Paper 2

Anti-Infectious Surfaces Achieved by Polymer Modification

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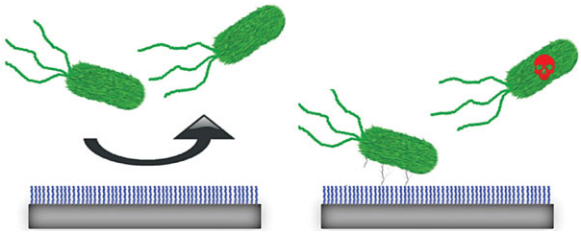
This review article presents the potential strategies of material surface modification by using polymeric approaches to design anti-infectious materials. Supported by recent examples such as utilizing either microbe lethal or repelling properties, different strategies to the design of antimicrobial surfaces are reviewed. The deposition strategies for creating polymer modified antibacterial surfaces are described.



1. Introduction

In natural environments and especially in hospitals, major issues arise from bacterial adhesion to surfaces. Surface adherent microorganisms usually grow and produce a matrix of extracellular polymeric substances to eventually form a biofilm,[1] within which bacteria are more resistant to usual treatments by antimicrobial agents than their planktonic counterparts, which thus raise undesirable economic and health concerns. When such biofilms are formed on either the surface of medical devices used in hospitals or on implants, patients might suffer from acquired infections like ventilator associated pneumonia, catheter associated urinary tract infection, and central line-associated blood stream infections just to cite few examples.[2] It has been estimated through statistics that in 2002 about 1.7 million persons in the USA suffered from such hospital acquired infections, which did lead to 99 000 deaths.[3] In 2005, hospital acquired infections were the main cause of death and in 2006 the number of patients suffering from these infections was more than from any other disease.[4] There is thus an ever growing demand for materials that could resist bacterial infections and a keen interest in designing antimicrobial surfaces which are both biocompatible and resistant to biofilm formation.[5] So far, the efforts to inhibit biofilm growth on medical devices and food conditionings to prevent pathogenesis have been mainly focused on avoiding the biofilm formation, and in particular on both the prevention of initial microbial adhesion as well as the inhibition of the proliferation of adherent bacteria.[6] The composition and structure of the biofilm have been systematically studied and reported to understand its assembly and functions to identify an effective way to inhibit or break down biofilm formation.[7] In this review article, we therefore at first give a short overview of relevant biofilm properties such as composition, structure, and function before focusing on the fight against biofilm formation based on approaches based on surface modification with polymers.

Figure 1a schematizes the current general model of the biofilm structure formation onto a solid surface. Biofilm formation is initiated by the attachment of bacteria to a surface. A micro colony then forms through division of the cells, and production of the biofilm matrix is induced. The extracellular polymeric substances produced by bacteria after adhesion to surfaces and their function in relevance with biofilm formation are summarized in Table 1. Other bacteria can then be recruited as the biofilm expands owing to cell division and further production of matrix components takes place. As shown in Figure 1b, the major matrix components are composed of polysaccharides, proteins, and DNA that are distributed between the cells in a non-homogeneous pattern, setting up differences between regions of the matrix. The classes of weak physicochemical interactions and the entanglement of biopolymers that dominate the stability of the extracellular polymer

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substances that compose the EPS matrix are depicted in Figure 1c whereas in Figure 1d, the result of a molecular modeling simulation shows the interaction between the alginate exopolysaccharide (right) and the lipase extracellular enzyme (left) of *Pseudomonas aeruginosa* in aqueous solution. The colored spheres represent 1,2-dioctylcarbamoyl-glycero-3-O-octylphosphonate in the lipase active site (as assigned in the crystal structure), the green spheres represent Ca^{2+} ions. The structure is stabilized by the interaction of the positively charged arginine and histidine amino acids (indicated in blue) with the polyanionic alginate. Water molecules are not shown.[7d] The major components of the EPS differ from one type of microorganism to the other. Whereas the alginate exopolysaccharide is produce by *Pseudomonas aeruginosa*,[7d] polymeric β-1,6-N-acetyl-D-glucosamine (poly-β-1,6-GlcNAc) is involved in the biolfilm formation of several microbes as evidenced, for the first time by chromatography and mass spectrometry subsequent to hydrolysis of the glycosidic linkages of poly-β-1,6-GlcNAc with hexosaminidase or dispersin B (DspB).[8] As reported in this publication, prevention of biofilm adhesion could be observed with *Escherichia Coli*, *Staphylococcus epidermidis* as well as with *Yersinia pestis*, *Actinobacillus actinomycetemcomitans*, and *Pseudomonas fluorescens*. Both growth of cultures in the presence of DspB or treatment of preformed biofilms with this enzyme resulted in the inhibition of biofilm formation, which clearly indicated that poly-β-1,6-GlcNAc plays a crucial role in the formation and stabilization of various bacterial biofilms.

Knowing the biophysical and biochemical properties of bacteria is important to understand their interaction with materials, which is a crucial stage in biofilm formation. In fact, because of their small size, low density, surface charge, and variable degrees of cell surface hydrophobicity, bacteria are considered as living colloids that interact with surfaces according to the Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory.[9] The forces to which bacteria are subjected to any separation distance are therefore the sum of the van der Waals forces, electrostatic, and acid–base interaction as well as Brownian motion. In particular, van der Waals forces, which result from induced dipole interactions between the colloidal particles and molecules present at the surface, are generally attractive. Upon approaching the surface, microorganisms will thus be attracted or repelled, depending on the resultant of these different non-specific interaction forces. As the magnitude of these contributions depends on the properties of the two interacting partners, the rate of microbial adhesion to surfaces is highly influenced by surface hydrophobicity and the charge of the bacterial outer membrane and, of most interest, by the physicochemical properties of the materials.[6] Over the course of bacterial adhesion, which involves reversible and irreversible adhesion processes, surface properties thus have a strong influence. The common factors of influence are related to surface topography and surface chemical composition.[10] Since these factors combine with bacterial properties to influence bacterial adhesion, no general rules exist which would be relevant for all bacterial species and strains, due to the wild variety of their properties. Nevertheless, some trends have been highlighted. Among surface physicochemical properties, effects of surface hydrophobicity and surface charges have been widely studied in the frame of the DLVO theory[9,11] demonstrating their influence as components of the free energy.[12] However, limitations of the DLVO theory to describe the early adhesion process were clearly evidenced, due to partial consideration of the role of bacterial organelles[13]

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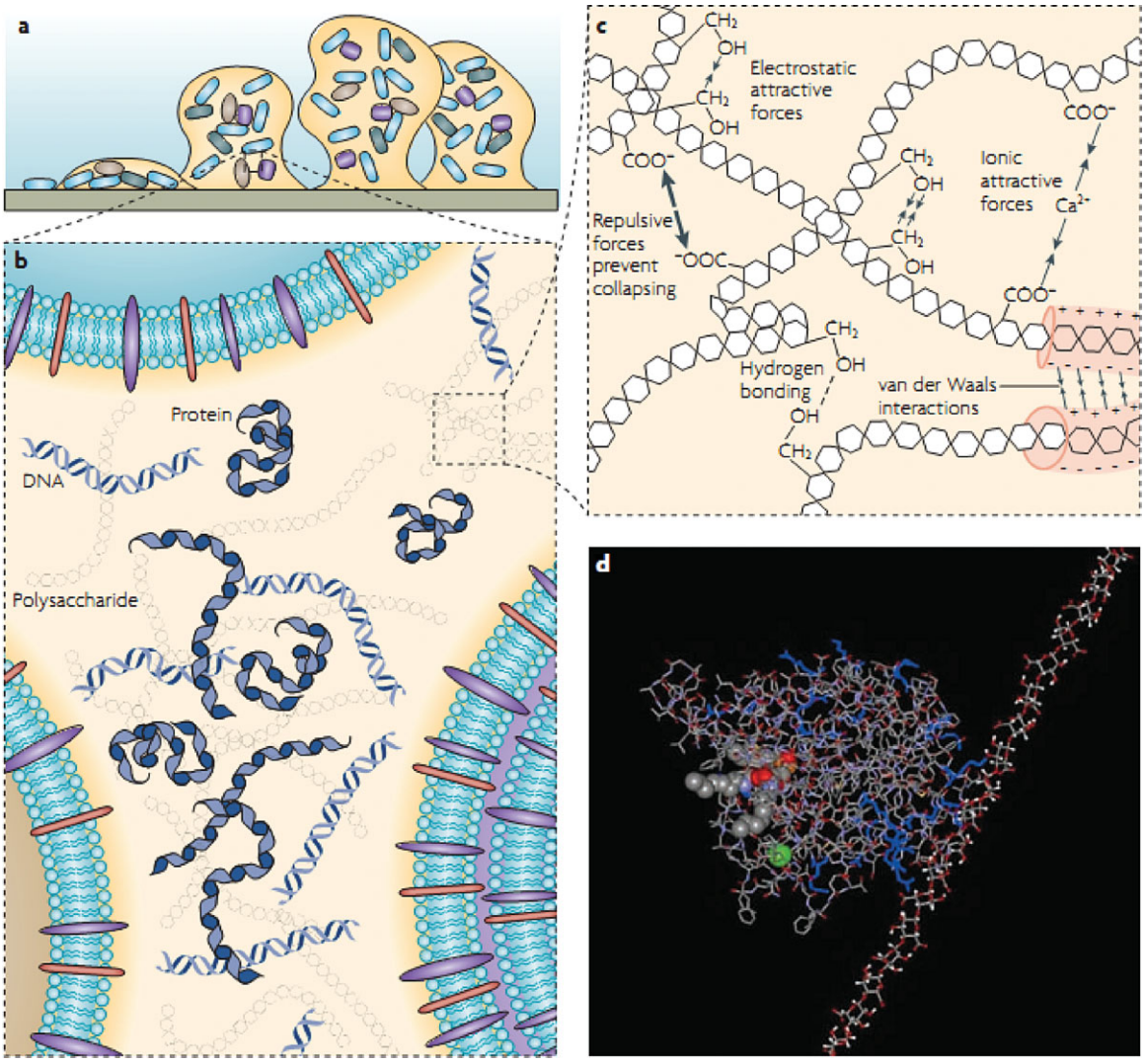

Figure 1. The extracellular polymeric substances (EPS) matrix at different length scales. (a) a model of biofilm onto a solid surface, (b) major components of the biofilm matrix, (c) model of weak physicochemical interaction in biopolymer, (d) molecular modeling simulation of the interaction between an exopolysaccharide and the lipase extracellular enzyme in aqueous solution. Reproduced with permission.[7d] Copyright 2010, Macmillan Publishers Limited.

and sensing ability of bacteria.[14] Experimentally, some studies have suggested that the hydrophobicity of adherent bacteria correlates with the one of the surface.[15] This rule is therefore often accepted, although contradictory results were published as recently reviewed.[10a] In the case of charges, bacteria are thought to be attracted by oppositely charged surfaces.[16] Nevertheless, some studies have highlighted that bacteria adhesion, in response to surface charge, is a complex topic involving surroundings ionic strength and incubation time. Influence of surface topography which is the most frequently described only by the mean roughness parameter, has been in comparison rarely studied.[17] Exact correlation between topography and bacterial adhesion is not clear, although it is usually accepted that topographical features with dimensions similar to bacterial size favor bacterial adhesion, probably by providing protective effects.

In fact, microorganisms universally attach to surfaces and produce extracellular polymer substances, resulting in the formation of a biofilm. Biofilms pose a serious problem for public health due to the increased resistance of biofilm-associated organisms to antimicrobial agents and the potential for bacteria to cause infections in patients with indwelling medical devices and in various other infection schemes. The prevention of bacterial attachment to surfaces is thus generally thought to be the effective way

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Table 1. Function of extracellular polymeric substances (EPS) in bacterial biofilm growth Reproduced with permission.[7d] Copyright 2010, Macmillan Publishers Limited.

Function	Relevance for biofilms	EPS components involved
Adhesion	Allows the initial steps in the colonization of abiotic and biotic surfaces by planktonic cells, and the long-term attachment of whole biofilms to surfaces	Polysaccharides, proteins, DNA and amphiphilic molecules
Aggregation of bacterial cells	Enables bridging between cells, the temporary immobilization of bacterial populations, the development of high cell densities and cell-cell recognition	Polysaccharides, proteins and DNA
Cohesion of biofilms	Forms a hydrated polymer network (the biofilm matrix), mediating the mechanical stability of biofilms (often in conjunction with multivalent cations) and, through the EPS structure (capsule, slime or sheath), determining biofilm architecture, as well as allowing cell–cell communication	Neutral and charged polysaccharides, proteins (such as amyloids and lectins), and DNA
Retention of water	Maintains a highly hydrated microenvironment around biofilm organisms, leading to their tolerance to dessication in water-deficient environments	Hydrophilic polysaccharides and, possibly, proteins
Protective barrier	Confers resistance to nonspecific and specific host defenses during infection, and confers tolerance to various antimicrobial agents (for example, disinfectants and antibiotics), as well as protecting cyanobacterial nitrogenase from the harmful effects of oxygen and protecting against some grazing protozoa	Polysaccharides and proteins
Sorption of organic compounds	Allows the accumulation of nutrients from the environment and the sorption of xenobiotics (thus contributing to environmental detoxification)	Charged or hydrophobic polysaccharides and proteins
Sorption of inorganic ions	Promotes polysaccharide gel formation, ion exchange, mineral formation and the accumulation of toxic metal ions (thus contributing to environmental detoxification)	Charged polysaccharides and proteins, including inorganic substituents such as phosphate and sulfate
Enzymatic activity	Enables the digestion of exogenous macromolecules for nutrient acquisition and the degradation of structural EPS, allowing the release of cells from biofilms	Proteins
Nutrient source	Provides a source of carbon-, nitrogen- and phosphorus-containing compounds for utilization by the biofilm community	Potentially all EPS components
Exchange of genetic information	Faciliates horizontal gene transfer between biofilm cells	DNA
Electron donor or acceptor	Permits redox activity in the biofilm matrix	Proteins (e.g., those forming pili and nanowires) and, possibly, humic substances

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Table 1. Continued

Function	Relevance for biofilms	EPS components involved
Export of cell components	Releases cellular material as a result of metabolic turnover	Membrane vesicles containing nucleic acids, enzymes, lipopolysaccharides and phospholipids
Sink for excess energy	Stores excess carbon under unbalanced carbon to nitrogen ratios	Polysaccharides
Binding of enzymes	Results in the accumulation, retention and stabilization of enzymes through their interaction with polysaccharides	Polysaccharides and enzymes

to avoid the biofilm formation. In Figure 2 is schematized the bacterial biofilm growth[17b] whereas in Figure 3, a scanning electron micrograph of a developed bacterial biofilm is shown.[7c]

2. General Principles for Designing Antimicrobial Surfaces

When biofilms are formed on the surface it is extremely hard to remove them since adhered bacteria show greater resistance to all kinds of biocides. It is thus crucial to design surfaces which will not allow settlement of microbes at the very first place. Thus, keeping this strategy in mind, antimicrobial surfaces are designed with the principle that they should either repel the microbes or kill them on contact (Figure 4). Hence antimicrobial surfaces may broadly be divided into two categories with these two criteria (a) either microbe repelling, (b) or lethal surfaces. As described in this review article, repelling surfaces make it very hard for bacteria to attach by decreasing bacterial adhesion and are generally prepared by modifying the surface with either neutral polymers like poly(ethylene glycol), which prevent bacterial adhesion by steric hindrance or charged, anionic polymers, which repel the negatively charged cell membrane.[18a] Contact killing surfaces could be designed by modification of the surface with cationic polymers, which

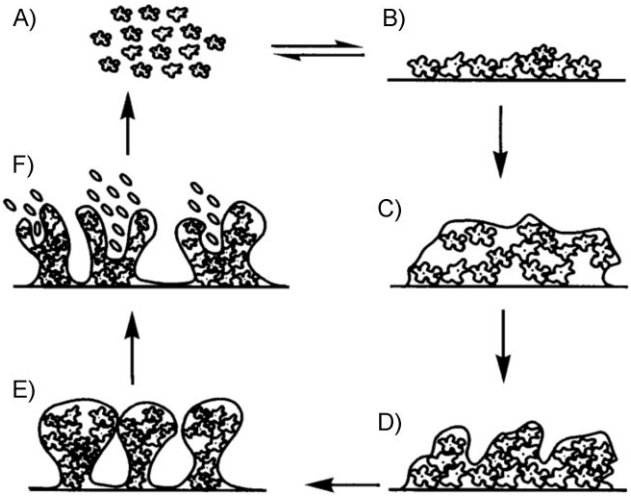

Figure 2. Biofilm growth cycle. (A) Planktonic bacteria, (B) reversibly attached to a surface suitable for growth, (C) Bacteria begin secretion of the EPS and attachment becomes irreversible, (D) The maturing biofilm begins to take a three-dimensional shape, (E) The biofilm fully matures, and a complex architecture is observed, (F) Bacteria disperse from the biofilm to reinitiate biofilm colonization of a distal surface. Reproduced with permission.[17c]

Figure 3. Scanning electron micrograph depicting a developed biofilm (A), the substratum (B), and an attached cell (C). Reproduced with permission.[7c] Copyright 2001, Infectious Diseases Society of America.

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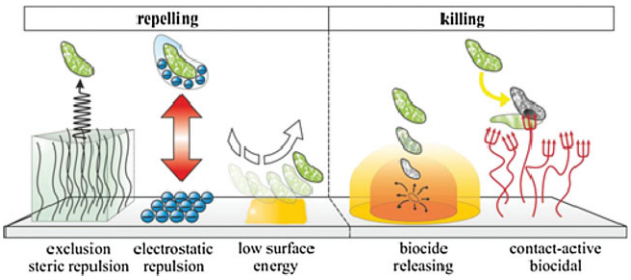

Figure 4. Diagrammatic representation of repelling and killing bacteria surfaces. Reproduced with permission.[90]

strongly interact with the cell membrane and cause its disruption.[18b] N-halamine based polymers constitute another class of macromolecules, which interact with cellular receptors leading to microbial cell activity inhibition or inactivation. Composite polymers incorporate either antibiotics along their backbone or metallic particles like silver, copper, and titanium dioxide known for their antibacterial activity.[18c]

Depending on the mode of modification of the surface with polymers three schemes may be applied (Figure 5). In the first strategy, the antimicrobial polymer film is formed on the surface by simple physical absorption without any covalent attachment. In this context, antimicrobial coatings have been developed by simple dip coating or a painting methodology as assessed in various reports.[19] Alternatively, this scheme also involves preparation of antimicrobial surfaces by the layer by layer assembly, which is based on the electrostatic attraction of oppositely charged polyions.[20] Although this scheme for immobilizing polymers is very simple and do not require any tedious chemical strategy, there is a high risk with such coatings of biocide leaching out to the surrounding in some instances, which may lead to a loss of antimicrobial activity over a short time. To overcome this issue, another scheme for designing antimicrobial surface coatings is the covalent attachment of the biocidal polymer to the surface. Polymers are immobilized covalently on the surface by either classical free radical polymerization or by a simple coupling reaction on activated surfaces.[21] The antimicrobial surfaces created by this methodology do not allow the biocide to leach easily and long-term non-leachable antimicrobial coatings could be designed.

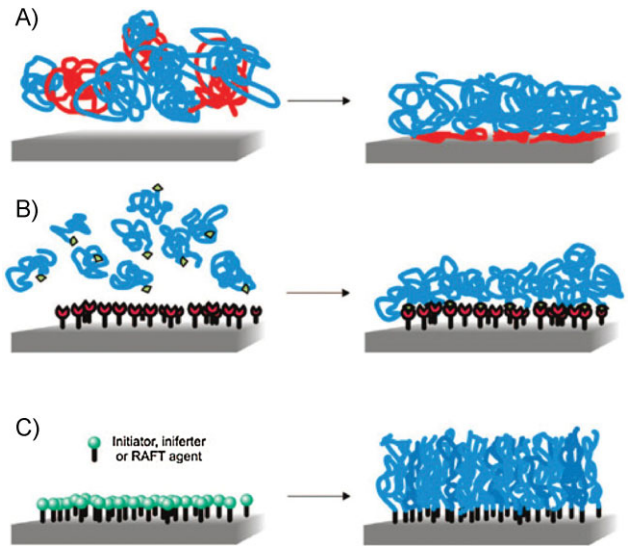

Figure 5. Main polymer immobilization schemes (A) Physical adsorption by non-covalent sometimes multi-dentate interactions, e.g., LbL films, block copolymer coatings, etc. (B) Grafting-to methods by creating covalent bonds with the surface, e.g., PEIs (poly(ethylene imine)), cationic polymers, (C) Grafting-from or surface-initiated polymerization via synthesis of antimicrobial coating from initiators revealed at the surface by ATRP e.g., PVP, PDMAEMA, methacrylates. Reproduced with permission.[91]

However, surfaces created by such strategies fail to strictly control the monomer distribution, polymer molecular weight, topology, and density of functional groups that would allow rational and easy modification of polymers to enhance their antimicrobial activity. Hence to overcome these limitations a third scheme of polymer immobilization on surfaces is achieved by grafting from strategies. In this scheme, antimicrobial polymers are synthesized from the initiator present on the surface by atom transfer radical polymerization (ATRP) or reversible addition fragmentation chain transfer polymerization (RAFT) resulting in highly monodispersed non-leaching antimicrobial surfaces.[22] In the following sections of this chapter, we will discuss the research results achieved with these modes of polymer modification of surfaces in details.

3. Polymers for Immobilization to Design Contact Killing Surfaces

3.1. Cationic Polymers

Microbial cells generally possess a net negative charge due to the presence of the teichoic acid membrane protein in Gram-positive bacteria and negatively charged phospholipids at the outer membrane of Gram-negative bacteria. Due to this reason, cationic polymers can get effectively adsorbed at the bacterial cell surface. When such cationic polymers are sufficiently amphiphilic, they can easily penetrate through the cell membrane and subsequently cause its disruption leading to leakage of cytoplasmic constituents, which ultimately induces cell death. Thus, cationic polymers have been used for designing highly potent antimicrobial surfaces, which can kill bacteria just by contact. The hypotheses of action of these polymers in

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killing bacteria have been confirmed by numerous investigations employing live/dead two colors fluorescence assays, transmission electron microscopy (TEM), atomic force microscopy (AFM), monitoring of loss of bacterial cell constituents, and leakage of dye from model liposomes which mimics the bacterial cell membrane.[23] Cationic polymers most probably kill bacteria by damaging the cell membrane wall through lysis, which induces the release of the cellular constituents in solution. Most cationic polymers are generally based on quaternary ammonium, guanidinium, phosphonium, or sulfonium groups grafted on the polymer backbone.[24] Various studies have shown that the antimicrobial efficacy of cationic polymers is directly proportional to the constitutive alkyl chain length, hydrophobicity, and amount of cationic groups. Speier and Malek for the very first time demonstrated in 1980s that surfaces modified with polycations can kill bacteria on contact.[25] In the following section of this review, we will illustrate how contact killing antimicrobial surfaces are designed by various strategies using cationic polymers as described in various research reports.

3.1.1. Antimicrobial Surfaces Formed by Simple Dip Coating or Layer by Layer Assembly (Physical Absorption) of Cationic Polymers

It has been demonstrated that dipping glass slides or PE slides into organic solutions containing sufficiently long hydrophobic N-alkyl-PEI for a brief period followed by solvent evaporation leads to the creation of highly effective bactericidal surfaces, which kill up to 100% *Staphylococcus aureus* and *E. coli* reaching the surface.[19] A very novel strategy to design bactericidal surfaces by mere painting with biocidal polymers is been reported as well in this article. When glass slides were painted with a solution of branched or linear *N*,*N*-dodecyl methyl-PEI in butanol it also rendered the resulting surface resistant to influenza viruses, which were killed with 100% efficiency. Since influenza viruses have an envelope composed of a lipid membrane, polymers such as hydrophobic cationic copolymers penetrate through the membrane resulting in inactivation of the virus. Klibanov recently developed permanent microbicidal coatings derived from hydrophobic polycations either covalently attached or deposited onto different surfaces and demonstrated that when microbes come in contact with these surfaces the polycations penetrate through the cell membrane causing disruption of the lipid bilayer and thus avidly killing microbes.[26] Being these surfaces designed by a non-releasing antimicrobial strategy, the antimicrobial activity of these surfaces is retained permanently.

Han et al. recently devised a very efficient strategy for immobilizing amphiphilic polycations by dip coating.[27] In this method, amphiphilic polycations were synthesized via polymerization of monomers containing dodecyl quaternary ammonium, methoxyethyl, and catechol groups in different molar ratios. While quaternary ammonium imparted bactericidal properties, methoxy ethyl provided the optimal amphiphilic balance, a prerequisite for desired antimicrobial effects. The adhesive and cross-linking property of the catechol groups enabled the immobilization of the polymers into smooth thin films which further prevent polymer leaching that imparts highly potent bactericidal property for longer time to these surfaces.

A non-water-soluble natural antibacterial peptide, gramicidin A, was successfully incorporated into polyelectrolyte assemblies to elaborate biocidal thin films.[20] For this, a double strategy was used. The first step consisted in the complex formation between a peptide and a non-denaturing anionic amphiphilic polysaccharide, carboxymethylpullulan. In a second step, the negatively charged complex was assembled layer-by-layer with the cationic poly(L-lysine) to form bio-functionalized thin films. The antibacterial activity of the resulting bio-functionalized films was evidenced against a Gram-positive bacterium, *Enterococcus faecalis*.

3.1.2. Natural Cationic Polymers to Design Antifouling Surfaces

Due to its non-toxicity, biodegradability, biocompatibility, antitumor, and antibacterial activities as well as antioxidant and mucus adhesiveness properties, chitosan, a deacetylated derivative of chitin, has attracted much of attention in particular for application in the food, pharmaceutical, and cosmetic industries.[28] In addition, chitosan has demonstrated superior characteristics to prepare antifouling substrates. Chitosan-based materials such as gels and other formulations have thus been used as tissue engineering scaffold, wound dressing, and to immobilize coagulating agents that prevent bacterial contamination and biofilm formation. Considering these uses, chitosan could successfully be incorporated into seafood products to enhance both quality and human nutrition. Due to these outstanding characteristics, chitosan can thus be viewed either as a functional component to design bacteria repellent substrates or as a natural antibacterial polymer.[29]

The antifouling properties of chitosan-modified surfaces have been described by the ability of cationic chitosan to disrupt the negatively charged cell membranes as microbes settle on the surface.[30] The reported investigations clearly demonstrate that chitosan has a profound effect on the negative charge of the fungal cellular membrane, which may translate into interference with surface colonization or adhesion and cell–cell interaction during biofilm formation.[31] For example, a net positive charge of chitosan surfaces may keep yeast cells in suspension, preventing

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initial adhesion and subsequent biofilm formation.[32] At the molecular level, the binding of the positively charged chitosan to DNA and inhibition of messenger RNA synthesis could occur through chitosan penetration toward the nuclei of the microorganisms and interfere with the synthesis of messenger RNA and subsequent protein coding.[33] It is most likely that the interaction between positively charged chitosan molecules and negatively charged microbial cell membranes leads to the leakage of proteinaceous and other intracellular constituents, causing cell death, which consequently inhibits and degrades the biofilm.[34]

Both natural and modified chitosan polymers could be used to infer antibacterial and antifouling activities to materials of interest. The following examples evidence the antimicrobial effect of chitosan nanoparticles on *Streptococcus mutans* biofilms.[35] These were prepared from various molecular weights (MW) chitosan of different degrees of deacetylation (DD). The method to prepare the nanoparticles is based on the electrostatic interaction between chitosan and tripolyphosphate (TTP) as shown in Figure 6. As a result, complexes of nanometer size prepared from high MW chitosan show low antimicrobial effect (20–25% of cells damaged); whereas those prepared from low MW chitosan shows higher antimicrobial effect (more than 95% of cells damaged). This study demonstrates that there is a strong effect of low molecular weight chitosan on the antibacterial activity of the resulting nanoparticles. Though no clear picture of the mechanism of the chitosan molecular weight effect on antimicrobial activity of the nanoparticles could be evidenced, it may be possible that the lower molecular weight nanoparticles have a systematically reduced number of TPP molecules available per molecule which may thus be more susceptible to disaggregation within the biofilm. It could as well be due to the difference in the size of the chitosan particles prepared from different molecular weight chitosan as reported elsewhere, resulting in the difference of antibacterial efficiency.[36]

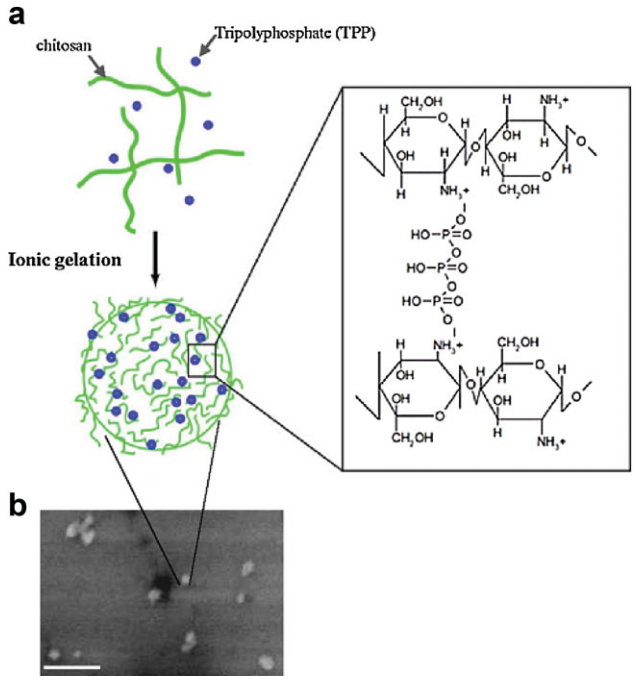

Figure 6. Formation of the chitosan–tripolyphosphate complex by ionotropic gelation. (a) Schematic illustration of the chitosan–TTP complex and (b) SEM image of the resulting structures. Bar, 200 nm. Reproduced with permission.[37] Copyright 2011, American Society for Microbiology.

The coating of catheters with chitosan and inhibition of *C. albicans biofilm* formation *in vivo* has been reported as shown in Figure 7. [37] As a result, the fungal burden in chitosan-treated catheters was statistically significantly lower than that in untreated control catheters. For instance, biofilms grown in the absence of chitosan show a network comprising yeast cells and hyphae surrounded by moderate amounts of exopolymeric matrix components as shown in Figure 7B–D. However, chitosan-treated catheters do not enable *C. albicans* biofilm formation (Figure 7E–G). Instead, they show minimal adhesion of red blood cells and fibrous debris.

In several other reports, chitosan-coated surfaces demonstrated antifouling properties against bacteria and fungi in vitro, including *Candida albicans*.[38] Quaternized chitosan inhibited biofilm formation by *Staphylococcus epidermidis* ATCC 35984 on titanium surfaces coated with the natural chitosan polymer.[39] In their study, the ability of a water soluble hydroxypropyltrimethyl ammonium chloride chitosan (HACC) to both prevent biofilm development and test the susceptibility of a preexisting biofilm to HACC on a titanium surface was assessed. In the biofilm prevention test, the scanning electron microscopy (SEM) results indicated that biofilm formation was significantly prevented by treatment with an increased substitution degree and concentration of HACC (Figure 8). In the biofilm susceptibility test, the biofilm formation and viability were inhibited only at high concentrations of HACC (18 and 44%). These results indicate that pre-coating of orthopedic implants with HACC has thus the potential to block bacterial colonization and biofilm formation.

There have been several systems designed to immobilize and to maintain the localized concentrations of antibiotics on material surfaces over long durations. Although numerous antimicrobial medical devices have been reported to enable control on biofilm formation and reduction of infection rates for short-term uses,[40] most of these systems cannot provide long-term protection because of the high release rate of antibiotics from the devices. After a short period of time (e.g., days), most of the drugs are released, and inhibitory effects are lost. It is therefore crucial to extend the antimicrobial duration of medical devices surfaces. Cationic surfactants including

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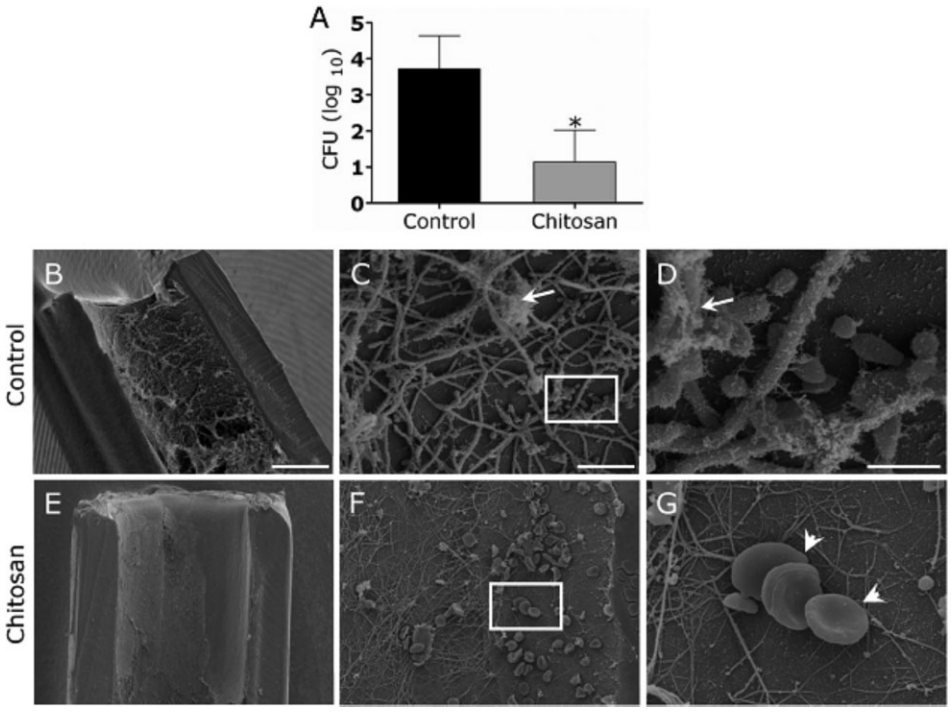

Figure 7. Reduction of *Candida albicans* SC5314 surface-associated growth on chitosan-coated catheters. A, Mean fungal burden in catheters infected with 1×10^6 cells mL^{-1} *C. albicans*. B-D, scanning electron microscopic examination of untreated *C. albicans* biofilms; E–G, scanning electron microscopic examination of *C. albicans* biofilms treated with 5 mg · mL^{-1} chitosan. *C. albicans* biofilm formed on the luminal surface of the untreated catheter. Scale bar for panels B and E, 200 mm; scale bar for panels C and F, 20 mm; scale bar for panels D and G, 5 mm. Reproduced with permission.[37] Copyright 2010, Infectious Diseases Society of America.

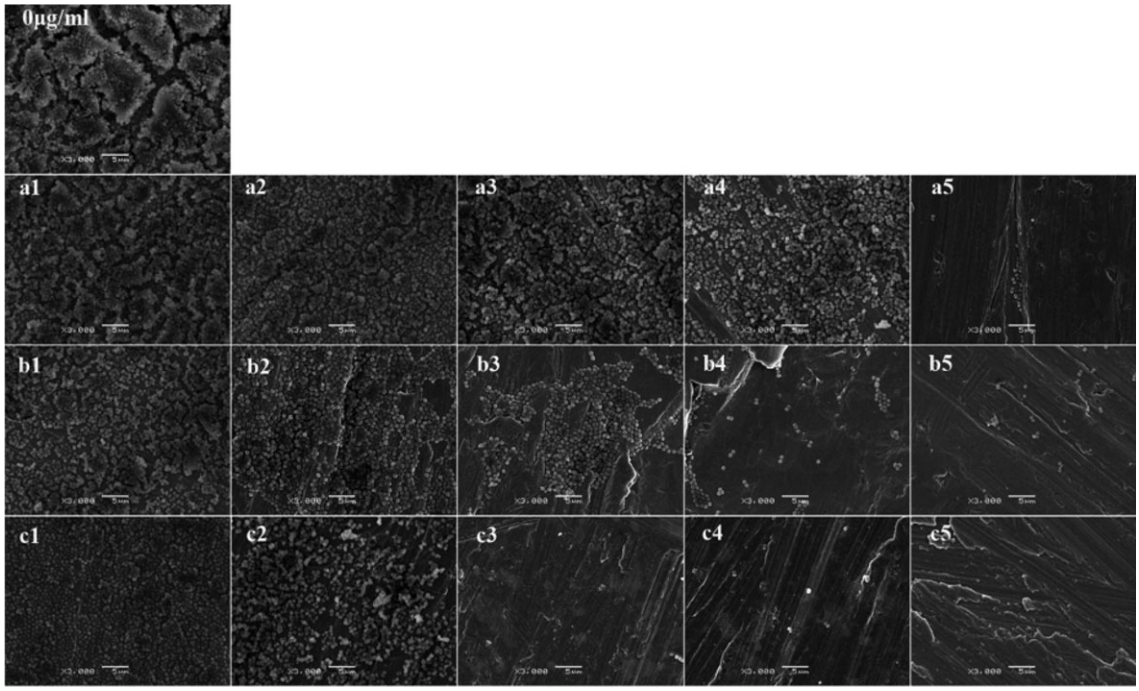

Figure 8. Scanning electron micrographs of biofilms formed by the *S. epidermidis* strain ATCC 35984 incubated on a titanium surface for 24 h coated with HACC 6% (a), 18% (b), or 44% (c) at the following concentrations: 0 μg · mL^{-1}, 4 μg · mL^{-1} (1), 32 μg · mL^{-1} (2), 64 μg · mL^{-1} (3), 128 μg · mL^{-1} (4), or 256 μg · mL^{-1} (5). Magnification 3 000, scale bars of 5 μm. Reproduced with permission.[39] Copyright 2011, American Society for Microbiology.

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tridodecylmethyl ammonium chloride and benzalkonium chloride were therefore adsorbed on device surfaces, to enable the adhesion of anionic antibiotics through ionic interactions.[41] The bound antibiotics are then slowly released, extending the antimicrobial duration up to several days. However, the effectiveness of this process has been demonstrated in short-term applications because drugs can only be adsorbed onto the device surface, which usually is of limited capability for adsorbing antibiotics that prevents long-term antimicrobial activities.[42] Nonetheless, there is a drawback of using surfactant. Release of the cationic surfactants in the blood stream could be of a concern.[43] To solve these problems, new polymeric materials (e.g., polyurethanes) bearing cationic functional groups along their side chains have been recently synthesized to ensure slow release of anionic antimicrobial agents.[43b]

Along this line, chitosan is considered as the most suitable polymer to coat surfaces. For instance, chitosan-coated surfaces were systematically developed on the basis of rechargeable long-term antimicrobial and biofilm-controlling systems.[44] Indeed natural cationic chitosan strongly binds anionic antibiotics (e.g., rifampin) through the formation of ionic complexes. This system shows high efficiency in antibiotic binding, enhanced duration, and sustained release of rifampin. High antimicrobial and antibiofilm activities against Gram-positive bacteria including *Staphylococcus epidermidis* and *Staphylococcus aureus*, which are responsible for a wide range of medical device-related infections, have been demonstrated for periods longer than 30 d. One of the results of these promising investigations of inhibition study is shown in Figure 9. Inhibition by chitosan against *S. aureus* and *S. epidermidis* biofilm growth is shown in Figure 9A and C. The inhibition of rifampin-containing chitosan film against *S. aureus* and *S. epidermidis* growth is shown in Figure 9B and D. Besides, the released drugs can be reloaded on the

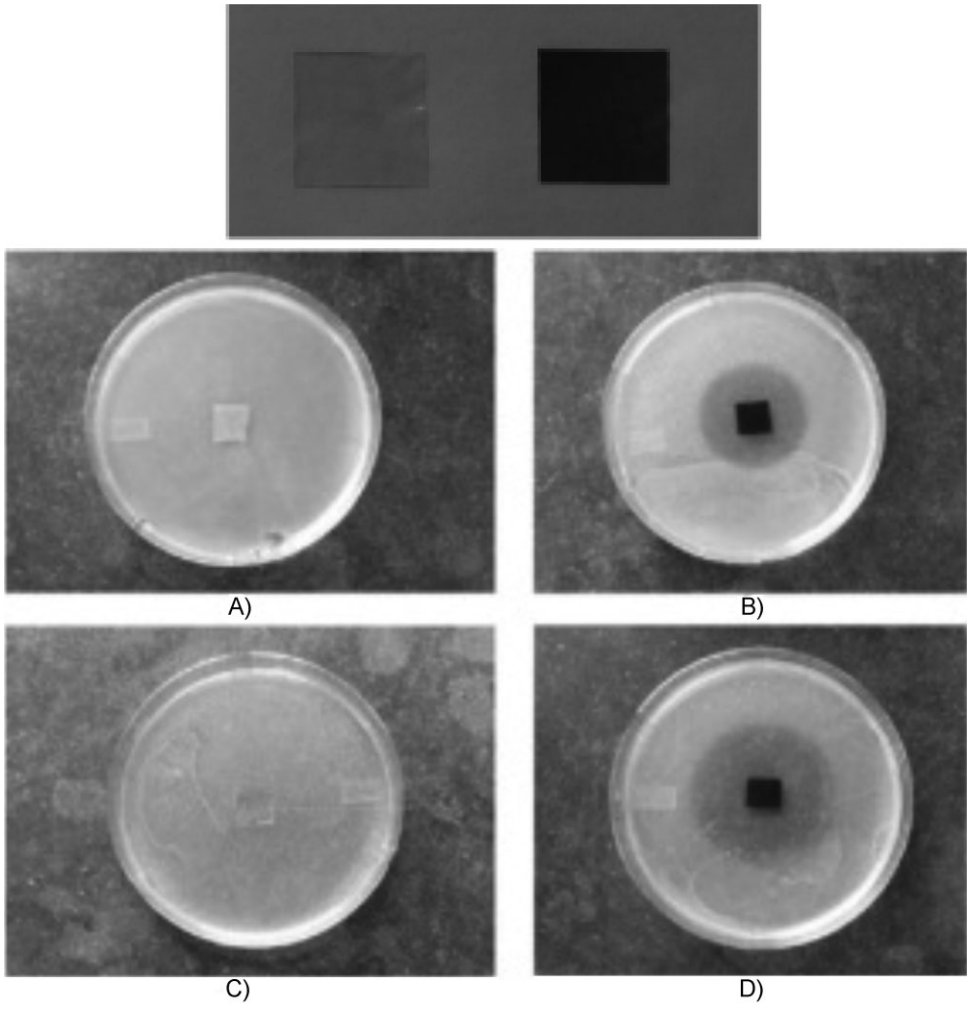

Figure 9. Chitosan films before (left) and after (right) rifampin treatment (rifampin treatment condition: rifampin concentration was 2 wt.-% in methanol; time 24 h; temperature 25 °C). Reproduced with permission.[44]

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chitosan-coated surface, which becomes positively charged subsequent to the drug release (free primary amine along the chitosan backbone, which can further be protonated by tuning the pH) through fresh drug binding and consequently enhanced antimicrobial durations.[44] In their novel system, drug release mechanisms and potential applications are also discussed. Chitosan strongly binds anionic antibiotics such as rifampin through ionic complex formation. Drug binding capacity is significantly affected by solvents and drug incubation time. The rifampin-containing chitosan samples demonstrate sustained drug release. The new system indeed provides potent, durable, and rechargeable antimicrobial and biofilm-controlling functions against Gram-positive bacteria including *S. epidermis* and *S. aureus*, which are among the most isolated species that are responsible for a wide range of medical device-related infections, particularly central venous catheter-related bloodstream infections. These attractive features make the new system potential candidate in the antimicrobial treatment for a broad range of highly needed long-term use medical devices.[35]

Aside from chitosan, new antifouling coatings have been prepared using a simple UV photochemical crosslinking from natural rubber precursors, i.e., 1,4 oligoisoprenes, bearing photosensitive functions and quaternary ammonium groups.[46] These increased the antifouling activity without any detectable release of biocidal molecules.

Biomimetic approaches have recently been reported. A novel copolymer (PMNC), inspired by both the adhesive nature of 3,4-dihydroxy-phenylalanine (DOPA), a component of mussel adhesive proteins, and the fouling resistance of the cell membrane owing to phosphorylcholine (PC) side groups that are a major component of the outer surface of the erythrocyte membrane.[47] The resulting polymer can be deposited on a variety of substrates by dip-coating. This approach appears as particularly relevant since it has been recently reported that phospholipids, triglycerides, and cholesteryl esters play a major role in non-specific adsorption.[48] García-Fernández et al.[49] applied antibacterial strategies from the sea to confer antifouling properties to polymer surfaces using a bioinspired biocide. Due to the catechol reactivity, chloride-dopamine was incorporated into hydrogels or codeposited as a thin coating film to prevent bacterial attachment.

3.1.3. Antimicrobial Surfaces Formed by Covalent Attachment of Cationic Polymers

One of the earliest examples to design contact active sterile surfaces by covalent attachment of polymers was reported by Klibanov and coworkers.[50] They covalently linked poly(4-vinyl-*N*-alkylpyridinium bromide) to amino-modified glass slides via acylation with acryloyl chloride followed by copolymerization with 4-vinylpyridine, and finally N-alkylation with different alkyl bromides. Through this approach the authors demonstrated effective retention of the antibacterial properties of these surfaces since the long chains of N-alkylated poly(4-vinyl pyridine) were of sufficient length and flexibility to penetrate the cell wall and disrupt the cell membrane, leading to cell death. The resulting surfaces, depending on the length of the alkyl chain, were able to kill up to (94 ± 4) % of sprayed *Staphylococcus aureus* cells. The surfaces modified with N-hexylated poly(4-vinylpyridine) were also very effective in killing Gram-positive bacteria such as *Staphylococcus epidermidis*, as well as Gram-negative bacteria like *Pseudomonas aeruginosa* and *E. coli*. The viability of the cells dropped more than 100-fold as compared to that on the original amino-modified glass. In a related study, the authors further extended this methodology to different polymer surfaces functionalized with hexyl PVP. Such polymer surfaces were able to kill 90–99% of Gram-positive and Gram-negative bacteria on contact.[51]

In another study, Lin et al. designed antibacterial surfaces by covalently attaching polyethylenimines (PEIs) to acylated amino-modified-glass slides and showed that these surfaces were equally lethal to both Gram-positive and Gram-negative bacteria.[21b] It was further illustrated by structure activity relationship (SAR) assays, that to create effective antibacterial surfaces, the immobilized polymer should have a long hydrophobic chain and should be positively charged. Further, they extended this strategy to textile surfaces like cotton, wool, nylon, and polyester and showed that these surfaces avidly kill bacteria. The bactericidal textiles were not only pathogenic to bacteria but also to fungi. The same research group investigated the kinetics of the mode of action of immobilized N-alkyl-PEI and demonstrated 100% inactivation of surface adhered bacteria. It was further evidenced that these surfaces selectively killed bacteria without causing any harm to mammalian cells making them highly useful for designing sterile medical devices for hospitals.

Another class of cationic polymers which shows highly potent antibacterial property and used for designing sterile surfaces are polymers containing quaternary ammonium or phosphonium groups along their backbone. In this regard, Kenawy et al. synthesized copolymers of 2-chlorovinylether and vinylbenzylchloride with grafted ammonium and phosphonium salts and demonstrated through transmission electron microscopy that phosphonium containing cationic polymers were more potent and cause disruption of the cell membrane of *S. aureus* leading to the release of potassium ions as assessed by potassium leakage assays.[23e]

Another strategy to design sterile surfaces is by silanization with quaternary ammonium containing silane agents. Andresen et al. designed permanent antimicrobial films by grafting octadecyldimethyl(3-trimethoxysilylpropyl)ammonium chloride (ODDMAC) quartenary

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ammonium compounds to micro-fibrillated cellulose surfaces via silanization.[21c] These surfaces were highly bactericidal and potent against both Gram-negative and Gram-positive bacteria. Zone of inhibition tests further showed that there was no release of ODDMAC to the surrounding making these antifouling surfaces useful for designing permanent antimicrobial surface coatings. In a seminal report, Saif et al. modified glass with quaternary ammonium terminated triethoxysilane by a sol–gel process using tetraethoxysilane (TEOS) as silanizing agent.[53] It was shown that there was a correlation between the surface concentration of quaternary ammonium silanes and the antibacterial activity. A concentration of 1.5×10^{-4} of quaternary ammonium silane per gram of coating was enough to kill nearly 95% of viable bacterial colonies after 48 h of exposure.

Another polymer which could be used for surface modification to create antimicrobial surfaces is poly-(diallyldimethylammonium chloride) (PDADMAC). Due to the presence of cationic quaternary nitrogens in its molecular structure, this polymer possesses very high adsorption ability to negatively charged particles. Thome et al.[53a] created PDADMAC coated surfaces by two strategies, either by grafting PDADMAC to the surface via radical polymerization of the monomer or by coupling PDADMAC to the activated surface which is plasma treated. It was shown that the surfaces were capable of reducing the settlement of bacteria such as *Micrococcus luteus* (Gram positive) and *E. coli* (Gram negative) by a factor of 105–106. Van der Mei et al designed PDADMAC coatings by immersing glass slides in different concentration of PDADMAC aqueous solutions.[53b] These coatings strongly enhanced adhesion of water borne pathogens. Indeed, *R. terrigena* and *E. coli* readily adhered in high numbers to p-DADMAC coated glass slides prepared from 1, 100, or 500 ppm aqueous solutions while *B. diminuta* adhered exclusively to strongly positively charged PDADMAC coatings prepared from 500 ppm solutions. Positively charged ammonium groups present in this coating thus drastically reduced the bacterial viability once these bacteria adhered to the surface

Grapski and Cooper designed series of quaternized poly(urethanes) (PU) and examined the resulting antibacterial activity against *Staphylococcus aureus* and *E. coli* by zone of inhibition experiments and fluorescence microscopy.[23b] The viability of the cells was shown to be dependent on the alkyl halide used for quaternization, the concentration of quaternized moieties in the PU, the Gram-type of the microorganism, and the contact time of the organism with the surface. Cationic PU with potent biocidal activity were designed by quaternization of the pyridine ring of *N*,*N*-bis(2-hydroxyethyl)isonicotinamide (BIN) which was incorporated as the chain extender in a series of poly(tetramethylene oxide)-based PU block copolymers.

In more recent studies, highly effective antimicrobial surfaces were designed by incorporating polymer surface modifiers (PSM) in biocidal polyurethane (PU).[54] In this context, Kurt et al designed antimicrobial coating by dipping the glass slides in a solution of 2 wt.-% PSM with a conventional PU and subsequently evaporating the resulting solution. PSM were prepared by random copolymerization of 1,3-propylene oxide soft blocks and grafted with alkylammonium and either trifluoroethoxy or pegylated side chains which confer the desired antimicrobial properties. In another elegant approach Makal et al. designed PSM modified PU surfaces which can be further activated.[55] The PSM modified PU was activated by conversion of near-surface amide groups to chloramide with 3 wt.-% hypochlorite. The resulting surfaces revealed somewhat increased hydrophobicity and exhibited potent biocidal activity against both Gram-positive (*S. aureus*) and Gram-negative (*Pseudomonas aeruginosa* and *E. coli*) bacteria. In a very recent study, multifunctional self-decontaminating PU coatings were designed by cross-linking diol-functionalized quaternary ammonium bromide salts with commercial poly(isocyanate).[56] Due to the presence of biocidal tethers there was no loss of biocide in the environment. Very effective non-leaching antimicrobial surfaces resulting from the microphase separation between the coated biocidal polymer and the PU constitutive of the coating as confirmed by AFM were thus designed.

3.1.4. Antimicrobial Surfaces Designed by Grafting from or Grafting to Techniques

An active area in the preparation of antifouling surfaces is in the synthesis of polymer brushes.[57] These enable, through the control of the brush hierarchical architecture, the structural regulation of functionalities.[58]

The Matyjaszewski and Russell laboratories jointly developed a strategy of ATRP to synthesize antimicrobial polymer films directly from the surface through living radical polymerization of initiators present on the surface.[58] The resulting surfaces are modified with polymers of controlled molecular weight and optimal chain length as well as high monodispersity, which impart these coatings very effective and ideal biocidal properties. Lee et al. for the first time used ATRP to polymerize antimicrobial polymers directly on the surfaces of glass or polymer.[22a] The tertiary amine 2-(dimethylamino)ethyl methacrylate was polymerized directly onto Whatman #1 filter paper or glass slides via ATRP by immobilizing an ATRP initiator on the surface prior to addition of monomers which polymerize on the surface thereafter. Subsequent to the polymerization step, the tertiary amino groups were quaternized using an alkyl halide to yield large concentrations of quaternary ammonium groups exposed on these modified surfaces which confer very high biocidal activity. Besides, the same surface could be used over and over again without significant loss of

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antimicrobial activity as assessed by their antibacterial activity against *E. coli* and *B. subtilis*.[22a] Murata et al. prepared antimicrobial polymer brushes on inorganic surfaces though surface initiated ATRP of 2-(dimethylamino)ethyl methacrylate (DMAEMA) followed by quaternization of the tertiary amino groups of the poly(DMAEMA) with alkyl bromides.[59] In a recent study, an improvement in antifouling and antibacterial property has been illustrated by covalent binding of 2-hydroxyethyl methacrylate (HEMA) and 2-(dimethylamino)ethyl methacrylate (DMAEMA) brushes onto the poly(vinylidene fluoride) (PVDF) membrane surfaces via surface-initiated ATRP.[60] In another study Huang et al. designed antimicrobial poly (propylene) (PP) plastic by chemical attachment of poly (quaternary ammonium) (PQA) to the surface of PP.[22b] This was done by first growing poly(2-(dimethylamino) ethyl methacrylate) (PDMAEMA) via ATRP from the PP surface followed by quaternization of the tertiary amine groups of PDMAEMA. Biocidal activity was tested against *E. coli*. The viability of the cells was shown dependent on the number of QA units which in turn was dependent on the molecular weight of the polymer immobilized on the surface. While almost 100% killing efficiency was achieved with high MW polymers ($\overline{M}_n > 10\,000\ g \cdot mol^{-1}$), a lower biocidal activity (85%) was observed for the surface grafted with shorter PQA chains ($\overline{M}_n = 1\,500\ g \cdot mol^{-1}$). Recently, PEG-like brushes of oligo(ethylene glycol) methyl ether methacrylate (MeOEGMA) have been systematically studied in respect with their growth and antifouling properties in various buffers.[61]

In another alternative strategy to design antimicrobial surfaces by the grafting from method, 2-(dimethylamino) ethyl methacrylate (DMAEMA) was polymerized from cellulosic filter paper via reversible addition-fragmentation chain transfer (RAFT) polymerization.[62] The quaternized PDMAEMA-grafted cellulosic fibers were shown to possess biocidal activity against *E. coli* and cellulose fibers with the highest degree of quaternization and fibers modified with the shortest alkyl chains showed maximum antibacterial activity. Another strategy, which combined ATRP and covalent immobilization through grafting onto, has been reported. Using this technique Huang et al. prepared antimicrobial glass surfaces which were shown to possess better biocidal efficiency than surfaces prepared by the grafting from method at comparable quaternary ammonium (QA) densities.[63] With this technique PDMAEMA/TMSPMA block and random copolymers were first synthesized by ATRP followed by covalent attachment of these polymers via reaction of the trimethoxysilyl polymer groups with surface silanol followed by quaternization of PDMAEMA amine groups. More recently Bieser et al. developed antimicrobial coatings by the "grafting from" approach on cellulose backbone by grafting antimicrobial N, N dimethyl dodecyl ammonium group (DDA) on (poly-2 ethyl-oxazoline) PEtOx side chains.[64] When microbial cells come and contact these surfaces they are avidly killed through disruption of the membrane. Due to cell lysis, the cellulase enzyme is released which degrades the top layer of cellulose that was contaminated by bacteria and allows the self-polishing of these surfaces. Table 2 summarizes the biocidal coatings obtained by surface modification with polymers along with their typical chemical structures which impart antimicrobial properties to the surface.

Biomimetic approaches are adopted as well to prepare bacteria repellent brushes by either RAFT polymerization[65] or ATRP.[66] A biomimetic modification route for the creation of antifouling methoxy-and hydroxyl-capped oligoethylene glycol methacrylate, 2-hydroxymethacrylate and carboxybetaine acrylamide brushes has been reported.[67] This route consists in the deposition of a polydopamine anchor layer prior to surface initiated-ATRP. In a similar approach, barnacle cement was used by Yang et al. to prepare brushes by RAFT polymerization.[68]

It has to be noted though that investigations on the structure-antifouling- activity relationships are still carried out to eventually achieve a comprehensive understanding of bacterial-surface interaction. Zhao et al. investigated the effect of the amide group on the hydration and antifouling activity of polyacrylamides and polyacrylates brushes as well as hydrogels and nanogels.[69] They demonstrated that the antifouling performance is positively correlated with the hydration properties.

3.1.5. *N*-Halamine Based Polymers for Creating Contact Active Surfaces which Lead to Cell Inhibition or Inactivation

Another class of polymers which is very effective in killing bacteria is constituted of cyclic N-halamine polymeric compounds. N-halamine polymers kill pathogens much faster than do the quaternary cationic polymers, can be regenerated by exposure to dilute halogen solutions, are generally inexpensive, non-toxic, and non-corrosive, since most do not release free halogen. In N-halamine, one or more halogen atoms are covalently bond to nitrogen atoms. The mode of action has been described as either direct transfer of active halogen from the haloamine groups to the cell wall of the microorganisms by direct contact followed by oxidation or dissociation into water followed by diffusion over the microorganisms. Active halogen species (oxidative halogens, i.e., Cl^+ or Br^+ ions) then interact with biological receptors (thiol or amino protein groups) upon contact with cells leading to cell inhibition or cell inactivation. Thus the mode of action does not arise from the polymer itself (as it is the case with cationic polymers) but mainly from the N-halamine functional group. There are several research reports describing the introduction of N-halamine into polymers to achieve antibacterial properties and many research groups have developed N-halamine

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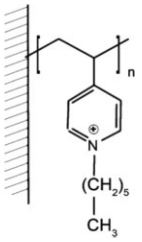
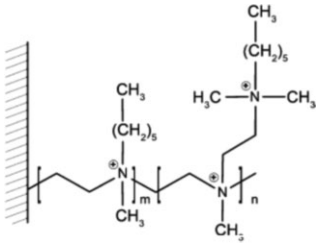
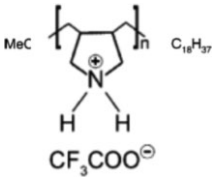
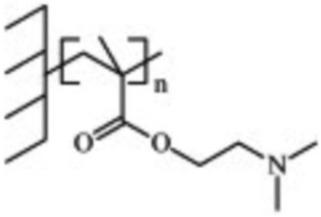
Table 2. Antimicrobial coatings obtained by surface modification with cationic polymers.

Name and typical structure of cationic polymeric coatings	Surface	Grafting strategy	Reference
P4VP polymeric coating 	Glass plastic	Covalent modification Covalent modification	[47,50]
PEI based polymeric coating 	Glass Textile	Covalent modification Dip coating	[21b][48,19,26]
Polymers with incorported quarternary ammonium ODDMACPDDMAC 	Cellulose Glass	Covalent modification Dip coating	[21c][54a][54b]
PDMAEMA 	Glass Inorganic surfaces Plastic (polypropylene)	ATRP (grafting from) RAFT (grafting from) ATRP + covalent (grafting onto)	[22a][60,63,59]

functionalized polymers and illustrated their highly potent antimicrobial properties.[70] Worley and coworkers[71a] illustrated that functionalizing surfaces with N-halamine siloxane polymers renders a surface of high biocidal activity against fungi, bacteria, yeasts, and viruses. A wide variety of surfaces can be modified by such coatings including cellulose, synthetic fibers, ceramics, plastics, polyurethanes, and metallic surfaces.

One of the polymers, a halogenated poly-styrene hydantoin derivative[71] is currently being marketed by HaloSource, Inc., all over India, China, and developing nations in an antimicrobial water filter application. The synthesis of hydantoin derivatives from commercial polystyrene appears as a very facile and inexpensive methodology (see Scheme 1). The polymer exhibits highly potent antimicrobial properties against a broad spectrum of

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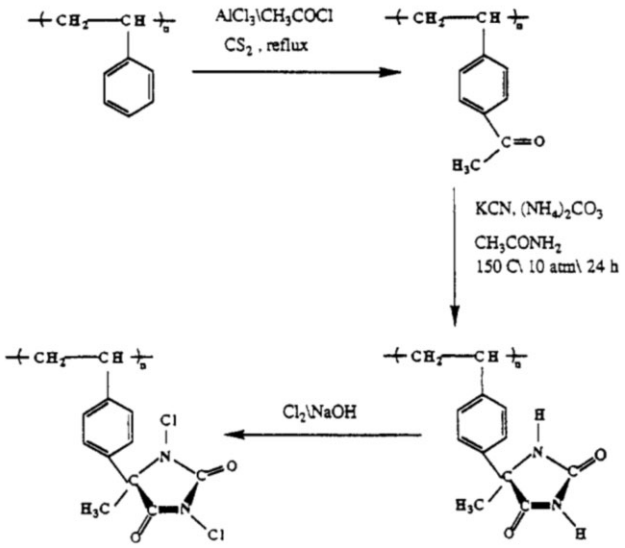

Scheme 1. Synthesis of poly(l,3-dichloro-5-methyl-5-(4′-vinylphenyl)-hydantoin), from commercial polystyrene by three steps methodology. Reproduced with permission.[71b] Copyright 1994, American Chemical Society.

pathogens including *S. aureus*, *P. aeruginosa*, *E. coli*, *C. albicans*, *Klebsiella terrigena*, *Legionella pneumophila*, and rotavirus, among others. Microrganisms get killed within few seconds of contact making it very advantageous for water disinfectant applications. Moreover the antimicrobial action is effective over a broad range of pH from 4.5 to 9.0 and in a wide range of temperature as well (between 4 and 37 °C). It is also effective in chlorinated waters. Further, since the polymer is insoluble in water it does not react with water, which makes the choice of this polymer a very safe option. The polymer is also very stable and all the studies so far for assessing its toxicity reveal that it is non-toxic and do not cause any adverse effect to humans and animal on contact.

The research group of Sun and Worley has also developed N-halamine siloxane and epoxy coatings of surfaces such as sand, textile, paint and cotton.[71b][71c] N-halamine based surface coatings thus reveal many advantages compared to other antimicrobial surfaces since they inhibit and kill a wide range of microorganisms without raising much of environmental concerns. Additionally, it has also been shown that it is very difficult for microorganisms to develop resistance against such surfaces.[71d] In a recent work, this research group also combined the unique antibacterial property of chitosan with the effectiveness of N-halamine by preparing N-halamine-modified chitosan films which were very effective in killing *S. aureus* (ATCC 6538) and *E. coli* O157:H7 (ATCC 43895).[71e] In another work, Chen and Han illustrated a highly efficient design of antibacterial surfaces based on N-halamine.[71f] PS-PtBA revealed its tert-butyl groups on the surface of PS when spin coated from toluene solutions. The tert-butyl groups were hydrolyzed into reactive carboxylic groups by exposition to TFA. Tert-butylamine molecules were conjugated with surface bound carboxylic groups via amide bonds using 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) as a linker. Antibacterial N-halamines were then generated by the chlorination of amide groups with a NaOCl solution. When these surfaces were challenged with *S. aureus* and *E. coli* there was a drastic reduction of bacterial colonization illustrating their antimicrobial activity (Figure 10).[71f]

In another report, an N-halamine precursor 5,5-dimethylhydantoin (DMH) was covalently attached to polyurethane (PU) and after subsequent bleach treatment DMH transformed into N-halamine.[72a] These N-halamine-based PU showed potent antimicrobial activity against variety of bacteria and prevented formation of fungal or bacterial biofilm. The most interesting property of this PU surface was also that if its antimicrobial property was lost it could be regenerated again by chlorination treatment (Figure 11).

Cao and Sun synthesized a novel N-halamine monomer, N-chloro-2,2,6,6-tetramethyl-4-piperidinyl methacrylate

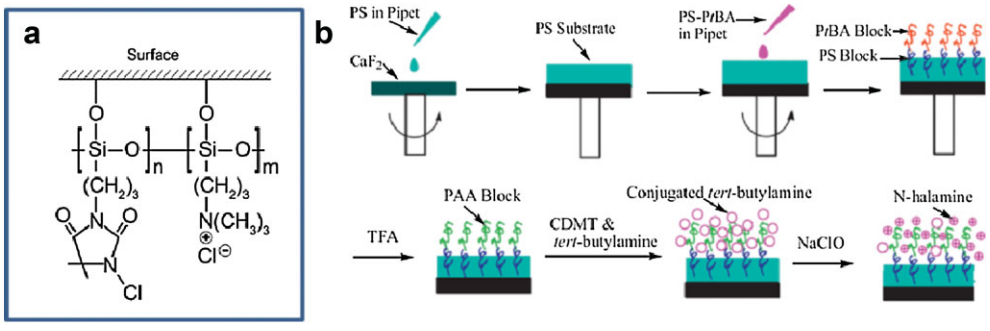

Figure 10. (a) Schematic representation of copolymer of an N-halamine siloxane with trimethyl-ammonium salt siloxane coated on a surface (b) Strategy to design N-halamine based antimicrobial coatings on a PS surface. Reproduced with permission.[71f] Copyright 2011, Elsevier.

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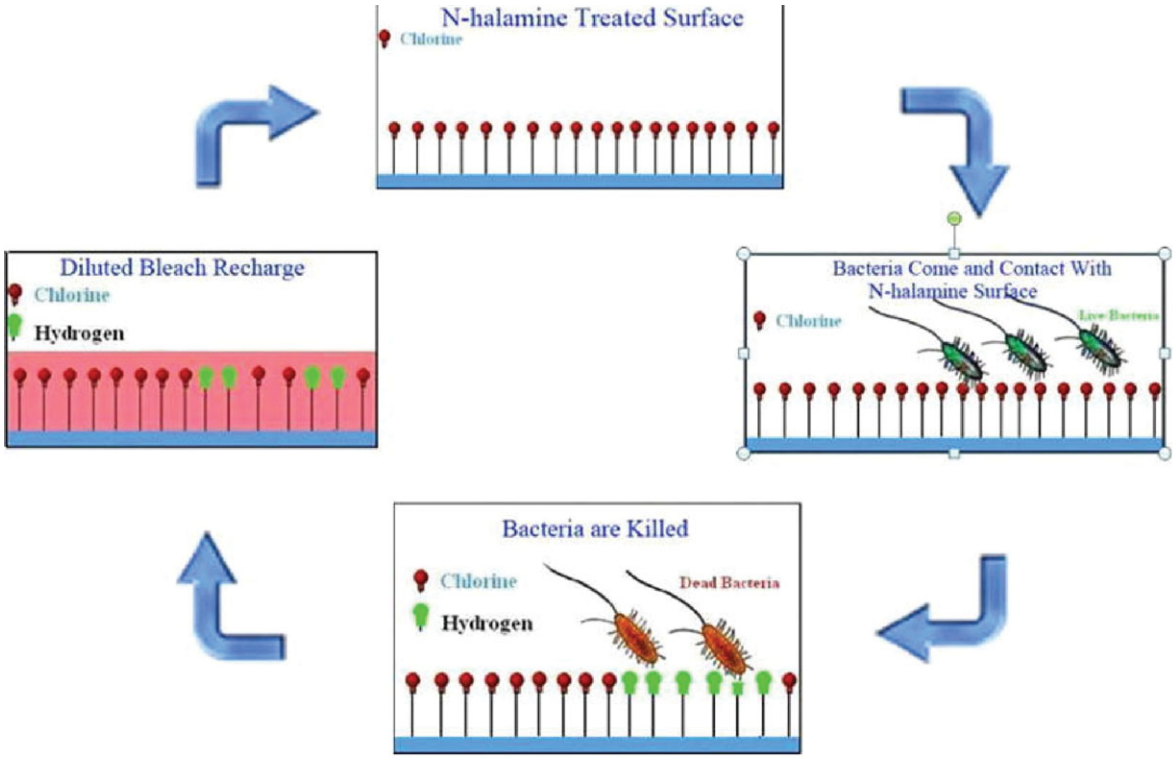

Figure 11. N-halamine based polyurethane surface which can kill bacteria and prevent biofilm formation and can be reused again after treatment with dilute bleaching solutions. Reproduced with permission.[73a] Copyright 2012, Elsevier.

(Cl-TMPM) to prepare water-based polymeric N-halamines by an emulsion polymerization technique.[73b] Polymeric N-halamine latex emulsions were mixed with commercial latex paints as antimicrobial additive which conferred to these paints a long lasting rechargeable antibacterial property.

In another work, researchers synthesized series of copolymers containing units of a novel hydantoinylacrylamide and the sodium salt of 2-(acrylamido)-2-methylpropanesulfonic acid.[73c] These copolymers were water soluble and used as antimicrobial additives/coatings in water based latex paints. Another N-halamine based antibacterial coating which was very stable toward hydrolysis and UV photodegradation was synthesized to design cellulose fibers having antimicrobial property. In this approach copolymer of 3-chloro-2-hydroxypropylmethacrylate and glycidyl methacrylate was coated onto cotton, and, after curing it was further treated with an aqueous solution containing the potassium salt of 5,5-dimethylhydantoin to form a coating which became antimicrobial upon exposure to household bleach (sodium hypochlorite). N-halamine based siloxane and epoxide coatings were also synthesized to give potent biocidal properties to sand, textiles, and paints.[73d] Readers are referred to a recent comprehensive review about N-halamine based polymers.[71a]

4. Polymers Immobilization to Design Microbe Repelling Surfaces

Coating surfaces with non-charged hydrophilic polymers results in reduced adhesion of bacteria on a variety of surfaces by steric hindrance. Most of the literature in this area reports on the use of poly(ethylene glycol) (PEG) and its derivatives for surface modification to develop such microbe repelling surfaces. PEG is composed of $-CH_2CH_2O-$repeating units with a hydroxyl group at each end of the polymer chain.[73] Although the exact mechanisms of action of PEG on repelling bacteria are still not clear there are several hypotheses to justify the bacterial repelling property of PEG-modified surfaces which include hydrophilicity, high polymer flexibility, large excluded volume, and associated steric hindrance effects, which contribute to both protein and bacterial repellence of such surfaces.[74] In the following sections of this review, relevant research activities carried out by various groups to achieve antifouling surfaces by PEG modification will be discussed in detail.

Desai et al. modified the surface of poly(ethylene terephthalate) (PET) films with poly(ethylene oxide) (PEO) and demonstrated that these films were resistant to bacterial adhesion. A significant reduction (between 70 and 95%) of the number of adherent bacteria could be

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observed as the antifouling efficacy was tested on three bacterial strains most commonly associated with implant infections, namely *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.[75] For a recent review of the preparation and characterization of non-fouling polymer brushes on PET film surfaces, the reader is referred to the one from Tan et al.[76] Park et al. modified poly(urethane) (PU) surfaces with PEG ($\overline{M}_w$ 1000, PEG1k) bearing terminal hydroxyl, amino and sulfonate groups, PEG3.4k and PEG modified with heparin, PEG3.4k-Heparin, respectively. They showed that the bacterial repelling property was dependent on the molecular weight (chain length) of PEG. Polymers of high molecular weight were more efficient in reducing bacterial adhesion. It was also shown that bacterial adhesion also depended on the strain of bacteria and media used as assessed by their investigations with two bacterial strains *S. epidermidis* and *E. coli* in tryptic soya broth (TSB), brain heart infusion (BHI), and human plasma. While a significant reduction in *E. coli* adhesion could be observed on all PEG1k, PEG3.4k, and PEG-heparin modified surfaces in TSB and BHI media, no adhesion reduction could be monitored with *S. epidermidis* either on PEG1k modified surfaces or in TSB media, regardless of the terminal functional groups of PEG1k. However, adhesion in plasma was reduced to different degrees, depending on PEG1k terminal groups (least adhesion on the sulfonated PEG modified surface). Surfaces modified with relatively longer PEG (PEG3.4k) and PEG3.4k-heparin minimized bacterial adhesion in both media.[77]

Vacheethasanee et al. described a series of surfaces modified with polymers to prevent bacterial adhesion.[78] The surfactant-like polymers consist of a poly(vinyl amine) and a poly(ethylene oxide) (PEO) backbone modified with hydrophobic hexanal (Hex) side chains (PVAm/PEO:Hex). The surfaces of pyrolytic graphite (HOPG) and polyethylene (PE) were modified with these polymers by a simple dip coating procedure. It was demonstrated that significant suppression of *S. epidermidis* adhesion occurred on all surfaces modified with this polymer.

Roosjen et al.[79] designed novel bacteria and yeast repellent surfaces by covalently modifying glass or silica surfaces with PEO-brushes. The anti-adhesive properties of these surfaces were tested for five types of bacteria (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus salivarius*, *E. coli*, and *Pseudomonas aeruginosa*) and two yeast strains (*Candida albicans* and *Candida tropicalis*). It was demonstrated that the more hydrophobic strain showed lesser reduction in adhesion as compared to others whereas typically more than 98% reduction in bacterial adhesion was observed on glass surfaces modified with PEO brushes for the four bacterial strains. The more hydrophobic *Pseudomonas aeruginosa* suffered lower surface repulsion. For yeast strains, adhesion or repellence was less effective than for bacteria as demonstrated with two additional strains: The hydrophobic *C. tropicalis* was less repelled than the more hydrophilic *C. albicans*. In a related study, the same research group also demonstrated the influence of the polymer chain length and temperature on microbial adhesion.[80] PEO brushes composed of polymers of the longer chain length were more effective in reducing microbial adhesion.

Recently, Dong et al. reported the design of antifouling polyester (PE) surfaces modified by grafting poly(ethylene glycol) of various molecular weights (PEG, MW 200, 400, 600, 2000, and 4600).[81] PEG-grafted PE surfaces showed significant inhibition of attachment and biofilm formation by bacteria as compared to unmodified surfaces. It was shown that the antifouling property depended on the molecular weight of PEG grafted on the surface and PEG 2000 showed maximum inhibition of bacterial adhesion.

Ho et al. described novel coatings which can effectively both repel and kill bacteria. This coating was based on a hydrophilic polymer network of poly(2-hydroxyethylacrylate) with PEI cross-linking points. The latter is capable of selectively taking up silver ions from aqueous solution and act as a template for the formation of silver nanoparticles.[82b] PEGylation of these co-networks results in materials that efficiently kill *S. aureus* cells and still repel them after exhaustion of the silver (Figure 12). A new antifouling polyester monomethoxy-poly(ethylene glycol)-b-poly(L-lactide)-b-poly(sulfobetaine methacrylate) coating on glass surfaces demonstrated bacterial repellence as well.[82]

Recently, Cottenye et al. designed model surfaces allowing independent variation of the material topography and chemical composition to study the adhesion and biofilm growth of *E. coli* as assessed in static biofilm and dynamic growth mode.[83] These surfaces were produced by covalent binding of short nucleotide sequences and subsequent immobilization of vesicular structures resulting from the self-assembly of the polymer-modified nucleotide sequence complementary to the surface-tethered one. This study led to evidences that oligonucleotide-modified surfaces, independent of the topographical feature used, enhanced curli expression without any increase in the number of adherent bacteria. The nanoscale mean roughness with a topographical feature of the surface modified with nucleotide sequences did not have any effect on bacterial adhesion and curli expression, which raised the question of the effect of the substrate mechanical properties on the bacterial response.

Besides the synthesis of novel copolymers and self-assembled structures thereof to prepare antifouling brushes,[84] peptide and their synthetic analogues are currently investigated as well to design antifouling surfaces.[85] Along this line, a whole research area is devoted to the preparation of antifouling membranes. Being that topic going beyond the scope of this review

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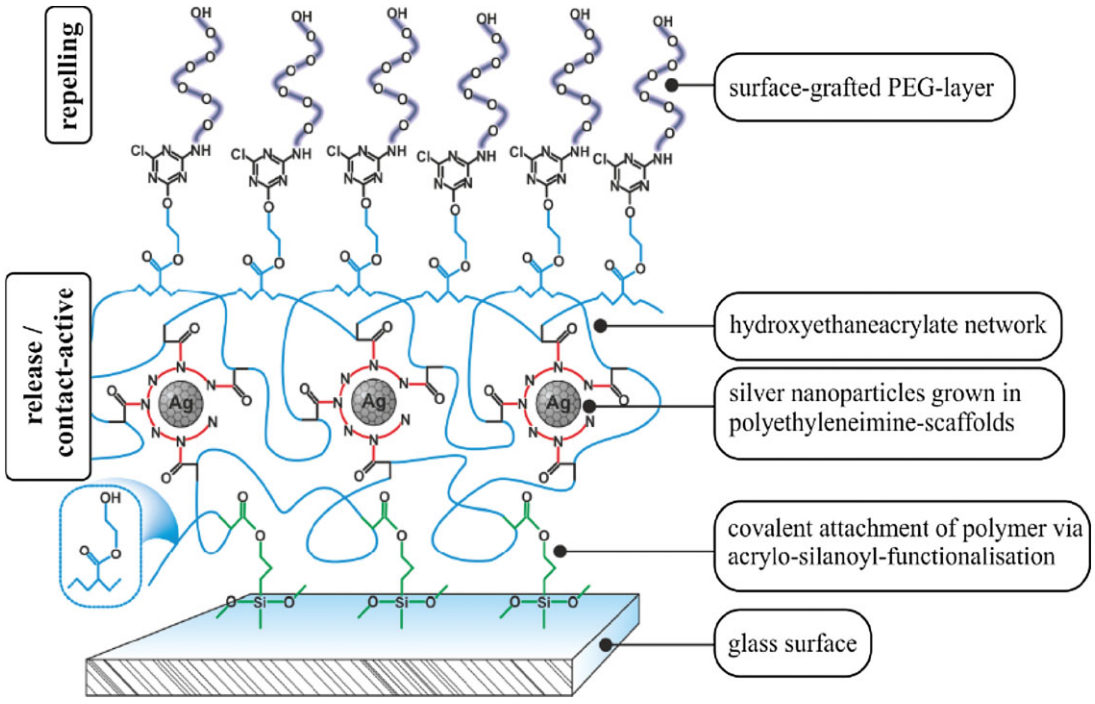

Figure 12. Design of repelling and releasing surfaces. Reproduced with permission.[50]

article, we give here few recent references to the reader as guide lines for further readings. The antifouling properties of ultrafiltration membranes have been improved[86] whereas external membrane surface coverage with brushes confer superior antifouling and self-cleaning abilities.[87] Macropourous antifouling membranes were prepared by the grafting of poly(2-hydroxyethyl methacrylate) and poly(N-isopropyl acrylamide) via a photo-induced surface initiated RAFT method.[88] Desalination and water treatment are areas of application in which fouling might become an issue and is fought through the polymerization of brushes as well.[89]

5. Conclusion and Perpectives

The ideal approach would create a permanently sterile, non-leaching material by functionalizing the surface with an antimicrobial compound. Polymeric chains, natural biocidals such as chitosan or to which antimicrobial moieties are attached such as antibiotics or silver ions, allow their permeation into and kill the cells of the pathogen. The effectiveness of such coatings is strongly dependent on the antibiotic release profile from the polymer. The use of biodegradable polymers enables the delivery of higher doses of antibiotics and depending of the polymer composition, crystalline state as well as inclusion of fillers and the degradation products are common metabolites and thus toxicity is of less concern. In parallel, much research has focused on developing polymers that resist bacterial adhesion to design passive coatings in analogy to their counterpart, which actively release antibacterial agents. Exploiting surface mechanical properties for preventing surface colonization by bacteria appears as a possible future strategy to explore for the prevention of biofilm formation and biofilm-related infections. Although some publications, yet few, evidence the possibility to control bacterial adhesion by tailoring the material softness, the potential impact of surface mechanical properties on bacterial behavior and colonization on material is usually neglected and almost completely unknown. Exploiting the surface mechanical properties for preventing surface colonization by bacteria could appear as an alternative promising future strategy for the prevention of biofilm formation and biofilm-related infections.

Abbreviations

EPS	extracellular polymeric substances
DspB	Dispersin B
PEG	poly(ethylene glycol)
ATRP	atom transfer radical polymerization
RAFT	reversible addition fragmentation chain transfer polymerization
PEI	poly(ethylene imine)
PVP	polyvinylpyrrolidone

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PDMAEMA	poly2-(dimethylamino) ethyl methacrylate
TEM	Transmission Electron Microscopy
AFM	Atomic Force Microscopy
SEM	Scanning Electron Microscopy
PE	polyethylene
TTP	tripolyphosphate
DD	degrees of deacetylation
HACC	hydroxypropyltrimethyl ammonium chloride chitosan
SAR	structure activity relationship
ODDMAC	octadecyldimethyl(3-trimethoxysilyl-propyl)ammonium chloride
TEOS	tetraethoxysilane
PDADMAC	poly(diallyldimethylammonium chloride)
PU	poly(urethanes)
BIN	N,N-bis(2-hydroxyethyl)isonicotinamide
PTMO	poly(tetramethylene oxide)
PSM	polymer surface modifiers
ATRP	atom transfer radical polymerization
DMAEMA	2-(dimethylamino)ethyl methacrylate
PP	poly(propylene)
PQA	poly(quaternary ammonium)
PDMAEMA	poly(2-(dimethylamino)ethyl methacrylate)
RAFT	reversible addition-fragmentation chain transfer
QA	quartenary ammonium
DDA	N, N dimethyl dodecyl ammonium
PEOx	(poly-2 ethyl-oxazoline)
CDMT	2-chloro-4,6-dimethoxy-1,3,5-triazine
PET	poly(ethylene terephthalate)
TSB	tryptic soya broth
BHI	brain heart infusion
PLL-g-PEG	poly((l-lysine)-grafted-PEG
PLL-g-PEG/RGD	poly((l-lysine)-grafted-PEG/Arg-Gly-Asp

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