditions against both *E. coli* and *S. aureus*. However, the efficiency in case of *E. coli* was lower; the Gram-negative bacterium, *E. coli* has a stronger cell wall than the Gram-positive bacterium, *S. aureus*.

Surfaces Functionalized with Antimicrobial Peptide (AMP): Antimicrobial peptides (AMPs) represent an attractive biomaterial for developing microbicidal and antifouling coatings because of numerous advantages: a number of AMPs have been shown to have a broad spectrum of antimicrobial activity, AMPs are rarely susceptible to the rise of bacterial resistance, and microbes are highly susceptible to AMPs even at low concentration.[249–255] To realize the antimicrobial and antifouling potential of AMPs, researchers have investigated the efficacy of surfaces functionalized with AMPs.[3,6,48,253,255–260]

Glinel et al. synthesized antifouling copolymer brushes based on 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) and hydroxyl-terminated oligo(ethylene glycol) methacrylate (HOEGMA) using ATRP.[48] These polymer brushes were then functionalized with the AMP magainin I via oriented chemical grafting on the hydroxyl groups present on poly(MEO_2MA-*co*-HOEGMA). The relatively long, water-soluble, and flexible oligo(ethylene glycol) side chains of poly(MEO_2MA-*co*-HOEGMA) brushes provide mobility and accessibility to magainin I, which could facilitate its interaction with the bacterial cell membrane and result in a microbicidal effect. The antimicrobial properties of these magainin I-functionalized polymer brushes were also improved by increasing the surface density of chemically grafted magainin I. In another parallel study, Bagheri et al. reported that AMPs show reduced antimicrobial activity upon immobilization; however, the authors suggest that the optimization of coupling parameters, such as the length of the spacer and the amount of target-accessible peptide, are critical in obtaining reasonable antimicrobial activity.[253] While other studies did not provide information on the mode of action of immobilized AMPs, the permeability tests done by Bagheri et al. clearly suggest that surface immobilization does not influence their mechanism of action, i.e., membrane permeabilization.[253]

Schneider and co-workers designed an antimicrobial hydrogel scaffold from the peptide MAX1, a 20-residue, water-soluble peptide.[256] MAX1 forms a hydrogel in the presence of cell culture media by folding itself into an amphiphilic β-hairpin conformation and subsequently forming a network of interconnected fibrils rich in β-sheets. The authors suggested that fibril formation is driven by the collapse of the valine-rich

hydrophobic faces of individual hairpins, which keep the cationic lysine residues surface-exposed. As a result, the hydrogel surface becomes lysine-rich and polycationic and accordingly exhibits antimicrobial properties.[256] Fluorescence imaging of hydrogel surfaces exposed to 2.5×10^3 CFU mL^{-1} of *E. coli* for 24 h confirmed bacterial killing. However, the effect was limited when the gel was challenged at a higher cell density (ca. 2.5×10^9 CFU mL^{-1}) because of the initial cellular debris sticking to the surface. The results of a membrane permeabilization assay, based on measurement of β-galactosidase released from lysed cells, suggested that the MAX1 hydrogel causes inner and outer membrane disruption upon cellular contact.[256]

Antimicrobial peptides protect a number of multicellular organisms (e.g., insects, frogs, and mammals) from the action of several pathogens.[252] With the increasing evidence that AMPs are less susceptible to bacterial resistance than traditional antibiotics, there has been a growing interest in rationally designing new AMPs which have limited similarity to naturally occurring peptides but exhibit strong bactericidal activity.[261,262] Recently, in silico peptide design strategies have been devised based on combination of quantitative structure-activity relationship (QSAR) and machine learning techniques[262] or Teiresias pattern discovery tools.[261] These studies demonstrated that a new set of AMPs can be designed to obtain either comparable or superior activity relative to conventional antibiotics and natural AMPs. These synthetic AMPs could be used for generating antimicrobial surfaces.

Nanomaterials: Several nanoparticles are known to possess antibacterial activity.[189,263–271] Apart from silver nanoparticles (which we have already discussed), other nanoparticles made of TiO_2, silicon dioxide (SiO_2), magnesium oxide (MgO), copper oxide (CuO), and zinc oxide (ZnO) have been reported to possess good biocidal activity. Quantum dots of cadmium selenide and cadmium telluride are also known to have antimicrobial activity.[272–274] Additionally, a number of recent studies reported carbon nanotubes to have strong bactericidal activities.[50,275–277]

Kang et al.[50] reported that highly pure single-walled carbon nanotubes (SWNT) possess strong antimicrobial activity against *E. coli* K12 cells. For these studies, SWNTs with tube diameters ranging from 0.75 to 1.2 nm were prepared and used to form films by filtering them onto poly(vinylidene fluoride) (PVDF) membrane filters. These nanotubes showed very good bactericidal activity against *E. coli* cells when suspended in solution or deposited as films. A fluorescence-based assay was used to measure the biocidal activity. Propidium iodide (PI, red dye) was used to stain dead cells and 4′-6-diamidino-2-phenylindole (DAPI, blue dye) was used to stain live cells. PI staining indicated severe damage to the bacterial cell membrane. The metabolic activity of the cells on the SWNT films was also tested by 5-cyano-2,3-ditolyl-tetrazolium chloride (CTC) staining, and it was found that only 6% of the cells were stained by CTC and hence metabolically active. SEM images (**Figure 14**) showed a marked difference in the morphology of cells on nanotube films compared to the ones on bare PVDF membrane. The authors hypothesize that the cylindrical structure and high aspect ratio of the nanotubes enable them to physically pierce the cell membrane and cause permanent damage.[50] An estimation of the efflux of cytoplasmic material (plasmid DNA and RNA) was performed for cells incubated with and without SWNTs in solution. The tests showed a fivefold increase in plasmid DNA concentration and a twofold increase in RNA concentration in the cell solution containing the nanotubes, presumably due to the efflux of cytoplasmic material through the damaged cell membrane. Thus, SWNTs offer a promising combination of unique mechanical properties along with strong antimicrobial activity, which makes them a great choice as a component of an antimicrobial coating.

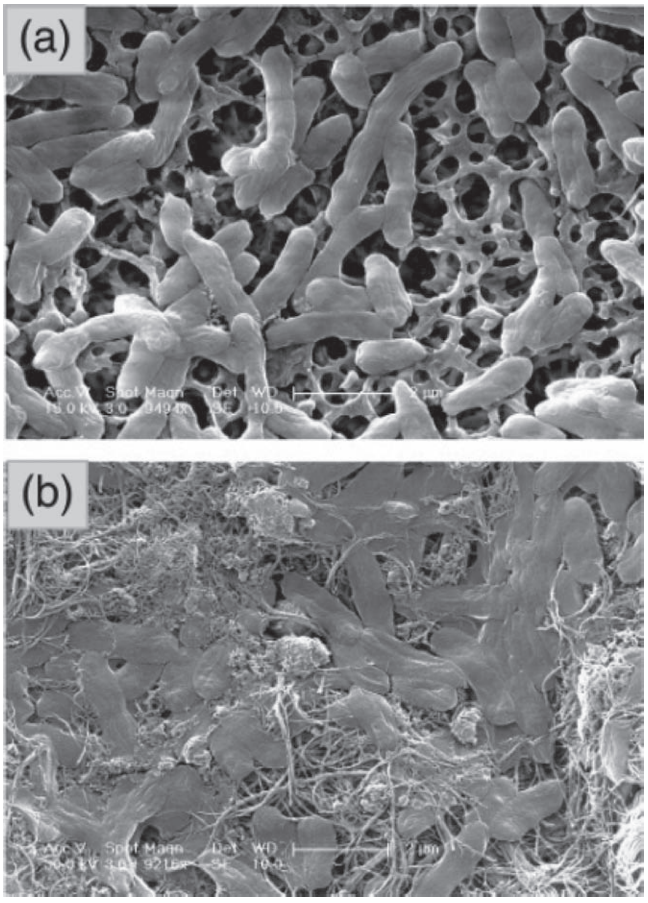

Figure 14. SEM images of *E.coli* on bare PVDF membrane (a) and SWNT deposited PVDF membrane (b). Reproduced with permission.[50] Copyright 2007, American Chemical Society.

Nanomaterials may also be of use in the packaging industry, particularly the food industry, for their bactericidal properties.[13] ZnO nanoparticles exhibit good biocidal activity on both Gram-positive and Gram-negative bacteria.[278,279] The bactericidal activity of these nanoparticles depends on their size as well as their concentration.[280,281] Li et al. discussed the fabrication of antimicrobial packaging material by coating ZnO nanoparticles on polyurethane plastic films[282] and poly(vinyl chloride) (PVC) films.[13] The films were more active against *S. aureus* (Gram-positive) compared to *E. coli* (Gram-negative) but did not show any antifungal activity. The exact mechanism for the antibacterial activity of these films is not well understood. There have been studies on the generation of ROS by nanoparticles like TiO_2 and ZnO, but this is a light or UV-activated phenomenon and is discussed in another section. The antibacterial activity of

the films may be due to the release of Zn^{+2} ions or damage to the bacterial cell membrane due to interaction with the ZnO nanoparticles.[13]

Recent years have also witnessed the development of nanoparticle-integrated textiles,[283] which have antimicrobial properties and can be used as hygienic clothing, bandages for wound healing, and in hospital settings that are susceptible to bacterial invasion. Copper and its compounds have been known to show bactericidal,[284,285] fungicidal, and antifouling properties for decades.[286] Perelshtein et al. reported the deposition of both ZnO[287] and CuO[288] nanoparticles on the surface of cotton fabrics using an ultrasound process. These fabrics exhibited biocidal activity against both *E. coli* and *S. aureus*. Based on these studies, nanoparticles seem to present great potential as an antimicrobial material. However, some recent reports suggest that higher doses of nanoparticles, quantum dots, and carbon nanotubes are associated with toxicity to human beings and might pose a serious environmental concern.[289–293]

Enzyme-Based Antimicrobial Coatings: Another approach to confer antimicrobial properties to a coating material is by incorporating enzymes that either generate antimicrobial agent or kill the bacteria on contact. In order to achieve a microbicidal effect, two types of enzymes can be used: reagent–requiring and reagentless. The first type of enzyme utilizes reagents from the surrounding solution to produce an antimicrobial agent.[36,52,294–296] In contrast, reagentless enzymes can directly produce a bactericidal effect, often as a result of cell wall degradation. Reagentless enzymes are generally active against Gram-positive bacteria because the cell wall is directly accessible.[14,52,297–309] Although several studies report the effectiveness of both types of enzymes in solution, there are a few reports showing microbicidal efficacy of enzyme-based coatings.[14,52,296–298,308,309]

Luckarift et al.[308] utilized the biomineralization property of lysozyme to synthesize bionanocomposites of silica and TiO_2, which show antibacterial effect as a result of surface-exposed lysozyme molecules. Lysozyme is a muramidase that can hydrolyze the cell wall of Gram-positive bacteria, e.g., *Micrococcus lysodeikticus* (*M. lysodeikticus*). The enzymatic activity of lysozyme was tested using two assays, one involving the use of a synthetic membrane mimic and the second based on the ability to lyse *M. lysodeikticus* scells. Interestingly, even though lysozyme was involved in biomineralization and, hence, in the formation of silica and TiO_2 composites, it retained a significant amount of hydrolytic activity (ca. 85% activity retention in silica composites based on an assay involving a synthetic substrate). The bacteriolytic activity measured based on lysis of *M. lysodeikticus* was comparable to that obtained from a substrate-based activity assay.

Nepal et al. used layer-by-layer assembly to prepare multilayer films containing carbon nanotube conjugates of lysozyme and DNA (**Figure 15**) that showed strong antimicrobial activity against *M. lysodeikticus*.[298] The films were formed as a result of electrostatic interactions between positively charged lysozyme and negatively charged DNA. Multilayer films terminating in a lysozyme-based layer exhibited strong antimicrobial activity, while those with the DNA-nanotube layer on top did not show any effect against *M. lysodeikticus*. The antimicrobial effect seen in this case was reported to be due to contact and not a result of enzyme leaching from the film.[298]

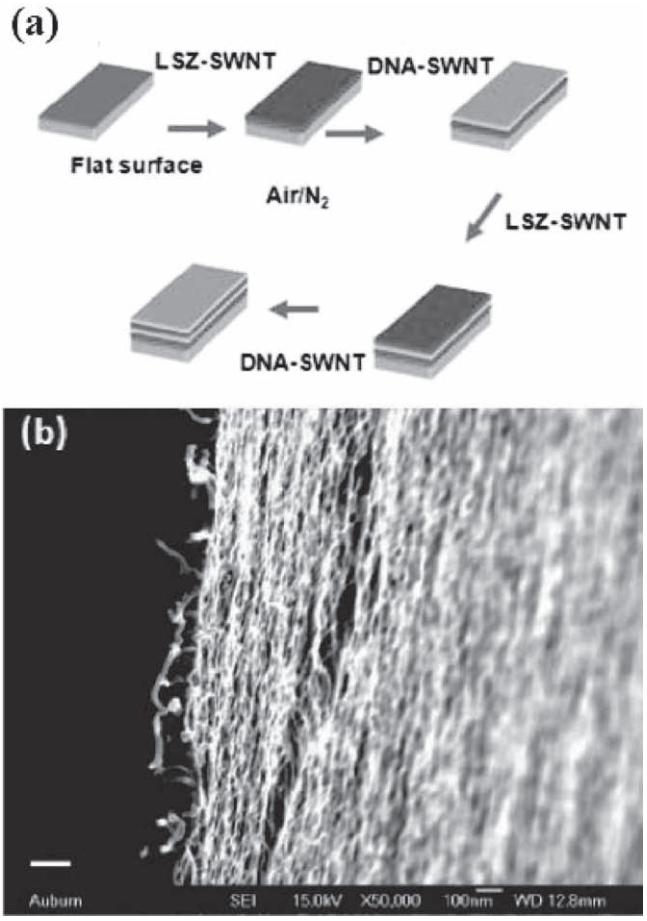

Figure 15. Lysozyme-containing antimicrobial surface. a) Schematic of layer-by-layer assembly of lysozyme-SWNT and DNA-SWNT. b) SEM image of layer-by-layer assembly of lysozyme-SWNT/DNA-SWNT of the 68th layer. Reproduced with permission.[298] Copyright 2008, American Chemical Society.

Dinu et al.[52] reported the preparation of polymer- and latex-based paint composites containing nanotube conjugates of a perhydrolase S54V (denoted as AcT). These perhydrolase-containing composites were shown to generate peracetic acid in the presence of propylene glycol diacetate and hydrogen peroxide (**Figure 16**). Peracetic acid is a potent oxidant which exhibited excellent and rapid disinfecting activity against *Bacillus cereus* spores. The enzyme lost its native activity to some extent when immobilized onto nanotubes, owing to unfavorable nanotube-enzyme interactions; the extent of loss of activity was reduced by incorporating a PEG-based linker between the enzyme and the nanotube.[52] Polymer- and latex-based paint composites containing these conjugates also showed significant amount of activity retention. Paints containing 0.15% (w/w) AcT conjugates produced 12 mM of peracetic acid within 20 min, which was more than the critical concentration required for a bactericidal effect.[52]

Photoactivated Antimicrobial Agents: Many light-activated antimicrobial agents work by producing ROS (Figure 9). Some examples include TiO_2, porphyrins, toluidine blue, rose bengal, and methylene blue.[24] Rose bengal and toluidine blue have

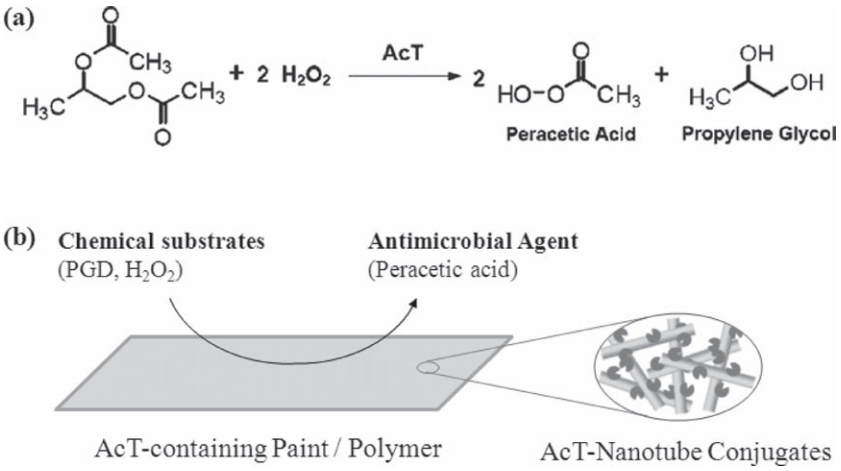

Figure 16. a) AcT-catalyzed perhydrolysis of propylene glycol diacetate to generate peracetic acid. Reproduced with permission.[52] Copyright 2010, Wiley-VCH. b) Antimicrobial polymer or paint films containing AcT-nanotube conjugates.

been immobilized in a cellulose matrix,[118,126] while methylene blue and toluidine blue along with gold nanoparticles have been incorporated in polysiloxanes and polyurethanes,[127] which are commonly used catheter materials. Also, porphyrins have long been used as antimicrobial agents.[10,310–313]

Bozja et al. reported the fabrication of porphyrin-grafted nylon fibers.[10] The compounds used were Protoporphyrin IX (PPIX) and Zinc protoporphyrin IX (Zn-PPIX). PAA was first grafted on nylon fibers and the porphyrin compounds were in turn attached to PAA resulting in the formation of nylon-grafted porphyrin. These nylon samples showed excellent biocidal activity against *S. aureus*. The bactericidal efficiency increased with an increase in the intensity of incident light. The authors claimed that these materials would be readily effective in normal indoor lighting which has an illumination of about 30 000 lux. The samples with Zn-PPIX showed more activity than those with PPIX against *S. aureus*. However, both the compounds did not show any substantial activity against *E. coli*. The above results and some other reports show that porphyrins work better in the case of Gram-positive bacteria, which have weaker cell walls compared to Gram-negative bacteria.[10,311–313]

Apart from being a self-cleaning material, TiO_2 has also been widely used as a photoactivated antimicrobial agent. Matsunaga et al.[314] reported the biocidal activity of platinum-loaded TiO_2 particles in the presence of UV or visible light. TiO_2 has been found to be active against a number of bacteria like *E. coli*,[315] *S. aureus*,[316] MRSA,[317] and *B. anthracis*.[318] There are some studies that indicate that the cell death due to TiO_2 occurs as a result of photocatalytic degradation of the cell wall and cell membrane or by direct inhibition of coenzyme A (CoA), which then restrains cell respiration.[24,51]

Moudgil and co-workers[51] prepared TiO_2-coated multiwalled carbon nanotubes. The modified material was used against *B. cereus* and found to be twice as effective as commercially available TiO_2. Xu and co-workers produced wrinkle-free fabrics with tethered TiO_2 and silver nanoconjugates.[8] Silver-anchored TiO_2 was synthesized using the procedure of Zhang et al.[319] The conjugates were tethered to the fabrics and showed very good biocidal activity against *E. coli*, when tested in sunlight, artificial sunlight, and in the dark. SEM studies showed deactivated cells on the surface of the modified fabrics as compared to bare fabric surfaces, which showed healthy cells. The advantage of using conjugates of silver and TiO_2 is that the antimicrobial activity is present both under light and in dark conditions. The fabrics were shown to retain their antimicrobial activity even after repeated washing and thus are highly suitable for practical applications.

Even though TiO_2 presents an excellent material, both as a self-cleaning and an antibacterial agent, there are some problems associated with TiO_2. The main problem associated with TiO_2 is that its absorbance peak lies mainly in the near-UV region. Researchers have attempted to increase the efficiency of TiO_2 and shift its absorbance to the visible region by doping it with nitrogen[320] or metals such as gold, silver, platinum, palladium, copper, etc.[51,321–328] Dopants help to narrow the band gap of TiO_2, which causes its absorbance to shift towards the visible spectrum, thereby increasing the efficiency of the modified TiO_2.

A common problem associated with TiO_2 and other light-activated materials is that their incorporation in organic materials leads to the degradation of the matrix because of ROS generation. It has long been observed that the exposure of TiO_2-based paints to sunlight leads to the formation of a white powdery material on the surface; this phenomenon is referred to as the chalking effect.[146] Recently, Parkin and co-workers[329] developed inert silicone polymer coatings (commonly used in catheters) with covalently bound alternative photoactive materials (methylene blue and toluidene blue O). These polymeric materials have high bactericidal efficiency even on exposure to a low power laser for 4 min. Recently, Lin et al.[330] fabricated PVC films dip- coated with TiO_2 that showed promising bactericidal effects. Incorporation of photoactive materials and generation of ROS at the surface increases the potency greatly in contrast to their bulk incorporation within a hydrophobic polymer owing to the short diffusional distances of the ROS within the polymer, which prevents them from reaching the surface.[329]

3.2.3. Release- and Contact-Based Antimicrobial Coatings

There are a few studies that report antimicrobial coatings that work through both contact-based as well as release-based mechanisms.[53,160,193,255,331] Li et al.[53] fabricated dual-function antimicrobial materials based on silver ions or nanoparticles and immobilized quarternary ammonium salts (QAS). Multilayers of PAH and PAA containing embedded silver ions or nanoparticles were deposited. Following the incorporation of silver ions or nanoparticles, multilayers were capped with alternate layers of PAH and silica nanoparticles at the top. The silica nanoparticles had modified surface chemistry, which was exploited to tether QAS. QAS is a contact-based antimicrobial agent, as opposed to silver which produces its antimicrobial

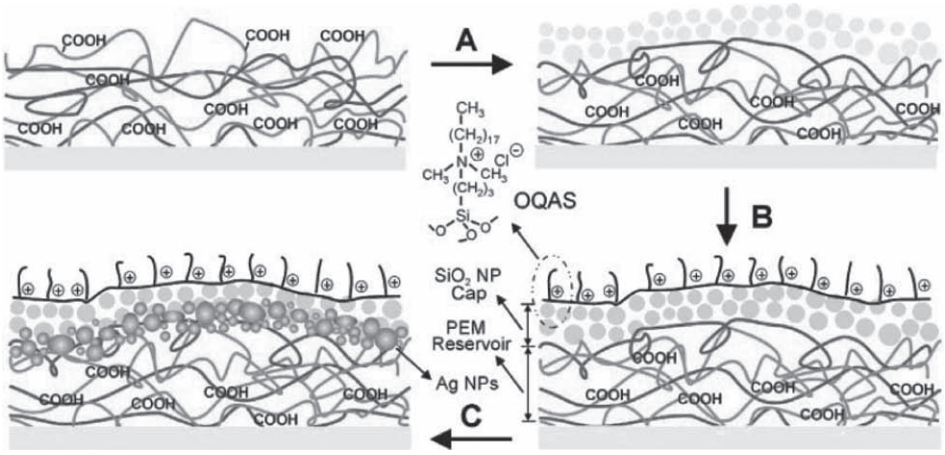

Figure 17. Scheme showing the design of a dual functional antibacterial coating with both quarternary ammonium salts and silver. The coating starts with the layer-by-layer deposition of a reservoir made of bilayers of PAH and PAA. A) A cap region made of bilayers of PAH and SiO_2 NPs is added to the top. B) The SiO_2 NP cap is modified with a quaternary ammonium silane, OQAS. C) Ag^+ ions are loaded inside the coating using the available unreacted carboxylic acid groups in the multilayers. Reproduced with permission.[53] Copyright 2006, American Chemical Society.

effect through a release-based mechanism and thus is depleted slowly. This fabrication strategy (illustrated in **Figure 17**) gave rise to a more lasting microbicidal effect because it retained significant antimicrobial activity due to the QAS even after the depletion of the embedded Ag ions or nanoparticles.

Sambhy et al.[193] reported another type of dual-action antibacterial material based on silver bromide nanoparticles and a cationic polymer, poly(4-vinyl-*N*-hexylpyridinium bromide). Silver bromide is sparingly soluble in water and thus releases silver at a more controlled rate than other, soluble, salts of silver. The cationic polymer was shown to be nontoxic[332] to mammalian cells but exhibited biocidal activity by causing bacterial membrane disruption on contact.[47,228,333]

The cationic antimicrobial agents have strong biocidal activity but are fouled by the deposition of dead microbes, which may block the functional groups and reduce their antimicrobial activity. On the other hand, surfaces modified with PEG or other zwitterions can only inhibit the attachment of microbes without exterminating pathogenic microbes. Cheng et al.[160] reported a strategy for developing biocompatible polymer surfaces that not only have strong biocidal effect but also release the dead bacteria, thus combining the advantages of the cationic antimicrobial polymers and overcoming some of their shortcomings. A switchable polymer surface was designed that contains a cationic precursor, poly(*N*,*N*-dimethyl-*N*-(ethoxycarbonylmethyl)-*N*-(2′-(methacryloyloxy)ethyl)-ammonium bromide) (pCBMA-1 C2). The pCBMA-1 C2 has potent biocidal properties and can kill more than 99.9% of *E.coli* K12 in 1 hour. In an aqueous environment, the pCBMA-1 C2 is slowly hydrolysed into non-fouling zwitterionic polymer, pCBMA, which releases 98% of the dead bacteria deposited on the initially cationic surface. The zwitterionic surface generated after hydrolysis prevents the nonspecific adsorption of proteins in addition to bacteria. This strategy of switching from a cationic surface to a zwitterionic surface may be used to protect implants from infection and to destroy pathogens in an infected area.

Ho et al. reported another method that makes use of both release-based and bacteria repelling approaches.[331] The authors designed a polymeric coating wherein silver nanoparticles were generated in situ and embedded in the polymeric matrix. Additionally, the surface of the coating was modified with microbe-repelling PEG groups.[331] This dual functionality was achieved by using a copolymer of PEI that is derivatized with double bonds and 2-hydroxyethyl acrylate (HEA). While PEI from the copolymer facilitated silver nanoparticle formation, the hydroxyl functional groups of HEA could be exploited for the covalent attachment of PEG. The authors showed that the size, distribution, surface availability, and hence the antimicrobial activity of the silver nanoparticles can be controlled by manipulating the weight fraction of PEI in the film.[331] In order to impart adhesion-resistance activity to the silver-nanoparticle-containing polymer films, PEG was grafted onto the surface. As far as antimicrobial effect was concerned, for short incubation periods (≤24 h) no colonies were seen when the film was rich in silver. On the other hand, the effect diminished when films were incubated in a *S. aureus* suspension (in PBS) for longer periods, possibly due to exhaustion of silver from the film. Nonetheless, PEGylated surfaces allowed four to eight times fewer microbes to adhere than non-PEGylated samples, which may help lower the risk of infection in case of exhaustion of silver.[331]

Antimicrobial multilayers containing an AMP, gramidicin A, were prepared by the layer-by-layer assembly of cationic PLL and an anionic complex prepared by interfacing gramicidin A with hydrophobically modified carboxymethylpullulan (CMP), which is an amphiphilic and anionic polysaccharide.[255] While CMP allows efficient solubilization of gramicidin A in aqueous solution without denaturation, layer-by-layer assembly of this complex along with cationic PLL allows the formation of an active antimicrobial coating. Gramicidin A-containing films had strong antimicrobial activity, which is suggested to be a result of the dual mechanism, i.e., contact-mediated killing and release of gramidicin A in the surrounding solution.[255] The advantage in this case was that the films retained the microbicidal activity after use, indicating that the peptide did not leach completely.

4. Marine Antifouling Coatings

After the recent ban on tributyltin (TBT)-releasing paints and coatings by the International Maritime Organization (IMO) and the Marine Environmental Protection Committee (MEPC),[17,334,335] the generation of tin-free antifouling coatings has become more important.[17,18,335,336] As a result, several tin-free self-polishing and antifouling coatings replaced the conventional TBT-based coatings.[17,18,20] However, the mechanism of action remained the same: release of a biocidal agent. Hence, biocide-releasing and self-polishing coatings can be categorized as conventional and derived coating formulations and the details of these coatings can be found in recent reviews.[17,18,20] The biocides used in preparing tin-free self-polishing coatings include oxides of copper and zinc and isothiazolone.[20,337,338] Initially, water-insoluble paint matrices (e.g., vinyl, epoxy, acrylic, or chlorinated rubber polymers) were used for incorporating the biocidal agents; however, water-soluble matrix paints (e.g., rosin) were developed in order to maintain the antifouling efficiency over a longer period, by incorporating a binder that dissolves in seawater. Further improvements in the antifouling effect were achieved by using booster biocides or co-biocides (e.g., copper and zinc pyrithiones) and by controlling the degradation rates of the main binder resin.[17,339] Thus, factors such as binder systems and co-biocides play a key role in complementing the biocidal action of copper oxide. While TBT-based self-polishing coatings are being replaced by other biocide-releasing coatings, the environmental toxicity of these compounds is also under scrutiny.[17,18,20] Therefore, there is a significant interest in developing non-toxic technologies.

The focus of this part of the review is on recent and unconventional techniques that are being investigated to develop non-release coatings. These approaches are mainly based on controlling physicochemical and mechanical properties, which impact the interactions between marine organisms and the surface. These properties include surface free energy, wettability, elasticity, and surface topography.[336]

4.1. Amphiphilic Antifouling Polymer Coatings

While highly hydrophilic (PEG-based) coatings inhibit the attachment of proteins, bacteria, and marine organisms, the attached species get easily released from hydrophobic polymer-based (fluoropolymer and PDMS) surfaces.[336] Amphiphilic copolymers, on the other hand, possess both hydrophobic and hydrophilic blocks. Due to their dual nature, the adherence of proteins or glycoproteins to amphiphilic copolymer-functionalized surface via either hydrophobic or hydrophilic interactions becomes energetically unfavorable, thereby weakening the interactions of the organism with the surface.[340]

Gudipati et al. synthesized amphiphilic copolymers with optimal nanoscale heterogeneity in terms of composition and topography by crosslinking a hyperbranched fluoropolymer (HBFP) and PEG in different weight percentages.[340] The topographical heterogeneity was attributed to the phase segregation between HBFP and PEG components. Analysis of the surface by XPS indicated that low surface energy fluoropolymer groups were present at the polymer-air interface.[341] On the other hand, when HBFP-PEG coatings were soaked in water and were subjected to contact angle measurements, they exhibited lower contact angles than dry HBFP-PEG coatings. The change in hydrophilic behavior was attributed to surface reconstruction, which was believed to be driven by the swelling of the PEG domains and the energetic favorability offered by segregation of PEG groups to the solid-water interface. Similar, environment-dependent surface reconstruction by flipping of the side chains was reported by Krishnan et al.[56] in the case of comb-like block copolymer with amphiphilic side chains (**Figure 18**). When evaluated for their marine antifouling and fouling release properties using settlement and release assays involving *Navicula* diatoms and *Ulva* spores, amphiphilic coatings showed comparable, and in some cases better, antifouling and release properties than PDMS-based coatings.[56] A similar strategy was employed by Feng et al.[342] and Joshi et al.[343] in

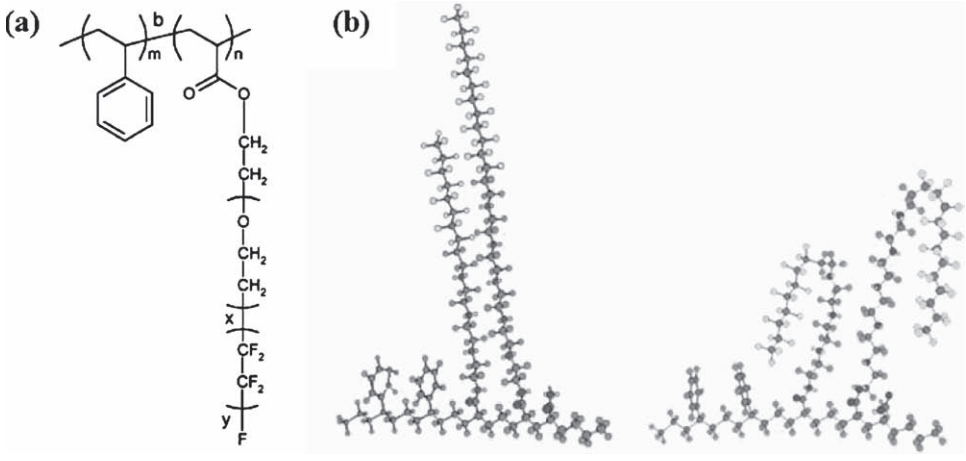

Figure 18. Anti-biofouling amphiphilic copolymers. a) Comb-like block copolymer with amphiphilic (ethoxylated fluoroalkyl) side chains. b) Proposed mechanism for surface reconstruction of the amphiphilic side chains upon immersion of the surface in water. The picture on the left indicates the orientation of the side chains in air whereas that on the right shows the effect of water immersion. Reproduced with permission.[56] Copyright 2006, American Chemical Society.

preparing anti-biofouling surfaces based on perfluoroether-PEG and polyurethane-polyol amphiphilic polymers. Recently, Ober and co-workers[344] studied the effect of the modulus of the base layer on the antifouling properties of amphiphilic block copolymers. They found that the use of a low-modulus thermoplastic elastomer base for amphiphilic copolymers enhanced the release of sporelings of *Ulva* by hydrodynamic shear.

4.2. Enzyme-Based Antifouling Coatings

Similar to microbial biofilm formation, several reports suggest that marine organisms use a variety of bioadhesives (i.e., proteins and/or glycoproteins) while adhering to synthetic surfaces such as ship hulls.[345–347] For instance, glycoproteins, carbohydrates, and proteins are prominently present in the case of fouling caused by spores of the green algae *Ulva*,[345] diatoms,[346] and barnacles,[347] respectively. Considering their proteinaceous nature, it is reasoned that these bioadhesives can be degraded by using proteases, which will in turn decrease the adhesion strength of the colonizing microorganisms. In various solution-based studies, protease-containing formulations (e.g., subtilisin-based Alcalase and Savinase) have been shown to be effective in reducing the adhesion of barnacle cypris larvae, diatom cells, algal spores, and fouling microorganisms.[348–352] Several of these enzyme-based formulations have been critically summarized and evaluated by Olsen et al. and Kristensen et al. in their reviews.[36,37]

Kristensen et al.[353] demonstrated the antifouling potential of coatings containing starch, glucoamylase, and hexose oxidase, which collectively lead to the generation of hydrogen peroxide (H_2O_2) in an aqueous environment (**Figure 19**). The coating was able to inhibit biofilm formation by most of the marine proteobacteria tested, as a result of H_2O_2 release.[353] However, similar results were not seen when the enzyme-containing coatings were tested in a biofilm reactor with filtered seawater, where a similar number of cells were seen on the control and H_2O_2-releasing surfaces. The authors suggest that this result is due to the physical adsorption of nonviable cells.[353] On the other hand, these enzyme-containing coatings showed an antifouling effect comparable to that of commercially available self-polishing coatings in raft trials performed in seawater. The effect was demonstrated in terms of reduced number of late-juvenile to adult barnacles attached to the coatings. While the enzymatic system remained relatively clean for 4 weeks, there was an increase in fouling thereafter based on visual inspection of the panels. The panels were then subjected to a shear stress test, suggesting decreased number of barnacles along with slime and diatoms attached to the enzyme-containing coating than the reference coating. The antifouling effect, observed in this case, has been attributed to significant release of H_2O_2 from the coating. However, a disadvantage of this method is that the concentration of H_2O_2 released has to be above the MICs for marine bacteria and diatoms, which can otherwise settle and attach to form a conditioning layer, resulting in further decreased H_2O_2 flux and increased fouling.

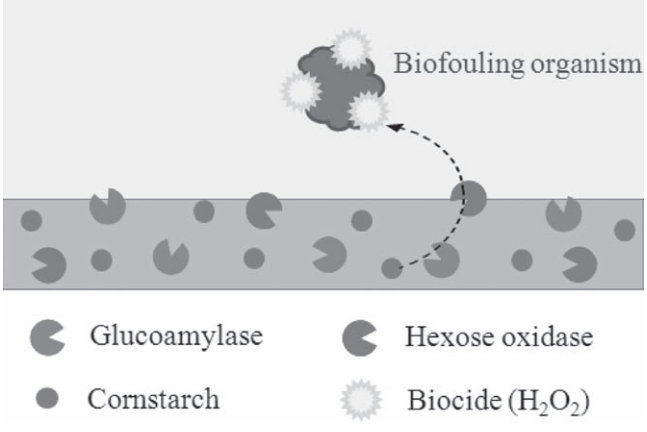

Figure 19. Enzyme-containing antifouling coating. Biodegradation of cornstarch by glucoamylase and hexose oxidase leads to generation of biocidal hydrogen peroxide (H_2O_2) in an aqueous environment.[36]

As suggested earlier, fouling organisms mostly use their superficial biological molecules such as proteins to make the first contact with a synthetic substrate. Consequently, the use of proteases to control the fouling of surfaces has been explored in several studies.[54,349,351,352] Recently, Tasso et al.[54] prepared maleic anhydride-based copolymer thin films that were surface-functionalized with a protease in order to realize the antifouling and fouling-release potential of protease-based coatings. The hydrophilic and high-swelling poly(ethylene-*alt*-maleic anhydride) (PEMA) copolymer was used to obtain high enzymatic activity. As a result, these enzyme-containing coatings were found to be effective in inhibiting settlement and attachment of two of the major marine foulants, green algae (*Ulva linza*) and diatoms (*Navicula perminuta*). The spore-release effect correlated well with the catalytic activity of the immobilized protease Subtilisin A (based on hydrolysis of a peptide-based substrate), suggesting that the mechanism of spore-release is through proteolytic degradation of the bioadhesive used by spores.[54] While the antifouling potential of proteases has been demonstrated in various solution-based studies, exclusivity of enzymatic mechanisms had been questioned.[36] However, Tasso et al. utilized maleic anhydride copolymer as an efficient enzyme immobilization platform to achieve covalent surface attachment of purified Subtilisin A, thereby avoiding spurious effects caused by stabilizers or preservatives.[54] While the polymer–enzyme formulation used by Tasso et al. showed an excellent antifouling potential against attachment of *Ulva* spores, the general applicability of protease-based coatings would be governed by physicochemical properties of the polymer of interest, enzyme stability in polymeric environment, and, most importantly, its surface availability and accessibility.[54]

Olsen et al.[36] have critically reviewed the obstacles that enzyme-containing antifouling formulations face in realizing their practical applicability. Stability and potential self-degradation of enzymes (i.e., proteases) is one of the major pitfalls to be resolved in order to demonstrate the feasibility of such coatings. While immobilization on a microscale support has been a conventional approach for stabilizing the enzymes, nanomaterials were recently shown to be excellent enzyme supports in terms of stabilization and enzyme loading.[16,52] Moreover, incorporation of enzyme-based nanomaterial biohybrids were shown to have negligible effects on paint properties,[52] thereby

demonstrating nanobiocomposites to be an acceptable formulation to impart antifouling characteristics to the coated surface.

4.3. Antifouling Surfaces with Microtopography

With the increasing knowledge of the effect of surface chemical composition and topography on wettability, various self-cleaning surfaces have been conceptualized and prepared for different applications.[132,354–359] Several naturally occurring superhydrophobic surfaces (e.g., lotus leaf) are composed of hydrophobic materials with some topographical texture, which contributes to the observed self-cleaning effect.[141–145] In the context of biofouling, nature-inspired coatings with rough topographies have been designed for underwater applications.[55,360–362]

Petronis et al.[360] designed and prepared silicone surfaces with microstructures and well-defined surface chemistry for antifouling purposes. Specifically, the surface consisted of arrays of pyramids or riblets with a scale ranging from 23 to 69 μm in height and from 33 to 97 μm in periodicity. Field experiments were performed to test the resistance of these surfaces to macrofouling by barnacles. Riblets, which mimic the topography of shark skin, appeared to be more resistant to fouling than pyramids, though they had the same inclination angles and surface area.[360] Recently, Brennan and colleagues extensively investigated the effect of topographic features on marine biofouling.[361,362] Several design patterns, including channels, ridges, pillars, pits, and ribs (Sharklet AF, biomimetic topography inspired by shark skin), were fabricated on PDMS elastomer using standard photolithography techniques. Based on their studies of the performance of several microtopographies, they concluded that an effective coating should possess topographical features that are smaller than either the dimension of marine organisms or the parts of organisms that explore the surface while settling.[361,362] For instance, *Ulva* zoospore assays on these engineered microtopographies suggested that the Sharklet AF topography, which had feature dimensions smaller than the spore body, significantly reduced settlement density by ca. 86% relative to that on a smooth PDMS coating.[361] The *Ulva* zoospore settlement assay used in these studies involved the incubation of slides with the spore suspension for 1 h followed by fixation with 2% glutaraldehyde and spore quantification.[361,362] De Nys and co-workers[363,364] reported that the adhesion strength is governed by the number of attachment points of the marine organism to the coating, which depends on the difference between the dimensions of the organism and the characteristic length scale of the coatings. As a result, one can expect weak attachment and, therefore, an antifouling effect if the settling organism is slightly bigger than the topographical features. Based on these previous results, Efimenko et al.[55] suggested that coating with a topographical pattern with a single length scale is unlikely to prohibit marine biofouling at large, which involves a very diverse range of marine organisms (e.g., bacteria, algae, diatoms, and barnacles). Therefore, the authors reasoned that a coating with a hierarchically wrinkled surface topography (HWST) having patterns of different length scales ranging from tens of nanometers to a fraction of a millimeter can be employed as antifouling coating for underwater applications (**Figure 20a**).[55] In their

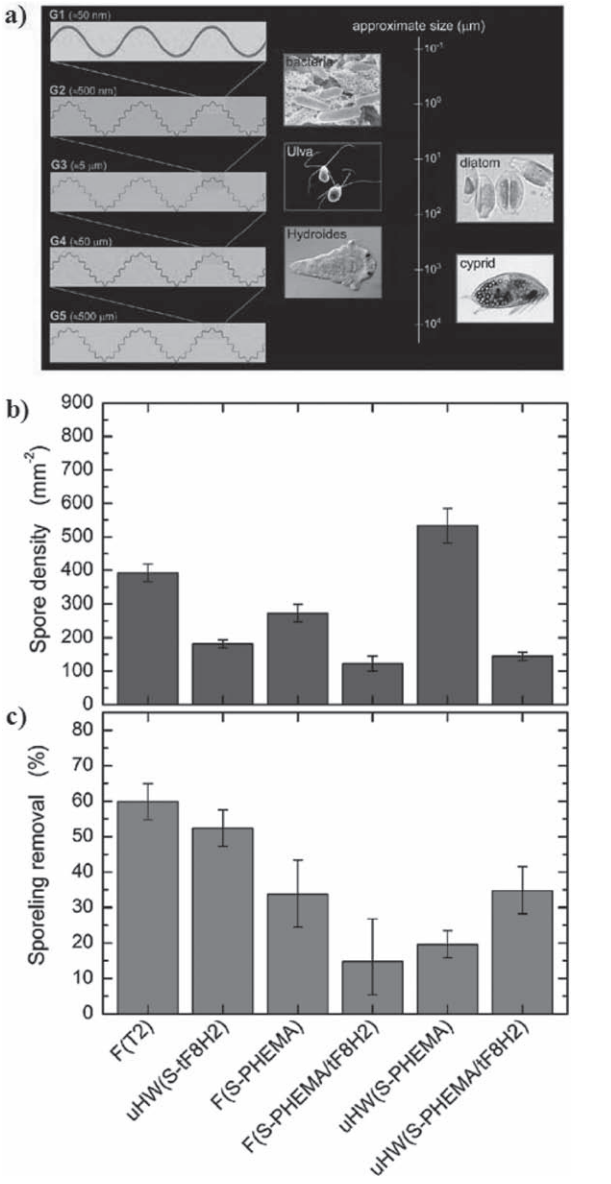

Figure 20. Hierarchically wrinkled (HW) coatings for marine antifouling. a) Schematic depicting the structure of HWST coatings comprising nested wrinkled topographies from tens of nanometers to a fraction of a millimeter. The right panel shows typical dimensions of selected marine organism. b) Settlement of zoospores on and c) removal of sporelings of *Ulva* from various substrates: F(T2), flat Silicastic T2; uHW(S-tF8H2), uniaxial HWST Sylgard-184/tF8H2; F(S-PHEMA), flat Sylgard-184/PHEMA, F(S-PHEMA/tF8H2), flat Sylgard-184/PHEMA-tF8H2; uHW(S-PHEMA), uniaxial HWST Sylgard-184/PHEMA; uHW(S-PHEMA/tF8H2), uniaxial HWST Sylgard-184/PHEMA-tF8H2. uHWST = uniaxial HWST, tF8H2 = 1H, 1H, 2H, 2H-perfluordecyltrichlorosilane, PHEMA = poly(2-hydroxyethyl methacrylate), Reproduced with permission.[55] Copyright 2009, American Chemical Society.

design, individual wrinkle generations were arranged in nested pattern, where each larger pattern resides underneath and represents a scaled-up version of the smaller generation (Figure 20a). Their field experiments revealed that these patterned coatings inhibited biofouling to a significant extent compared to their smooth counterparts, for a prolonged period (up

to 18 months). The importance of surface chemistry in inhibiting biofouling was found to be species-dependent. While barnacle biofouling was minimized on topographically corrugated coatings without tuning the surface chemistry, a combination of surface composition and topography was required to inhibit the attachment of spores and sporelings of *Ulva* (Figure 20b,c). As shown in Figure 20b, the spore settlement density was lowest on the wrinkled fluorinated and on the smooth and wrinkled amphiphilic surfaces. The wrinkled fluorinated surface also showed release properties that were comparable to those of the PDMS standard, Silastic T2 (Figure 20c). On the other hand, the percent removal of sporelings from hydrophilic and amphiphilic surface was low, irrespective of the nature of topography (Figure 20c).

5. Conclusion and Future Prospects

We have reviewed the key approaches for designing antifouling surfaces, with special emphasis on advances made in the past decade. Most approaches for preventing biofouling caused by proteins, microbes, and marine organisms involve at least one of the following two mechanisms: resistance to adhesion of biocontaminants or degradation of the biocontaminants.

The most common approach for preventing the adhesion of proteins or microbes involves the functionalization of surfaces with PEG or oligo(ethylene glycol) groups. Major advances in recent years include the development of new approaches for the functionalization of surfaces with PEG, the development of alternatives to PEG for resisting protein and microbial adhesion, and approaches based on the control of surface modulus and topography. Even though most of these surfaces resist the adsorption of proteins and bacteria, they do not address the issue of deactivating the microbes, which is more vital for combating microbial contamination. As far as coatings for marine antifouling applications are concerned, surfaces with low surface energy or with an optimized surface topography show promising results. However, more studies testing the cost effectiveness, durability, and stability of such coatings under seawater are necessary.

Important approaches for killing or degrading bacteria include the design of surfaces that release antibiotics or silver, surfaces functionalized with polycations or antimicrobial peptides (AMPs), photoactive surfaces, and coatings containing enzymes. Antibiotics- and silver-releasing systems have long been used to kill microbial contaminants. Their widespread use, however, has led to emergence of antibiotic- or silver-resistant bacteria. Moreover, antibiotic- and silver-containing coatings act through a release-based mechanism and are therefore exhausted over time. Recently, approaches involving generation of microbicidal coatings based on AMPs, enzymes, polycationic polymers, and photoactive materials have gained significant attention and are promising. However, even these materials have certain drawbacks. For example, biomolecules such as AMPs and enzymes may lose activity upon incorporation into coatings. Nonetheless, the activity of AMPs and enzymes can be improved by using appropriate linkers and nanomaterials as immobilization supports. Surfaces functionalized with amphipathic polycations show promising antimicrobial activity on contact. Even though these coatings cannot prevent the deposition of cell debris, the antimicrobial properties can be recovered using surfactant wash. The efficacy of photoactive materials, such as TiO_2, is largely limited by the intensity of incident radiation. The absorbance of TiO_2 lies predominantly in the UV region, and hence researchers are working on the production of TiO_2 doped with other substances such as platinum, silver, copper or nitrogen to improve its efficiency in the visible region. Porphyrins represent an interesting alternative to TiO_2 because they have photoactive antimicrobial effects in the visible region. However, the antimicrobial activity may be lost with continued irradiation due to photobleaching.

In summary, we have reviewed several strategies for designing effective antifouling coatings. Most of these strategies are helpful in combating the problem of fouling but several of them are also associated with shortcomings related to stability, toxicity, or the method of fabrication. The active pursuit of new strategies and materials to overcome these shortcomings over the last decade is encouraging and gives hope that better materials will be developed in the future.

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