Review

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Development of biomaterial surfaces with and without microbial nanosegments

Abstract: Infections by microorganisms are a major problem in public health throughout the world. Artificial materials, including biomedical goods, inherently lack defense against microbial development. Therefore, microbial cells can adhere on any type of artificial surface, particularly in a moist environment, and start to multiply to form a huge population. In this review, we will discuss a strategy for designing antimicrobial polymers and antimicrobial surfaces. Generally, there are five types of antimicrobial polymers: (a) polymeric biocides, (b) biocidal polymers, (c) biocide-releasing polymers, (d) bioactive oligopeptides, and (e) antimicrobial surfaces. Antimicrobial surfaces preventing the growth of microorganisms are a promising method to inhibit the spread of microbial infections. The antimicrobial surfaces can reject the attachment of microbes and/or kill microbes in the vicinity and can be designed to kill microbes on contact. It is recommended that the material surface not release biocidal substances, therefore preventing exhaustion of biocide release to kill microbes. Furthermore, the antimicrobial surfaces are desired to be nontoxic to human cells. The development of contact-active antimicrobial surfaces by grafting antimicrobial nanosegments onto the material surface will be an important topic in the future.

Keywords: antimicrobial; biomaterial; nanosegment.

DOI 10.1515/polyeng-2015-0046
Received February 10, 2015; accepted March 16, 2015; previously published online April 17, 2015

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1 Introduction

Infections by microorganisms are a major problem in public health throughout the world [1]. Hence, control of the growth of all types of microbes, such as bacteria, fungi, and viruses, in the human body as well as in the human environment is necessary for the survival of humans. These control mechanisms, including the human immunological system and environmental systems, often do not work well, which leads to microbial infections. The majority of deaths of humans were reported to be caused by microbial infections [2]. Recently, preventing and treating

microbial infections is becoming more difficult because of antibiotic-immune patients and the multiple drug resistance (MDR) capacity of certain microbial cells [2]. MDR is currently one of the most severe global healthcare problems in human society [3, 4]. According to one report, in 2010, two million people acquired nosocomial infections in hospitals in the USA, and nearly 100,000 people died from the infection [5] However, at present, in the USA, the frequency of nosocomial infections has gradually declined, although approximately 70% of these infections become resistant to at least one antibiotic, and this trend is starting to increase.

Artificial materials, including biomedical goods, inherently lack defense against infectious agents. Therefore, microbial cells can adhere on any type of artificial surface, particularly in a moist environment, and start to multiply to form a huge population. Catheters used for a long time can lead to dangerous implant-associated infections. Nearly one-half of nosocomial infections are due to the use of medical implants, and these infections can be extremely serious because infectious agents with MDR usually cause them [1].

Any contaminated surface commonly starts to form a biofilm when the number of microbial cells on the surface increases. The biofilm created by microbial cells consists of a polysaccharide matrix with embedded cells (Figure 1) [6]. The construction of biofilms protects the microbial cells, allowing them to survive under their optimal conditions. These conditions generate a barrier to antibiotics and biocides, making the cells much less susceptible compared with microbial cells without the protection of biofilms [7]. Pathogenic and resilient infections are spread via the biofilms, in which several toxins secreted by the microbial cells accumulate at high concentrations in the closed system [8]. Furthermore, it has been reported that the bacteria within biofilms occasionally become multiresistant bacterial strains [9]. The enterohemorrhagic *Escherichia coli* epidemic, which is widely distributed in Europe, is one example of this situation [2]. Therefore, the prevention of microbial growth in different environments, such as hospitals, materials manufacturing and the food industry, is a recent key issue.

One of the methods used to prevent microbial infection and propagation is maintenance of sterile conditions on material surfaces using toxic disinfectants composed of H_2O_2, hypochlorite, and chemicals that generate reactive oxygen species. Alternatively, alcohols, ammonium compounds, and silver salts can be used for this purpose.

However, it is difficult to maintain sterile conditions for a long time, which leads to frequent usage of disinfectants. The frequent usage of disinfectants causes an environmental problem, as has been reported for the usage of triclosan in society [10]. Furthermore, the long-term usage of disinfectants leads to the development of microbial strains that are strongly resistant to disinfectants. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one example of a bacterium that causes nosocomial infections, and it is causing more deaths than human immunodeficiency virus is in the USA [11, 12].

The development of antimicrobial surfaces not only prevents biofilm formation by infectious agents, but also presents alternative methods to inhibit the further spread of microbial infections. The antimicrobial surfaces can reject the attachment of microbes and/or eradicate microbes in a particular environment. Material surfaces that release biocides such as antibiotics, active chlorine, triclosan, antimicrobial ammonium components, or silver can kill microbes even in the vicinity of the material surface. However, the problem with these material surfaces is that their release of these biocides can be exhausted and can also pose an environmental problem due to the release of the biocides into the environment. An alternative method is use of the surface of materials to catalytically synthesize biocides using externally induced electrical, chemical, or optical energy. However, the design and development of these surfaces are limited.

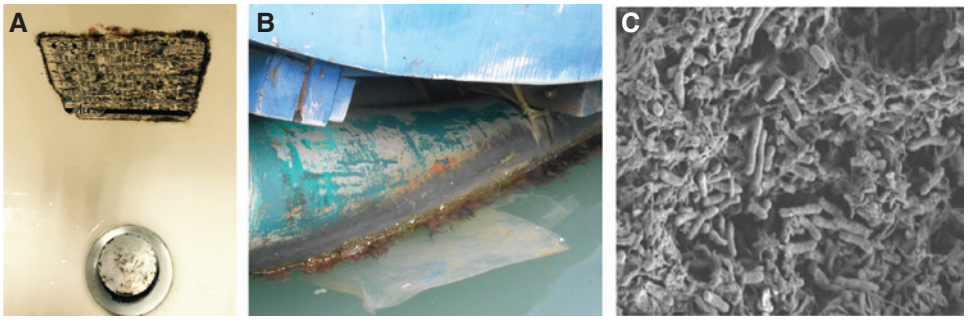

Figure 1: Biofilm morphologies: (A) mold found in a room, (B) algae found on a ship wall, and (C) bacterial biofilm found on a catheter. Adapted from [2] with permission under Creative Commons Attribution License.

Another possible way to develop antimicrobial surfaces is to design a material surface that kills microbes on contact, without releasing chemical substances, i.e., biocides, and that therefore does not easily undergo biocide depletion by release to kill microbes. These antimicrobial surfaces, i.e., contact-active surfaces, can be developed by grafting antimicrobial nanosegments onto the material surface [13]. However, only a few studies on the development of contact-active antimicrobial surfaces have been reported, and the preparation methods are only valid for specific material surfaces with special conditions of usage [13]. It will be necessary to develop a novel type of contact-active antimicrobial surfaces in which effective antimicrobial nanosegments are grafted at an optimal concentration. Furthermore, according one report, the nanosegments on the surfaces act differently toward microbial cells in aqueous solution, so their functional principle is still under investigation [2, 14]. Further analysis and development of the mechanism of killing microbial cells upon contact with antimicrobial surfaces are also necessary.

Free antimicrobial nanosegments (polymers) in aqueous solution have been developed by several researchers [2], although only a few cases have been reported, in which antimicrobial nanosegments were immobilized on a material surface to develop antimicrobial surfaces. Several types of polymers with antimicrobial characteristics have been developed, such as polymers with a salicylic acid group and a quaternary ammonium group. However, these polymers' functions are not fully understood, and the efficiency of the antimicrobial effect when these polymers are grafted onto a material surface is unknown. The number of FDA-approved disinfecting polymers has been extensively increasing, especially in biomedical usage in the past decade, indicating the need for both alternatives to antibiotics and ecofriendly disinfectants.

Generally, there are five types of antimicrobial polymers: (a) polymeric biocides, (b) biocidal polymers, (c) biocide-releasing polymers, (d) bioactive oligopeptides, and (e) antimicrobial surfaces (Figure 2) [2].

The polymeric biocides have biocidal groups that are conjugated to the polymer, analogous to a low-molecular-weight (MW) biocide, i.e., each monomer group is a biocide (Figure 2A). In general, the polymeric biocides are less bioactive than the corresponding low-MW biocides. This difference might be caused by the steric hindrance and low mobility of the polymer backbone.

The biocidal polymers do not necessarily have antimicrobial repeating units in their main chain (Figure 2B), and only a few biocidal polymers have been reported. This system does not work through the actual polymeric sites; rather, the polymer functions as a carrier of biocides that can be transferred to the targeted microbial cells. The biocide-relating polymers are the most active system because they can release their biocides very close to microbial

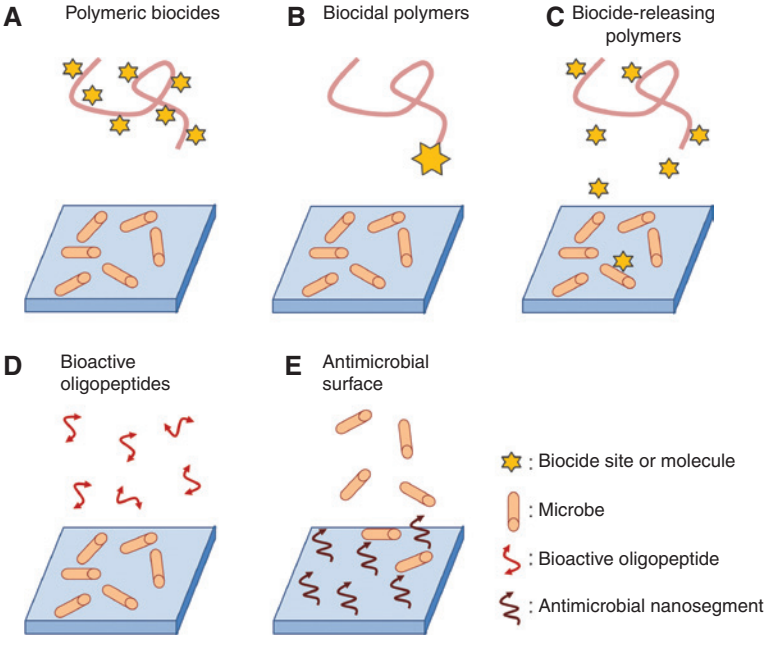

Figure 2: Antimicrobial polymers and their antimicrobial activity: (A) polymeric biocides, (B) biocidal polymers, (C) biocide-releasing polymers, (D) bioactive oligopeptides, and (E) antimicrobial surface.

cells, with high local concentrations. However, there is a limited time period in which the biocide-releasing polymers are active, because the biocides run out after their full release from the polymers. The following section will outline the recent developments in the field of polymers, i.e., the use of antimicrobial nanosegments to develop and design contact-active antimicrobial surfaces including specific nanosegments (polymers).

2 Antimicrobial nanosegments (polymers)

2.1 Polymeric biocides

Polymeric biocides are defined as polymers consisting of bioactive repeating units. These polymers are composed of multiple interconnected biocides and are expected to work similarly to the biocide monomers. However, because of polymerization, the biocidal monomers often lead to inactive antimicrobial polymers. One example is crosslinked polymers prepared from the antimicrobial monomer of 4-vinyl-*N*-benzylpyridinium chloride, leading to the formation of non-biocidal polymers, which do not kill microbes but which do capture microbes effectively [15].

To attain high activity and less toxicity, polymerization of antibiotics is a well-accepted method. For example, the antibiotics cephradine and penicillin V are less toxic when bound to the polymer of polyethylene glycol (PEG)-lysine [16].

However, if antibiotics are conjugated via a hydrolytically labile bond, their full antimicrobial activity will be activated. Biocidal active polymers were reportedly formed by direct modification of vancomycin with polyethylene glycol methyl ether methacrylate and subsequent polymerization [17]. However, the polymeric biocides are generally described as extremely low-activity biocides compared with the unmodified antibiotic [18]. Another report suggested that polyacrylate nanoparticles attached to penicillin had better activity against MRSA than unattached penicillin did [19]. Recently, polymeric biocides were designed using antimicrobial side groups, consisting of hydrophobic groups with quaternary ammonium. These polymers are occasionally more effective than the monomers, and their active function originates not only from the biocidal groups, but also from the antimicrobial characteristics of the polymeric main chain [20].

2.2 Biocidal polymers

The antimicrobial effect of biocidal polymers is exerted by the whole polymer chain. Polycations with amphiphilic characteristics are typical examples of biocidal polymers, acting on the surface of microbial cells, which carries a negative net charge.

Positive charges adhered on materials, bacteria and fungi surfaces have been used antimicrobial agents by themselves. The main positively charged sites in these natural or synthetic polymers are generally quaternary ammonium groups that generate quaternary ammonium compounds. The advantage of amphiphilic cationic polymers when compared to small amphiphilic molecules is their enhanced antimicrobial activity. In general, most of these polymers exhibit low toxicity to human cells, which is a major requirement for biomedical applications [21].

Some polymeric quaternary ammonium compounds such as polyquaternium-1, a quaternary ammonium polymeric compound, and myristamidopropyl dimethylamine (Figure 3) were known to induce lysis of spheroplasts of *Serratia marcescens* and *Aspergillus fumigatus*, but not those of *Candida albicans* [22].

Some polyions have antimicrobial and cell lysis abilities. Poly(*N*,*N*-dimethylaminoethyl methacrylate) (PDMAEMA) based-copolymers as well as poly (diallyldimethylammonium chloride) also show antimicrobial activity [23–26]. Quaternization of PDMAEMA or its copolymers by changing to alkyl quaternized derivatives is further used to improve antimicrobial activity. This is designed to enhance positive charge density and amphipathic characteristics of these polymers [21, 23, 24].

Chitosan derived from a deacetylation reaction of chitin has attractive antimicrobial activity due to its biodegradable characteristics. The native chitosan has extremely low solubility in aqueous solution at natural conditions (i.e., pH=7) (Figure 3). Chitosan is going to be soluble in aqueous solution and exhibit its antimicrobial activity only when chitosan has the positive charges which are carried by the protonated amine groups of chitosan in acidic conditions. Therefore, chemical modifications of chitosan are extensively studied to enhance its antimicrobial activity. Quaternization on the nitrogen atom of chitosan may be the most common method to render water-soluble and antimicrobial chitosan-derivatives at physiological pH conditions. Several reports have been published for antimicrobial activity of chitosan derivatives [27, 28]. Li et al. [28] prepared an antimicrobial hydrogel based on dimethyldecylammonium chitosan (with high quaternization)-graft-poly(ethylene glycol)

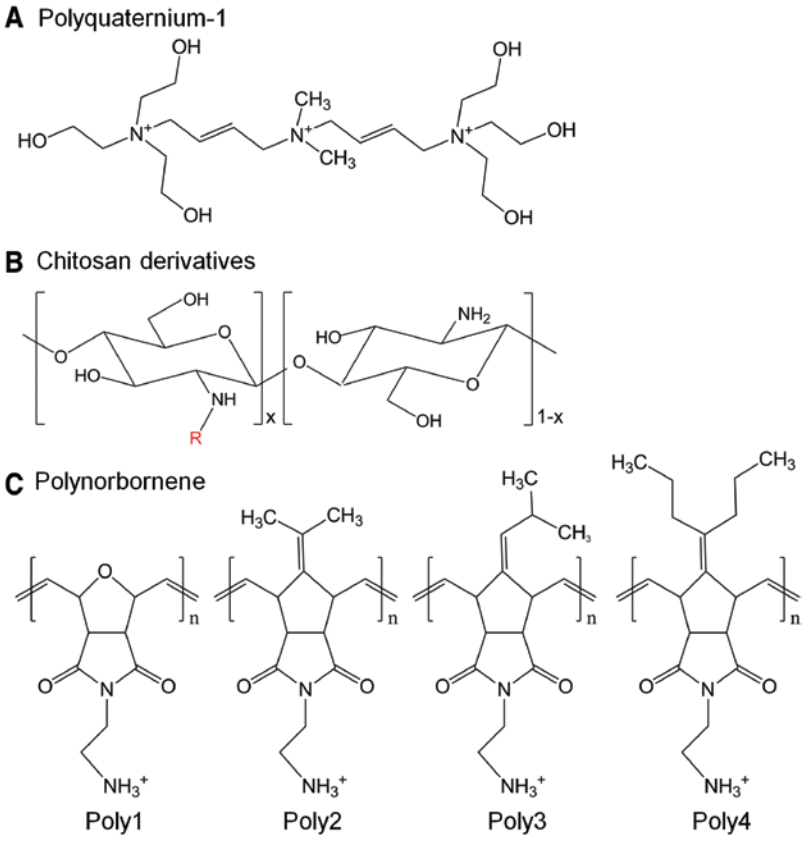

Figure 3: Chemical scheme of (A) polyquaternium-1, (B) chitosan derivatives, (C) polynorbornene derivatives, Poly1, Poly2, Poly3, and Poly4 [30]. R is alkyl and/or aromatic containing group.

methacrylate and poly(ethylene glycol) diacrylate, which had high antimicrobial activity against *E. coli*, *Fusarium solani*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The mechanism of the antimicrobial activity of the polycationic hydrogel was considered to be generated by attraction of sections of anionic microbial membrane into the internal nanopores of the hydrogel, leading to microbial membrane disruption that caused death of the microbe [28].

Ilker et al. [29, 30] synthesized several types of polynorbornene derivatives that are peptide-mimetic antimicrobial polymers and investigated the antimicrobial activity induced by the polynorbornene derivatives (Figure 3). The cationic polynorbornene derivatives showed antimicrobial activity depending on their molecular structure. Poly1, a cationic polymer with no substantial hydrophobic group, did not show any antibacterial activity in accordance with the lack of activity against phospholipid membranes [29, 30]. Antimicrobial activity is going to increase with an introduction of optimal size of hydrophobic side group. Poly2, having an isopropylidene side group, showed antimicrobial activity with a minimum inhibitory concentration (MIC)=200 μg/ml for *E. coli*. Poly3 displayed an extensive increase of antimicrobial activity (MIC=25 μg/ml for *E. coli*.). However, Poly4, which has the biggest hydrophobic side group, showed less antimicrobial activity (MIC=200 μg/ml for *E. coli*.) [30]. This study indicates that the balance between electrostatic interaction (cationic group in polynorbornene derivatives) and hydrophobic interaction is important for antimicrobial activity of cationic polymers.

Antimicrobial polymers occasionally are not composed of polymerized cationic biocides. Therefore, biocidal polymers containing no biocidal repeating units have been developed, with the antimicrobial activity originating from the whole molecule. The following are several examples of polymers with a quaternary ammonium unit on the side or main chain. These examples reveal that certain polymers involve biocidal repeating units in the polymer chain, whereas other polymers only need a quaternary ammonium unit.

Magainin and defensin are antimicrobial peptides (AMPs). The function of AMPs is based on the following two features [31–33]: (a) a highly stiff backbone and (b) side units composed of rigid molecules, with one

positively charged side unit and one hydrophobic side unit (Figure 4). This arrangement is highly efficient for disruption of the cell membrane of microbes because the whole backbone of AMPs can be inserted. This intrusion causes destruction of the membrane, breaking it apart, which results in spontaneous cell death [34].

Poly(phenylene ethynylene)-based conjugated polymers with positive side groups and rigid backbones have been designed based on the structures of magainin and defensin (Figure 4). These polymers show low toxicity and high antimicrobial activity. Certain random peptide sequences have been designed based on AMPs and are prepared using beta-lactams with ring-opening polymerization [35]. These polymers are considered to be mimics of the structure of magainin (Figure 4). The peptides such as magainins involve induction of a globally amphiphilic helix folding pattern upon interaction with a bacterial membrane (Figure 4). The globally amphiphilic conformation is responsible for disruption of the bacterial membrane. By contrast, the random peptide sequences generate microbial activity by the induction of globally amphiphilic but irregular conformations in the presence of a bacterial membrane [35].

Moreover, poly(phenylene ethynylene) and a random copolymer class of dimethylaminomethyl styrene and octylstyrene antimicrobial polymers with protonated tertiary and primary amino groups have been recently described [36]. Copolymers of dimethylaminoethylacrylamide and aminoethylacrylamide with n-butylacrylamide containing quaternary ammonium derivatives were synthesized, and these copolymers showed antimicrobial characteristics and were less toxic to blood cells [37]. Furthermore, Timofeeva et al. [38] developed poly(diallylammonium) salts containing secondary and tertiary amino groups [38]. These polymers have excellent antimicrobial activity against *C. albicans* and *S. aureus*. In addition to these polymers, quaternized and hyperbranched polyethylenimine (PEI) was found to exhibit excellent antimicrobial properties [39].

2.3 Biocide-releasing polymers

Vogl and Tirell [40] first reported the polymerization of biocide-releasing molecules using salicylic acid, but they did not report antimicrobial characteristics. However, these authors showed that polyester releases salicylic acid during degradation. Nearly the same effect was reported for acrylate polymers with salicylic acid side units or poly(anhydride esters) based on salicylic acid [41].

Tributyltin esters of polyacrylates are biocide-releasing polymers that kill microbial cells in the environment at trace concentrations [2]. Eknoian et al. [42] prepared a series of antimicrobial polymers conjugated to *N*-halamine groups, allowing long-term storage of active chlorine. This active chlorine is directly transferred to microbial cells, which are killed by oxidization of the lipids in the microbial membrane [43].

Coneski et al. [44] and Stasko and Schoenfisch [45] introduced another type of biocidal polymer that releases nitric oxide (NO). Varying the composition and curing temperatures of the polyesters resulted in polyesters with tunable thermal and degradation properties. Post-polymerization coupling of aminothiols to terminal carboxylic acids generated thiol-containing polyesters, with thermal and degradation characteristics similar to those of the parent polyesters. After nitrosation, these polyesters were capable of releasing up to 0.81 μmol NO cm^{-2} for up to 6 days. The antimicrobial activity of the polyesters was shown to reduce 80% of *P. aeruginosa* adhesion compared to unmodified polyesters [44]. This polyester contains diazeniumdiolate groups that can be generated by the addition of NO when the amino acids in the polyester are under high pressure, although the antimicrobial polyester fails to remain active for long-term use.

Tea is a natural source of polyphenols [46]. Kenawy et al. [47] synthesized polymers containing polyphenols, whose microbial activity revealed that the polymers released polyphenols to kill microbial cells.

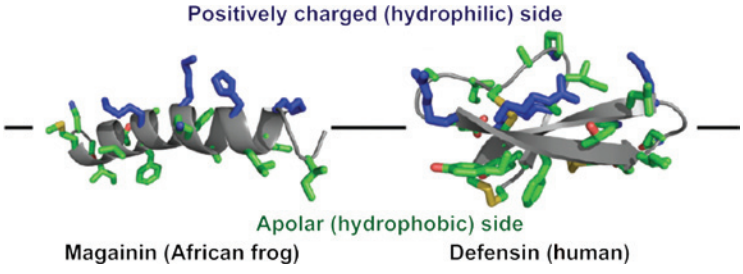

Figure 4: Conformation of magainin (α-helix) and defensin (β-sheet) peptides, in which the positively charged groups are marked in blue and the nonpolar groups are marked in green. Copyright 2010. Adapted from [31] with permission from the American Chemical Society.

Chemburu et al. [48] designed positively charged polyelectrolytes based on poly(phenylene ethynylene) derivatives. These polymers were reported to be significantly active in the presence of light, producing and releasing singlet oxygen ($_1O_2$) and presenting antiviral properties [49]. However, the polymers were found to also work in the dark due to their antimicrobial oligopeptide-mimicking component [50].

2.4 Bioactive oligopeptides

AMPs play a central role in the development of antibiotics that work in concert with the innate immune system of the organism [1, 31, 34, 50–54]. Currently, AMPs that are already approved in medical usages are gramicidin, nisin, daptomycin and polymyxins, and some of their derivatives [53, 55]. AMPs can be categorized as either non-ribosomally synthesized or ribosomally synthesized peptides. The ribosomally synthesized peptides are typically generated in the ribosomes of the eukaryotic cells, whereas non-ribosomally synthesized peptides are prepared in the cytosol of bacteria or fungi with the aid of peptide synthetases [53]. Alamethicin and gramicidin are categorized as non-ribosomally synthesized peptides. The alamethicin holds hydrophobic regions and negatively charged cytotoxic peptide regions, which leads to the self-organization for hexameric clusters of helices that traverse the bilayer and surround an aqueous pore in the bacteria [56]. Gramicidin A has hydrophobic sites and a helical transmembrane channel in the structure. The cation-selective right-handed helix conformation can be embedded into the bilayer membrane of bacteria as a single-handed head to head dimer [57]. Gramicidin A derivatives with the D-leucines at positions 10, 12 and 14 replaced by lysines have improved solubility in water and become cationic without altering the channel structure. These derivatives achieved bacterial specificity and low toxicity against mammalian cells [58].

Ribosomally synthesized AMPs are 12 to approximately 80 amino acid residues in length and assume several active conformations, such as α-helices (cecropin or magainin) and disulfide-rich β-sheets (defensin or bactenecin) (Figure 4).

Typical AMPs form extensively amphiphilic conformations, which are critical for penetrating into and/or disrupting the membrane around the microbial cytoplasm, leading to microbial death [34, 59]. Furthermore, AMPs can work via several different antimicrobial mechanisms together with the components of the innate immune system. Most AMPs have additional antimicrobial functions [60], and bacteria can respond to AMPs [51] and occasionally develop different resistance to their toxic influence [61].

AMPs have been generated by several methods, such as methods considering the amphiphilicity of native AMPs [62, 63], insertion of D-amino acids and/or acyl nanochains [64], and cyclization [65]. The introduction of β-peptide structures, which allow conformations of "12-helices" and "14-helices," is another way to design novel AMPs [66, 67].

Liu and DeGrado [68], Porter et al. [69], and Arvidsson et al. [70] independently reported that β-peptides allowed to form amphiphilic 14- or 12-helices had extensive antimicrobial ability. Based on their work, several different helical peptides using β-peptides or α/β-peptides have been developed [71]. In addition to the work of Patch and Barron [72], who designed amphiphilic, helical peptoids of antibacterial *N*-substituted glycine oligomers [72], Violette et al. [73] investigated antimicrobial foldamers with a urea backbone. It was reported that electrical charge, facial amphiphilicity, and a hydrophobic/hydrophilic balance are typically important factors in designing nontoxic antimicrobial compounds. However, there is no absolute requirement for any molecular characteristics [74].

3 Antimicrobial surface grafted with antimicrobial nanosegments

To restrict bacterial colonization without releasing antimicrobials into the environment, non-leaching microbicidal surfaces treated with antimicrobial polymers have been designed. These polymers can destroy airborne and waterborne microbes.

Several strategies can be considered for synthesizing non-leaching microbicidal molecules. Examples include covalent grafting of antimicrobial polymers on a surface and painting of polymers onto a surface (Figure 5). Because the behavior of antimicrobial molecules immobilized on a surface differs from that of the same molecules in aqueous solution, the working ability of the non-leaching antimicrobial coating materials and the methods of surface modification must be separately discussed [75].

Cationic polymers are known to hold antimicrobial activity as we discussed in Section 2.2. Therefore, the deposition or grafting of cationic polymers on material surfaces has been investigated to confer antimicrobial properties on the surfaces. Kügler et al. [76] investigated the effect of charge density for optimal antimicrobial activity on glass surfaces grafted with cationic quaternized

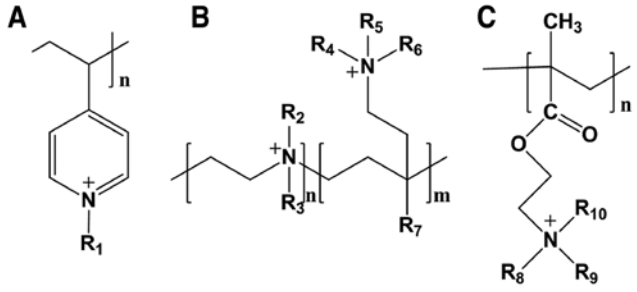

Figure 5: Chemical scheme of molecules covalently conjugated on the surface of (A) *N*-hexyl-polyvinylpyrrolidone (PVP), poly(4-vinyl-*N*-hexyl-pyridinium), (B) branched *N*-hexyl,*N*-methyl- polyethylenimine (PEI), and (C) quaternized poly(*N*,*N*-dimethylaminoethyl methacrylate) (PDMAEMA) [75]. R_1, R_2, and R_5=$CH_2CH_2CH_2CH_2CH_2CH_3$, R_3, R_4, R_6, R_7, R_8, and R_9=CH_3, and R_{10}=$(CH_2)_pCH_3$.

poly(vinylpyridine) chains by changing charge density from 10^{12} to 10^{16} positive charge/cm^2 on the surfaces. These ranges of positive charge showed a great influence on the killing efficiency of bacteria. Bacterial death was found to occur in <10 min in the quiescent state (in phosphate buffer saline) above a threshold value (10^{16} positive charge/cm^2 on SiO_2 beads for *Staphylococcus epidermidis*), whereas the death of *S. epidermidis* was observed on the beads surface having more than 10^{14} positive charge/cm^2 in the growth phase (in nutrient solution) [76]. This phenomenon also depends on the bacterial type. Only a few but alive bacteria were observed on uncharged SiO_2 beads, whereas the cationic surface treatment is effective in killing bacteria in different metabolic states, in either growth or quiescent conditions. This is because of the electrostatic attraction between the negative charges of the bacterial membrane and the positive charges of the beads surface. An electrostatic mechanism based on the exchange of counter ions between the functionalized cationic surface and the bacterial membrane explained the experimental results.

Their proposed mechanism is as follows. The bacteria having negative charge are holding cationic ions as their counter ions. When the bacteria adsorbed on the cationic surface, the negative charges of the bacteria envelop can be compensated with the cationic charges of material surface, which leads to the loss of natural counter ions of bacteria. This counter ion releasing triggers the bacterial death in this mechanism [76]. However, the penetration of cationic polyelectrolyte segments grafted on the surface into bacterial cells also provides another possible mechanism of antimicrobial activity of the surface grafted with cationic polymer [77].

Tiller et al. [13] investigated bactericidal polymers covalently conjugated with cationic *N*-alkyl-polyvinylpyrrolidone (PVP) bromides by graft copolymerization of 4-vinylpyridine with acryloyl units or covalent immobilization of partially *N*-alkylated PVP on a surface. *N*-hexyl-PVP coating led to only 62±8% death of *S. aureus*, whereas a surface with immobilized *N*-hexyl-PVP (MW 160 kDa) showed an extremely high percentage of death of several types of bacteria [13]. Low-density and high-density polyethylene, nylon, polypropylene, and poly(ethylene terephthalate) modified with hexyl-PVP showed certain antimicrobial activity [14]. Other polymers, such as hydrophilic PEI with branched nanosegments, were developed by Lin et al. [77, 78]. Materials coated with alkylated quaternized PEI (*N*-hexyl,*N*-methyl-PEI) (Figure 5) were also developed and showed antimicrobial activity toward several types of bacteria and fungi [77, 78]. Haldar et al. [79] and Park et al. [80] developed a technology, i.e., coating a surface with *N*-dodecyl,*N*-methyl-PEI, to kill *E. coli* and *S. aureus*. An enveloped influenza virus and bacteria were significantly killed (100%) on materials coated with *N*-dodecyl,*N*-methyl-PEI with a high MW (MW 750 kDa or 25 kDa), in which case the virucidal effect was generated on contact, with no leaching of these molecules [79].

Hu et al. [81] developed another technology, consisting of a method to prepare polymeric microbeads with a spherical morphology and a narrow particle distribution using poly(4-vinylpyridine)/poly(vinylidene fluoride). Microbeads in which pyridine groups were quaternized with alkyl bromides of different carbon chain lengths (from C4 to C10) were excellent antibacterial (against *E. coli*) and antifungal (against *Aspergillus niger*) agents that could be used for repeated treatment [81].

Murata et al. [82], Huang et al. [83], Huang et al. [84] and Lee et al. [85] developed a non-leaching antimicrobial molecule using atom transfer radical polymerization (ATRP), involving living radical polymerization, and prepared chains with regulated MWs and narrow polydispersities from a surface ("grafting from" method) or onto a surface ("grafting onto" method). The characteristics of the polymer distribution on a surface were observed to influence the killing activity of the treated surface.

Lee et al. [85] also described an antimicrobial surface. This antimicrobial surface was grafted with non-leachable PDMAEMA using the ATRP "grafting from" method. This technique involved localization of an ATRP initiator to a surface, followed by polymerization of PDMAEMA on the surface and finally quaternization of the tertiary amino groups of the obtained PDMAEMA with an alkyl halide, which generated a large amount of quaternized amine units on the surface (Figure 5) [85]. This antimicrobial material exhibited high killing ability against several types of bacteria, such as *B. subtilis* and *E. coli*. Additionally, no

loss of antimicrobial activity was noted after repeated use of the antimicrobial surface [85]. Huang et al. [84] investigated an antimicrobial surface (polypropylene) grafted with non-leachable quaternized PDMAEMA via the "grafting from" technique. The biocidal ability of the resultant surfaces toward *E. coli* was found to be regulated by the number with a similar grafting density on the surface. The biocidal ability was also found to depend on the MW [i.e., degree of polymerization (DP)] of the grafted molecules, and a minimal polymer chain size was necessary to kill microbes efficiently [84]. Murata et al. [82] also synthesized polymer brushes on inorganic surfaces using surface-initiated ATRP of PDMAEMA quaternized with alkyl bromides, which were prepared by using the "grafting from" technique. The macro chain length, density, and surface charge density were evaluated to determine the mechanism of the antimicrobial activity against *E. coli* [82]. Huang et al. [83] also used a special ATRP "grafting onto" technique to synthesize an antimicrobial material surface using PDMAEMA/poly(3-(trimethoxysilyl) propyl methacrylate) copolymers. This treated surface possessed higher antimicrobial activity than surfaces synthesized by the ATRP "grafting from" technique did at the same quaternized amino group densities. The results were explained by the inhomogeneous distribution of quaternized amino units, leading to areas of highly quaternized amino group units on the "grafting onto" surfaces [83].

Zwitterionic molecules such as phosphobetaine, sulfobetaine, and carboxybetaine have been reported to show resistance to nonspecific protein adsorption, bacterial adhesion and biofilm formation [86, 87]. These zwitterionic molecules possess mixed negatively and positively charged moieties within the same side chain of the polymer, which lead to the overall charge neutrality of the molecules. Therefore, Yu et al. [88] developed a non-leachable antimicrobial titanium (Ti) surface by grafting zwitterionic poly(sulfobetaine methacrylate) (polySBMA) via the ATRP with the "grafting from" method. In this graft polymerization of polySBMA, silane or dopamine was used as an anchoring intermediate immobilized on the Ti surface for surface-initiated ATRP polymerization, which generated two types of polySBMA-grafted Ti surfaces with distinct molecular structures and polymer packing (Figure 6) [88].

In fibrinogen adsorption experiments, a pristine Ti disk generated 320.2 ng/cm^2 fibrinogen adsorption, which was nearly the same as the amount of fibrinogen adsorption on tissue-culture polystyrene dishes (336 ng/cm^2).

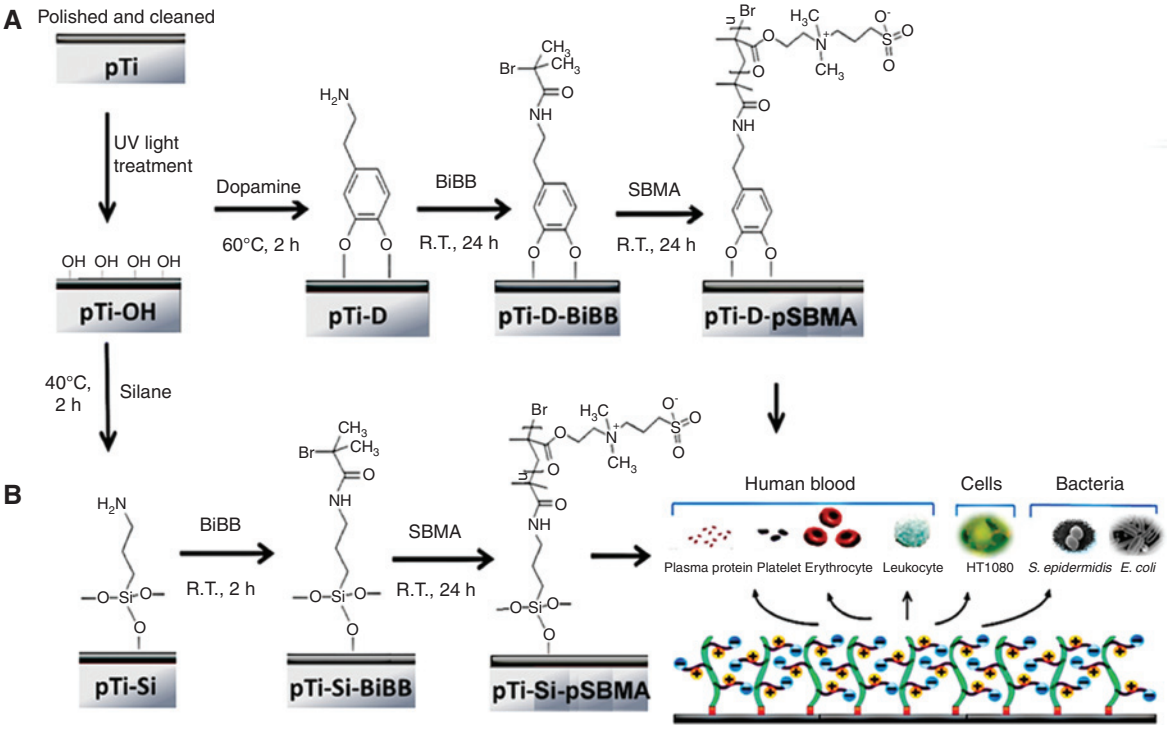

Figure 6: Schematic illustration of the preparation process of zwitterionic poly(sulfobetaine methacrylate) (polySBMA)-grafted Ti disks via the atom transfer radical polymerization (ATRP) method using either (A) dopamine or (B) silane as an anchoring agent. Copyright 2014. Reproduced from [88] with permission from the American Chemical Society.

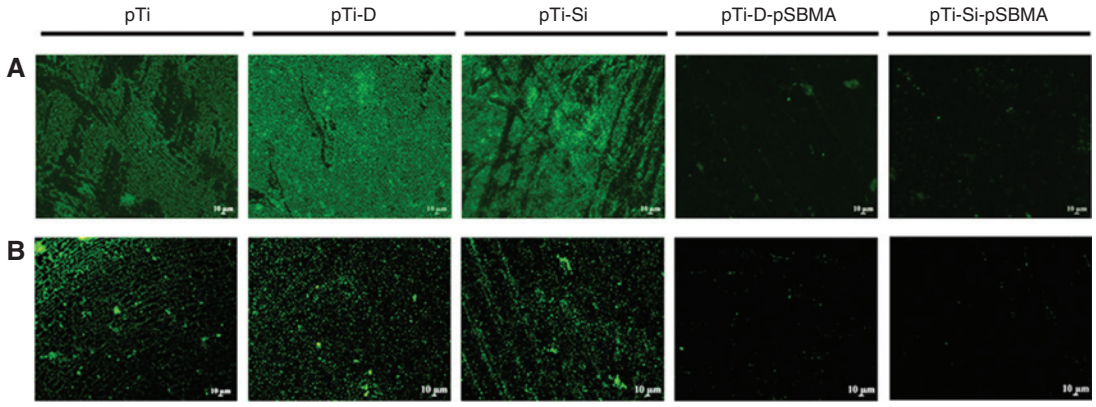

Figure 7: Fluorescence microscope images of *Escherichia coli* (A) and *Staphylococcus epidermidis* (B) attachment on five surface-modified Ti disks after 24 h of incubation; all images are magnified 100x. Copyright 2014. Reproduced from [88] with permission from the American Chemical Society.

In contrast, polySBMA-grafted Ti from dopamine-anchored surfaces (pTi-D-pSBMA) and polySBMA-grafted Ti from silane-anchored surfaces (pTi-Si-pSBMA) showed reduced fibrinogen adsorption of 29.9 ng/cm^2 and 82.7 ng/cm^2, respectively [88]. It is known that the surface hydration of the antifouling polymer brush is an important point affecting protein adsorption. The hydrophilicity of the Ti surface was measured as follows based on water contact angle analysis: pTi-D-pSBMA=pTi-Si-pSBMA>>Ti [88]. Hydrophilic surfaces showed better antifouling ability than less hydrophilic surfaces did in this study as well as in several reports published previously [89].

The antifouling surfaces of pTi-D-pSBMA and pTi-Si-pSBMA are expected to show antimicrobial characteristics, because it is known that a protein-resistant surface is required to resist bacterial adhesion. However, the fact that a surface which resists protein adsorption does not necessarily imply that this surface can resist bacterial adhesion and biofilm formation [88, 90]. Therefore, bacterial adhesion on a pristine Ti surface and a pSBMA-grafted Ti surface was evaluated using a Gram-negative strain of *E. coli* and a Gram-positive strain of *S. epidermidis*. Figure 7 shows fluorescent microscope observations of *E. coli* and *S. epidermidis* accumulated on Ti surfaces grafted with and without pSBMA [88]. Ti surfaces without pSBMA (Ti, pTi-D, and pTi-Si) displayed similar bacterial growth, indicating that the bacteria completely covered on those Ti surfaces. In contrast, very few bacteria were found to attach to both pTi-D-pSBMA and pTi-Si-pSBMA surfaces, with a reduction of approximately 95% relative to pSBMA-uncoated Ti surfaces [88]. These results demonstrate that pSBMA-grafted Ti surfaces resist protein adsorption as well as bacterial adhesion.

4 Conclusion

Currently, few studies have reported the development of contact-active antimicrobial surfaces, and the preparation methods are only valid on specific material surfaces with special conditions of usage. It will be necessary to develop a novel type of contact-active antimicrobial surfaces in which effective antimicrobial nanosegments are grafted at an adequate surface density. Furthermore, earlier reports have indicated that nanosegments on contact-active surfaces act differently toward microbial cells in aqueous solution, and their mechanism is currently under investigation. Precise analysis and development of the mechanism of killing microbial cells upon contact with antimicrobial surfaces will be performed in the future.

Acknowledgments: This project is supported by NSTIP strategic technologies program (no. 14-NAN 1251-02) in the Kingdom of Saudi Arabia. This work also proceeded in collaboration with Cathay General Hospital (103CGH-NCU-A3, CGH-MR-A10204 and CGH-MR-A10301).

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