Non-leaching surfaces capable of killing microorganisms on contact

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Health care associated infections and deaths have reached dimensions that rival traditional diseases and have become a serious financial burden on the health-care systems. Beyond simple hygiene, hopes are high that new materials capable of killing microorganisms on contact could ease the situation. This review highlights recent trends on surface-modifications capable of eliminating a microbial threat upon contact. Despite significant advances, the field still focuses on chemical synthesis and biological testing without breakthroughs in medical materials research.

1. Introduction

In 2002, an estimated 1.7 million people in the USA were infected with microorganisms during a stay in hospital, an infection that was lethal for 99,000 patients.[1–3] In statistics of the leading causes of death in the USA (2005), this figure would be ranked 6th and in terms of the total number of cases, the health care-associated infections overpass any of the current (2006) notifiable diseases in the USA.[4,5] After ventilator-associated pneumonia and catheter-associated urinary tract infections, central line-associated blood-stream infections are ranked third with an estimated 250,000 cases occurring annually in US hospitals.[6] To each of these cases, a mortality of 12%–25% is attributed. Furthermore, the marginal cost for each infection is estimated to $25,000 in 2001 leading to a multi-billion dollar burden to the US health-care system due to a prolonged stay of the patient in the hospital for 10–20 days.[6,7]

Among the causative pathogens, *staphylococci*, particularly *Staphylococcus epidermidis* and other coagulase-negative staphylococci, *Staphylococcus aureus*, *Enterococcus spp.*, and the yeast *Candida spp.* account for the majority of infections both of temporarily inserted and of permanently implanted materials.[8,9] Either in bacteria or fungi infections, the portal of entry is usually the skin. Once adhered to the surface of the foreign body, microorganisms multiply and accumulate in multilayered cell clusters, forming biofilms.[10] With regard to the impact of foreign body-associated infections, prevention is of utmost importance. Proper hygiene could reduce central-line associated infections by almost two thirds as a recent study of the Centers for Disease Control and Prevention impressively proved.[11] In parallel, however, action is needed to further lower the number of health care-associated infections and deaths.

Three major strategies have been developed to prevent implant-associated infections: (i) the design of medical devices that are resistant against microbial adherence; (ii) the design of devices with physically immobilized antibiotics and other

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antimicrobial agents and (iii) finally by the preparation of devices with covalently linked antibiotics in their surface.[12–15] Although anti-adhesive devices can reduce the adherence of microorganisms this approach does not kill them.[12] For example, the covalent attachment of poly(ethylene glycol) (PEG), one of the most effective molecules in reducing bioadhesion, reduced the level of *Pseudomonas sp.* adhesion by between 2 and 4 orders of magnitude as compared to the control (initial concentration of bacteria: 10^6 colony-forming units per mL).[16] The second approach raises some concerns since sufficient antibiotic or other antimicrobial agents (halogens, quaternary ammonium compounds, or heavy metals like silver or mercury) must be incorporated during the time the device is placed into the patient's body and the use of antibiotics as antimicrobial agents can contribute to the development of resistant pathogens.[8,12] The gradually decreasing level of the released compound may lead to sub-inhibitory concentrations of antimicrobial in the surroundings, which may provide conditions for development of microbial resistance.[13,17,18] In order to overcome these problems, the ideal strategy would be one where the antimicrobial agent is covalently immobilized onto the material surface rather than gradually released from it. However, for permanent antimicrobial effect, the killed microorganisms accumulated in the coated surface should be likely removed since this aspect might reduce the activity of the coating overtime (discussed below).

In the past decade, considerable research efforts were performed in the development of non-leaching surfaces capable of killing microorganisms on contact. In most cases, the technologies were used in non-medical applications such as food industry, industrial surfaces, textiles, furniture, shoe industry, *etc.* However, there is an increasing interest in introducing those technologies as coatings for medical devices, mainly because infections of medical devices has become a major global healthcare issue.[19] The increasing numbers of multidrug-resistant bacteria requires the development of new approaches to solve this threat.[20,21]

This review focuses on the most significant achievements in the last few years related to the design of permanent microbicidal medical devices.

2. Historical perspective on permanent microbicidal materials

The first examples of non-leaching surfaces capable of killing microorganisms on contact were described in the early 1970s.[22] Researchers from Dow Corning Corporation found that 3-(trimethoxysilyl)-propyldimethylalkyl ammonium chlorides with alkyl chain lengths varying from 6 to 22 carbons added to water gave a high degree of control over the growth of algae. Moreover, such compounds could also be immobilized to surfaces without losing their algicidal activity. Indeed, surface-bound 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride (Si-QAC) (Table 1) had algicidal, bactericidal and fungicidal properties.[23,24] In the late 1970's, after extensive toxicological testing, Dow Corning applied to the Environmental Protection Agency (EPA) for an industrial registration.[22] The free Si-QAC, also termed DOW CORNING 5700 (from which antimicrobial surfaces were easily accessible) was an IR-100 award winner for one of the best products to be commercialized in 1977.

In the early 1980's, Speier and Malek used a high throughput approach to demonstrate that solids upon which organic or inorganic cations had been chemisorbed indeed killed different microorganisms.[25] Remarkably, cations that had no antimicrobial activity in solution were capable of forming antimicrobial surfaces. Any substance which formed a cation other than a proton was found to actively kill microorganisms. Noncationic or acidic materials were inactive.

The first decade of the 21st century saw an increasing interest in the development of microbicidal coatings for medical devices. Current efforts focus on the development of new strategies for the immobilization of traditional antimicrobial agents (*e.g.* antibiotics, antimicrobial peptides) and the development of new antimicrobial therapeutics (*e.g.* polycationic polymers, peptoids, *etc.*).

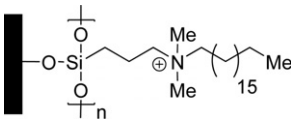
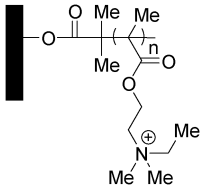
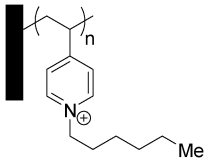
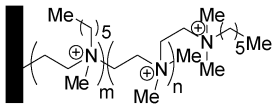
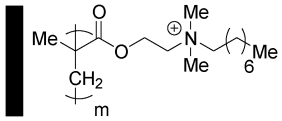
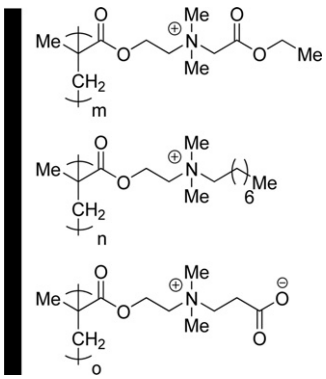
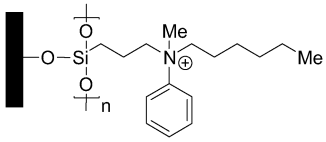
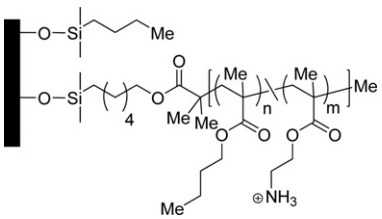
3.1 Non-leaching covalently immobilized polycationic chains

3.1.1 Non-natural polycations. A significant number of polycationic compounds with antimicrobial properties have been created in the last 10 years. Most of the compounds are relatively cheap, not susceptible to degradation and according to some studies they do not cause bacterial resistance.[26] Unfortunately, not much is known about their cytotoxicity and biocompatibility *i.e.*, their biological behavior when implanted *in vivo*.

Klibanov has developed antimicrobial surfaces which involve a covalent coating with long hydrophobic polycationic chains. The most important aspects of this technology have been reviewed recently in this journal.[27] The polymeric chains with an optimal balance of charge and hydrophobicity resisted to electrostatic repulsion and hydrophobic interchain aggregation and were able to penetrate microbial cell membranes. Surfaces containing immobilized long-chain alkylated polyvinylpyridines and alkylated polyethylenimines have been reported to be lethal to *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *E. coli*. For example, glass slides[28] (Table 1) or biomedical nonbactericidal polymers[29] covalently modified with poly(vinyl-*N*-hexylpyridinium bromide) was found to kill 90 to 99% of Gram-positive and Gram-negative bacteria deposited, through either air or water, onto their surfaces. Remarkably, these coatings do not promote microbial resistance.[27] Furthermore, mammalian cells are minimally affected by the polycationic antimicrobial surfaces when in contact for 2 h.[26] However, cytotoxicity tests for longer periods and *in vivo* biocompatibility tests should be performed in the future to give further insights about the promising potential of these surfaces.

A simple approach to render the medical device surface with antimicrobial properties involves the silanization with quaternary ammonium-containing silane agents. For example, the modification of microfibrillated cellulose with alkoxysilane octadecyldimethyl(3-trimethoxysilylpropyl)-ammonium chloride rendered the material highly microbiocidal[30] (Table 1). More than 99% of *S. aureus* or *E. coli* bacteria initially exposed to the material were killed after a 24 h contact. The bactericidal efficiency was found to be smaller toward *P. aeruginosa* (95% of the bacteria were killed) than toward *E. coli and S. aureus*. A similar approach has been described recently when glass was modified with a quaternary ammonium terminated triethoxysilane.[31] A concentration of 1.5×10^{-4} mol of quaternary ammonium silane per gram of coating was enough to kill nearly 95% of the viable colonies after 48 h of exposure.

Table 1 Schematic representation of non-natural polycations chemically immobilized onto different surfaces[a]

Chemical entity	Surface	Microorganism	Microorganism loading; killing percentage	Ref.
	28 surfaces were used	26 microorganisms were tested	2×10^2/cm^2; 95% killing in 30 min	24
	microfibrillated cellulose	*E. coli, S. aureus, P. aeruginosa*	1.0×10^4/cm^2; >99% killing in 24 h	30
	glass paper	*E. coli B. subtilis*	Between 1×10^6 and 4×10^7/cm^2; >99–100% killing in 1 h	32
	glass, polyethylene, polypropylene, nylon, poly(ethylene terephtalate)	*S. aureus, S. epidermidis, E. coli, P. aeruginosa*	NA; between 94 and > 99.8% killing in 2 min	28
	glass	*S. aureus, E. coli*	1.5×10^6/cm^2; 100% killing in 30 min	26
	glass, wood	*A. niger*	1.0×10^3/cm^2; 100% killing in 4 days	33
	Au	*E. coli*	NA; 99.9% killing in 1 h	95
	glass	*S. aureus, E. coli*	NA; between 96% and 99% killing in 2 min	31
	glass	*S. aureus, E. coli*	0.7×10^6/cm^2; 100% killing in 5 min	76

[a] NA = Not available.

In the previous examples, the active polycationic chains were synthesized either by classical free radical polymerization or simple coupling reactions and then applied to an activated surface. These types of reactions fail to strictly control the monomer distribution, polydispersity, molecular weight, polymer topology, and density of functional groups in a way that will allow rational modification of the polymer for increased antimicrobial activity[32] (Table 1). A reliable way to control these polymeric variables is performing the synthesis of polycationic chains by atom transfer radical polymerization (ATRP). Polymeric quaternary amines synthesized *via* ATRP have significant biocidal activity against the bacteria *Escherichia coli*,[32] *Bacillus subtilis*[32] and fungi including *Aspergillus niger*[33] (Table 1).

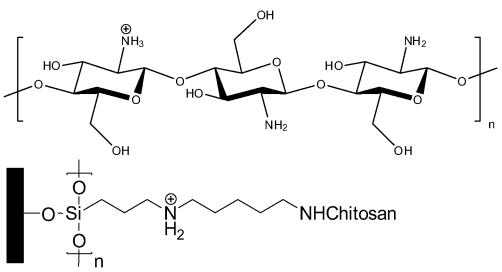
The precise antimicrobial mechanism of cationic polymers immobilized onto surfaces remains enigmatic. Some studies suggest that the mechanism of action involves the penetration of the long cationic polymer into the cell membrane with concurrent cell membrane disruption.[26,28,34] This will likely require polymer coatings of ~50 nm in order to effectively penetrate the cytoplasmic membrane of Gram-negative *E. coli* (46 nm[35,36]) or Gram-positive *B. subtillis* (45–55 nm[36]), and even longer chains when the cell wall is taken into account. However, short cationic agents such as 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride[24] or polymeric brushes formed by 2-(dimethylamino)ethyl methacrylate (thickness of 10 nm)[37] bound to a surface are highly antimicrobial. Other studies suggest that the mechanism of action involves ion exchange between the positive charges on the surface and structurally critical mobile cations within the membrane.[37,38] The loss of these structural cations results in a loss of membrane integrity.[37,38] Along these lines, it has been noted that surfaces with polymer-brushes terminated in long alkyl chains will probably not extend towards the water phase due to the unfavorable hydrophobic effect.[33] Further investigation will be needed to unravel the mechanism behind the antimicrobial properties of polycationic chains.

3.1. 2 Natural polycations. Chitosan is a polycationic polymer that can be an alternative to the non-natural polycationic polymers described above as antimicrobial agents for biomedical devices.[39] Chitosan is a natural polycationic polymer obtained by the *N*-deacetylation of chitin, the second most abundant natural polysaccharide obtained from the skeletal structures of crustaceans and the cell walls of fungi.[39] Chitosan is composed of β-(1–4)-linked 2-amino-2-deoxy-D-glucopyranose and 2-acetamido-2-deoxy-D-glucopyranose units and is non-toxic and biodegradable polymer. Several biomedical devices containing chitosan including wound dressings[40] and devices for artery closure have been approved by the FDA.[41]

The immobilization of chitosan to confer antimicrobial and cell adhesive properties to orthopaedic and craniofacial implant devices has been described (Table 2).[42] Coatings made from 91.2% de-acetylated chitosan were chemically bonded to titanium clippings *via* a silane-glutaraldehyde linker. The coatings were stable over 8 weeks in a cell-culture solution, and the attachment and growth of osteoblast cells was greater on the chitosan-coated samples than on the uncoated titanium.[42] Surfaces coated with chitosan do resist biofilm formation by bacteria and yeast.[43] Reductions in biofilm viable cell numbers ranging from 95% to 99.9% were observed for *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Candida albicans* on chitosan-coated surfaces over a 54-h experiment in comparison to controls.[43] As a comparison, coatings containing the antiseptic chlorhexidine did not significantly reduce *S. epidermidis* surface associated growth.[43]

The microbicidal mechanism of chitosan is not fully understood. Some studies indicate that this polycation interacts with the negative charge on the surface of the bacteria changing the permeability of the bacteria cell membrane. At certain concentrations, chitosan probably binds to the negatively charged bacterial surface causing agglutination and consequently permeability in the cell membrane.[39] The microbicidal effect of chitosan is largely affected by the pH of the surrounding solution.[44] For example, the deposition of chitosan onto the inner surface of oxidized poly(ethylene) tubing is ineffective in preventing *E. coli* adhesion present in bile fluids.[44] Chitosan was selected as an antimicrobial agent to prevent the general occlusion of plastic stents by biliary sludge (composed of bacteria and proteins). These stents are used to relieve the obstructive jaundice caused by biliary or pancreatic malignant tumors.[44] The

Table 2 - Schematic representation of a chitosan-based coating[a]

Chemical entity	Surface	Microorganism	Microorganism loading; killing percentage	Ref.
	TiO_2	*S. epidermis, S aureus, P. aeruginosa, C. albicans, K. pneumoniae*	$1.2 \times 10^5/cm^2$; >95% killing in 54 h	42, 43

[a] NA = Not available.

inefficacy of chitosan is likely a consequence of the deprotonation of amine groups in the polymer when in contact with bile fluids (pH above 7.0). Indeed, it has been shown that the microbicidal activity of chitosan is higher at pH 6.0 (pK_a value of chitosan is 6.2) than at pH 7.5.[39]

3.2 Non-leaching physically adsorbed polycationic chains

In general, the immobilization of polycationic chains described in the previous section requires several chemical steps which might be an obstacle for the scaling-up of the coating process. In addition, it requires the use of organic solvents that might affect the physical properties of medical devices and ultimately their biological performance. An alternative approach has been described recently. Antimicrobial surfaces could be fabricated by a simple dip-coating or a "painting" methodology. Instead of covalently immobilizing the polycations to the surface, the polymer was physically immobilized on the surface by non-covalent hydrophobic interactions. Klibanov *et al.* prepared polymeric coatings of branched *N*,*N*-dodecylmethyl–polyethyleneimine on glass surfaces by 'painting' them with a solution of the polymer in butanol[45] (Table 3). The coating was able to eliminate influenza virus with a 100% efficiency within minutes as well as *E. coli* and *S. aureus*.[45] Fuchs and Tiller developed a broadly applicable coating method based on emulsion polymerization using water-insoluble antimicrobial emulsifiers[46] (Table 3). They demonstrated that a water-insoluble diblock copolymer, PS-b-P4VMP, which consists of a hydrophobic (polystyrene, PS) and an antimicrobial hydrophilic (poly(4-vinyl-*N*-methylpyridinium iodide), P4VMP) block, can be used as an emulsifier in the aqueous emulsion polymerization of mixtures of styrene and butyl acrylate. Coatings prepared from stable aqueous suspensions of these polymers act as contact-active antimicrobial surfaces against *S. aureus*.[46]

The microbicidal mechanism of these paintings is likely to be the same as the one described for the covalent immobilization of polycationic polymers. It requires the physical contact of the microorganism with the painting. In addition, the experimental data indicate that the bactericidal effect is related to the molecular composition and organization in the top 2–3 nm of the surface and increases with increasing hydrophilicity and pyridinium concentration of the surface.[47]

Another process of preparing antimicrobial surfaces is by the layer by layer assembly which is based on the electrostatic attraction of oppositely-charged polyions. For example, cationic polyhexamethylene guanidine hydrochloride assembled with acetalyzed poly(vinyl alcohol)/sodium acrylate presents excellent antimicrobial activity against *S. aureus* and *E. coli*.[48] Furthermore, nanometric multilayer films obtained by the assembly of cationic poly(allylamine hydrochloride) (containing primary amines) and anionic poly(sodium 4-styrene sulfonate) under specific conditions have antibacterial properties against *Staphylococcus epidermidis* and *E. coli*.[49] Although the layer by layer coating technique is relatively simple to use on medical devices, it is unclear how stable they are over time in contact with biologic fluids. Also unclear is the full spectrum of activity of these coatings and their biocompatibility.

3.3 Antibiotic-based coatings

The modification of polymeric surfaces with antibiotics has been described recently (Table 4)[50–57] Typically the immobilized antibiotics act on the cell membrane of the microorganisms, inhibiting the proliferation or killing the microbes. With few exceptions,[58] the immobilization of the antibiotics onto surfaces requires multiple chemical steps and might create some challenges for the scaling-up of the process. Because many antibiotics are FDA approved, the modification of medical devices with these agents might accelerate their approval by regulatory agencies. This is an advantage relative to antimicrobial surfaces that rely on the immobilization of synthetic polycationic polymers (see above) not yet approved by FDA or EMEA. However, depending on the type of antibiotic used and the antibiotic surface density, there is a chance that antibiotic-modified surfaces might encourage

Table 3 Schematic representation of non-leaching physically adsorbed polycations

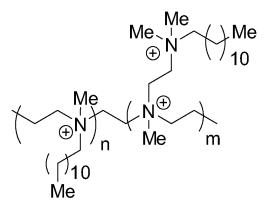
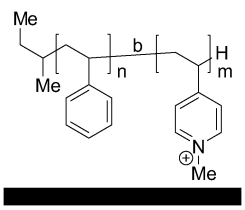
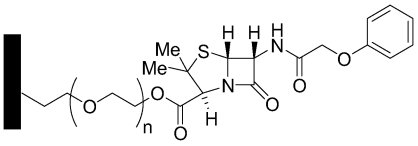
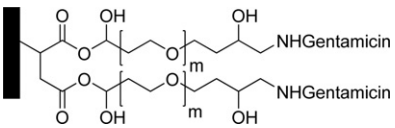
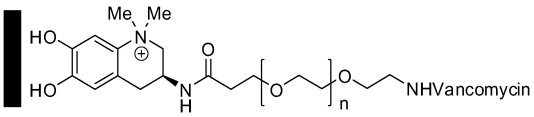
Chemical entity	Surface	Microorganism	Microorganism loading; killing percentage	Ref.
	glass	*S. aureus, E. coli, influenza A/WSN/33 (H1N1), influenza A/Victoria/3/75 (H3N2)*	$1.6 \times 10^3/cm^2$; 100% killing in 30 min	45
	glass	*S. aureus*	$2.1 \times 10^2/cm^2$; 99.9% killing in 5 min	46

Table 4 Schematic representation of antibiotic-based coatings[a]

Chemical entity	Surface	Microorganism	Microorganism loading; killing percentage	Ref.
	ePTFE	*S. aureus, P. aeruginosa*	NA; 100% killing in 24 h	50
	polypropylene	*P. putida*	NA; 60% killing in 5 h	53
	TiO_2	*B. subtilis*	NA; 100% killing in 6 h	58

[a] NA = Not available.

microbe resistance over time and the notion that surface-bound drugs are equivalent to their approved liquid formulations might be misleading. Great care has to be taken when a new antimicrobial system is designed and the biocompatibility of these surfaces with high concentrations of antibiotics should be thoroughly evaluated in animal models. On the other hand, surfaces with low concentrations of antibiotics might also present a risk factor. Subinhibitory concentrations of aminoglycosides antibiotics such as tobramycin were shown to actually promote biofilm formation of *P. aeruginosa*. as a specific, defensive reaction to the presence of antibiotics.[59] In a similar study, subinhibitory concentrations of antibiotics such as erythromycin and rifampicin were found to significantly alter the transcription pattern of the bacterium *Salmonella typhimurium*.[60] The same organism also shows increased resistance to oxidative stress and antimicrobial peptides when grown in the presence of sublethal concentrations of cationic antimicrobial peptides.[61]

Penicillin is an antibiotic effective against gram-positive bacteria including *Staphylococcus aureus*. This antibiotic inhibits the enzyme that crosslinks peptidoglycan monomers during the bacteria cell wall synthesis. The bacteria cannot compensate the osmotic stress any longer and rupture. Recently, penicillin was immobilized in expanded poly(tetrafluoroethylene) (ePTFE) utilized for vascular graft prostheses, heart patches or stapes prosthesis. For that purpose, ePTFE was modified by a microwave plasma reaction in the presence of maleic anhydride, followed by a surface hydrolysis to generate carboxylic acid groups, a subsequent esterification with poly(ethylene glycol) (PEG) and the final attachment of the penicillin to the terminal PEG hydroxyl group (Table 4).[50,51] These antibiotic-modified surfaces are highly effective in inhibiting bacteria growth due the mobility of the antibiotic molecule attached to PEG. When bacteria come in contact with the surface and attempt to grow, the peptidoglycan cell wall synthesis is immediately interrupted by the antibiotic molecules. More than 80% of bacteria were killed after 4 h. This immobilization platform was then extended to the antibiotic ampicillin (similar action mechanism to the penicillin) to develop antimicrobial surfaces that are effective against a broad spectrum of gram-negative and positive microorganisms including *Staphylococcus aureus*, *Bacillus thuringiensis*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas putida* and *Salmonella enterica*.[52]

Gentamicin is an antibiotic active against a broad spectrum of bacteria including Gram-positive and Gram-negative bacteria, whose mechanism of action relies on the inhibition of protein biosynthesis by binding to the 30S ribosomal subunit as well as on the resulting blockade of translocation on bacterial ribosomes. At higher concentrations, the antibiotic also damages bacterial cell membranes which causes the efflux of some ions and low molecular mass compounds from cytosol to the outside of cells.[55] To prevent the colonization of biomaterial after implantation, prosthetic vascular grafts made of poly(ethylene terephthalate) fibers were chemically modified with gentamicin.[55] Results confirmed that the modification of the prosthesis surface inhibits the growth of *E. coli*, *P. aeruginosa* and *S. aureus* strains in medium containing pieces of gentamicin-coupled prosthesis during at least 28 days of the experiment.[55] In contrast, a control medium containing pieces of prosthesis only soaked with gentamicin allowed a constant growth of bacteria.[55]

Orthopedic implants for hip replacement have infection ranges from 1%–5%.[19] Approximately 800,000 new hip arthroplasties are performed annually.[62] A new approach to limiting or even preventing bacterial colonization in orthopedic implants has been recently reported by the covalent immobilization of vancomycin onto titanium surfaces.[57,62,63] Vancomycin is an agent that displays low toxicity, exerts a broad spectrum of activity against Gram-positive bacteria and is active at the bacteria cell wall. Surfaces (rods; 1 mm diameter) challenged for 2 h with 1×10^4 cfu/mL *S. aureus* inhibited microorganism adhesion and

proliferation up to 91% as compared to the control (unmodified titanium).[62] Similar results were obtained for *S. epidermidis*.[64] For a period of at least 6 weeks, the covalently bound antibiotic was active against challenges with *S. aureus*. During this time, the antibiotic-modified Ti surface prevented bacterial colonization and biofilm formation. Remarkably, Ti surfaces that were pre-incubated with fetal bovine serum and challenged for 24 h with *S. aureus*[62] or *S. epidermidis*[64] showed minimal bacterial adherence, suggesting that serum protein coverage does not affect the activity of the tethered antibiotic. In addition, it was demonstrated that the surface with immobilized vancomycin did not foster the emergence of resistant *S. epidermidis* even after prolonged exposures. Unfortunately, surface cytotoxicity was not fully addressed by the authors. Another limitation of this technology is that vancomycin is active against Gram-positive but not Gram-negative organisms.

Recently, Gademann *et al.* reported an elegant solution, covering a titanium surface with vancomycin *via* a simple dip-coating process (Table 4).[58] Vancomycin was chemically linked to the anachelin chromophore. The catechol moiety of the anachelin chromophore part would bind to TiO_2 surfaces and thus render the surface antimicrobial to *Bacillus subtilis*.

Most of the coatings described previously are effective against bacteria but not fungi. However, fungi are the fourth most common cause of bloodstream infections in hospitalized patients.[9] Recently we developed an antifungal gel (amphogel) that can be used to coat medical devices.[54] Amphogel was formed by physically absorbing amphotericin B (AmB) into a dextran hydrogel. AmB is a FDA-approved, potent antifungal agent widely used in clinical practice. This agent has a broad spectrum of antifungal activity, and few resistant strains have been reported after 40 years in clinical use.[65] Dextran is a polymer widely used in medicine and known to be protein repellent.[66] Amphogel kills fungi within 2 h of contact and can be reused for at least 53 days without losing its effectiveness against *Candida albicans*. The killing process is initially (<24 h) mediated by AmB leached from the gel and afterwards (>24 h) by the contact of the microorganisms with the gel surface. The antifungal material is biocompatible *in vivo* and does not cause hemolysis in human blood. Amphogel inoculated with *C. albicans* and implanted in mice prevents fungal infection and biofilm formation.[54]

3.4 Antimicrobial peptide-based coatings

Antimicrobial peptides (AMPs) are 15–45 amino acid residues with broad-spectrum antimicrobial activity against bacteria, viruses, and fungi.[67] These agents have the ability to broadly distinguish eukaryotic cells from pathogenic invaders, and they raise few issues regarding the resistance of mutant microorganisms.[67] AmPs are much less likely to induce bacterial resistance than traditional antibiotics because they kill quickly and target the microorganism membrane non-specifically. Developing resistance to antimicrobial peptides would require bacteria to completely change their membrane structure.[67] These evolutionarily conserved peptides are usually positively charged to interact with negatively charged microorganism membranes, and have both a hydrophobic and hydrophilic side that enables the molecule to be soluble in aqueous environments yet also enter lipid-rich membranes. The mode of action of antimicrobial peptides includes formation of pores in the cell membrane resulting in the disruption of the membrane potential with eventual lysis of the cell.[67,68]

The immobilization of polymyxin B, a cyclic polycationic peptide with a molecular weight of ~1,200 on an alkyl acrylate polymer has been reported and the conjugate showed antibacterial action against *E. coli*. The authors demonstrated that the bioactivity was indeed due to the immobilized AMP and not AMP leached from the polymer.[68] A recent study has reported the immobilization of cathelin LL37, a cryptic antimicrobial peptide obtained by enzymic cleavage of its precursor cathelicidin which is stored in granulocytes.[69] The peptide was covalently immobilized onto a titanium metal surface by means of silanization. The application of a flexible hydrophilic poly(ethylene glycol) spacer and selective *N*-terminal conjugation of LL37 resulted in a surface peptide layer which was capable of killing bacteria on contact.[69] The *N*-terminal conjugation allowed optimal orientation of the peptide α-helices and the formation of membrane pores whereas the reaction with other amino acids in the peptide leads to random organization and loss of antimicrobial activity.

Several non-natural mimics of antimicrobial peptides with high activity have been developed in the last ten years, providing advantages in terms of chemical diversity and significant resistance to protease degradation.[70–73] There is a number of peptidomimetics that are being evaluated in clinical trials,[71] and therefore it would be interesting to evaluate their antimicrobial properties when immobilized onto surfaces. These synthetic mimics of antimicrobial peptides include β-peptides, peptoids and cyclic peptides.[74] Peptoids are non-natural mimics of polypeptides with the side chains appended to the amide nitrogen instead of the α-carbon. Antimicrobial peptoid oligomers (ampetoids) that were designed to mimic helical antimicrobial peptides were synthesized with a peptoid spacer chain to allow mobility and an adhesive peptide moiety for easy and robust immobilization onto substrates.[75] TiO_2 substrate were modified with the ampetoids and subsequently backfilled with an anti-fouling (AF) polypeptoid polymer in order to create polymer surface coatings composed of both AM (active) and AF (passive) peptoid functionalities.[75] Confocal microscopy results showed that the membranes of adherent *E. coli* cells were damaged after 2 h exposure to the modified substrate, suggesting that ampetoids retain AM properties.

Another group of antimicrobial materials that mimic host defence peptides is the facially amphiphilic antimicrobial polymers and oligomers.[74] They contain hydrophobic and hydrophilic side chains which can segregate to opposite regions, or faces, of the molecule forming a facially amphiphilic polymer. In a recent study, one of these polymers (poly(butylmethacrylate)-*co*-poly(Boc-aminoethyl methylacrylate) (Table 1) was immobilized onto silicon wafers and glass surfaces. The surface-bound polymers retained their antibacterial properties and killed *S. aureus* and *E. coli* 100% by contact in less than 5 min. Importantly, the antimicrobial properties of these polymers were independent of polymer chain length and grafting density.[76] In some cases, a polymer layer of 3 nm thickness was able to extend through the bacteria cell envelope, which is estimated to be ~30–37 nm thick, and kill the bacteria. This means that the mechanism for bacterial killing might not involve a physical

damage of the antimicrobial polymer in the cell membrane. Instead, might involve the release of Ca^{2+} and Mg^{2+} from the bacterial phospholipids membrane and the electrostatic compensation of the negative charge of the phospholipids membrane by the surface cationic charge.[38,76]

3.5 Materials incorporating non-leaching antimicrobial agents

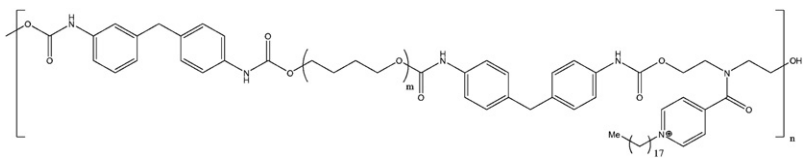
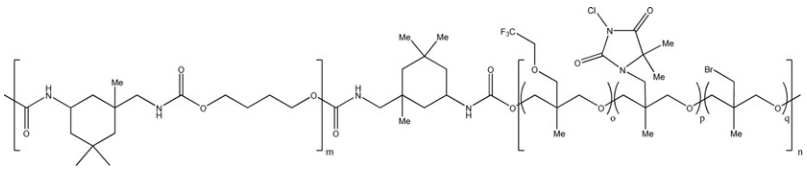
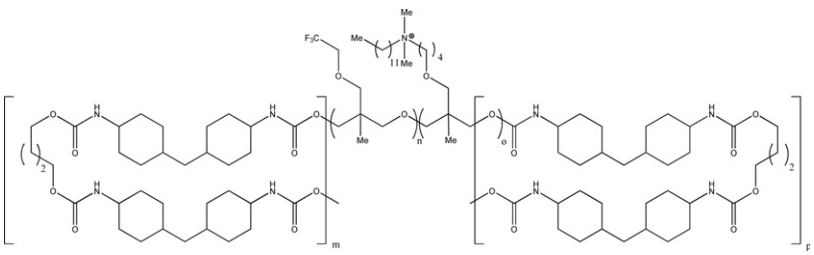
Another approach to make medical devices with antimicrobial properties is to incorporate antimicrobial agents in the bulk of the medical device. The agent might be (i) integrated in the original polymer used for the medical device,[77] (ii) blended with the original polymer and extruded in the desired shape,[78] or (iii) added to the original polymer as micro- or nanoparticles and crosslinked.[79,80] As compared to the coating of medical devices, this approach has several advantages: (i) no need to process the medical device after being made, (ii) simple control of the antimicrobial agent content in the medical device, and (iii) relatively easy to implement on an industrial scale. However, this approach has several limitations: (i) requires antimicrobial agents that are stable to extrusion temperatures or free radical chemical initiators, (ii) requires considerable amounts of antimicrobial agent to make the bulk of the medical device, (iii) the incorporation of the antimicrobial agent might affect the mechanical and biologic properties of the medical device, and (iv) the antimicrobial activity of the agent might be affected by the presence of other components in the bulk of the medical device.

Several examples have been reported in the literature for medical devices incorporating non-leaching antimicrobial agents. Polyurethanes composed of methylene diphenylene diisocyanate, poly(tetramethylene oxide) and the quaternized biocidal agent *N,N*-bis(2-hydroxyethyl)isonicotinamide) possess high antibacterial activity against Gram-positive *S. aureus* (up to 95%) but modest antibacterial activity against Gram-negative *E. coli* causing the death of 10% of adherent organisms[77] (Table 5). No leaching of the biocidal agent was observed from the polyurethane materials indicating that the killing activity of the material was by contact.[77] In fact, these quaternized polymers displayed long-term stability in an aqueous environment, exhibiting only small changes (less than 4%) in sample mass after 2-years immersion in water while keeping antimicrobial activity. The antibacterial effect of the polyurethanes can be also extended to Gram-negative bacteria. Polyurethanes containing hypochlorite activated dimethylhydantoin pendant groups or alkylammonium-functionalized soft blocks are effective contact biocidal surface against both Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa* and *E. coli*) (Table 5).[81,82]

The incorporation of biocidal groups in the medical devices can be done during the crosslinking of the material.[83] For example, biocidal quaternary ammonium groups attached to poly(methyl oxazoline) with a distal double bond were effective additives to render acrylate based polymeric networks contact-active antimicrobials against Gram-positive *S. aureus* bacteria.[83]

Composite resin materials are widely used in dental clinics for the replacement of hard tissues. Unfortunately these materials accumulate more dental plaque than other restorative materials which may result in secondary carriers. Two recent studies show that the incorporation of insoluble crosslinked quaternary ammonium polyethylenimine (PEI) nanoparticles in composite resin restorative materials (2% by weight) have a stable and

Table 5 Schematic representation of materials incorporating non-leaching antimicrobial agents[a]

Chemical entity	Microorganism	Microorganism loading; killing percentage	Ref.
	*S. aureus*, *E. coli*	NA; up to 95% killing in 30 min	77
	*S. aureus*, *P.aeruginosa*, *E. coli*	NA; >99% killing in 15 min	81
	*S. aureus*, *P.aeruginosa*, *E. coli*	NA; 100% killing in 30 min	82

[a] NA= Not available.

long-lasting (at least 1 month) antibacterial effect against oral bacteria, *Streptococcus mutants, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Pseudomonas aeruginosa*, and *Escherichia coli*.[79,80] Importantly, the antibacterial properties of the composite were not due to the leaching of the nanoparticles.[79] Furthermore, the results indicate that the incorporation of PEI nanoparticles did not affect the biocompatibility of the resin composite. No statistical difference was observed in macrophage viability when exposed to composite material with or without nanoparticles.[80]

4 Applications

It is unclear what level of antimicrobial efficacy is required for medical device coatings. This will likely depend on several variables including (i) the clinical history of the patient, (ii) the application site of the medical device, (iii) the number and type of microorganisms typically found at the implantation site, (iv) the contact to biologic fluids, amongst others. The design of most effective medical device coatings will require the determination of the full spectrum of biological activity. Studies with non-leaching immobilized non-natural polycations have shown a killing efficiency of 100% in 5–30 min for surfaces exposed to approximately 10^6 cells per cm^2 of *S. aureus* or *E. coli*.[26,32] However, these efficacy tests are performed against few bacterial species, and typically do not include fungi and viruses. Therefore, the advancement of this research field might benefit from a clear definition of standards and methodologies to determine the efficacy level of the antimicrobial surfaces.

For medical device applications, the antibiotic-based coatings might arrive sooner to the market than the other technologies, due to low regulatory hurdles. Yet, this will require an extensive *in vivo* evaluation of the biocompatibility and efficacy of the coating. The efficacy will likely depend on the type and density of antimicrobial agent used and the microorganism responsible for the infection.

A vast number of medical devices might benefit from antimicrobial surfaces or materials but at the top of the list we place catheters, cardiovascular devices and hip replacement devices. Each year, urinary catheters are inserted in more than 5 million patients in acute-care hospitals and extended-care facilities.[84] Catheter-associated urinary tract infection (CAUTI) is the most common nosocomial infection in hospitals and nursing homes, comprising >40% of all institutionally acquired infections.[84] The risk of an urinary tract infection is related to the duration of catheterisation and the standard of catheter care. In practice, 10–50% of patients undergoing short-term catheterisation (1–7 days) develop bacteriuria. Patients enduring catheterisation for 28 days or longer will have even high chances to be infected.[85] The routes of infections may originate from intraluminal migration of bacteria from the drainage spout or extraluminal ascendance through a thin liquid space between the catheter surface and the urethral mucosa.[84] In short-term catheterization, common organisms isolated from bacteriuria are *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus epidermidis*, *enterococci* and *Candida* species.[84,86] A recent US study to examine the current practices used by hospitals to prevent hospital-acquired urinary tract infections showed that 30% of the hospitals used antimicrobial catheters (in most cases based on the release of silver ions).[86] Unfortunately the improvement in the use of these devices was not reported in the same study. Permanent antimicrobial surfaces might be an alternative to the current antimicrobial catheters based on the release of silver ions.

Diseases of the heart and the circulatory system (cardiovascular disease or CVD) are Europe's leading causes of death responsible for over 4.3 million deaths each year.[87] CVD itself is the most common cause of death responsible for 1.92 million deaths per year. Over one in five women (22%) and over one in five men (21%) die from the disease.[87] Significant damage to the heart used to be untreatable but with modern medicine cardiac surgeries, pacemakers, defibrillators, and ventricular assist devices have become commonplace and life prolonging.[88] Unfortunately, significant infection rates have been reported for cardiovascular implants. The incidence of infections for pacemakers (temporary and permanent) is relatively high with 0.13–19.9%, for defibrillators 0.00–3.2%, for left ventricular assist devices 25–70%, and for ventriculoatrial shunts 2.4–9.4%.[89] Modern antimicrobial materials are expected to alleviate the death toll and the suffering in heart patients. On the other hand, infections of peripheral vascular stents is surprisingly low with only 1 case for 10,000 implants (with an estimated >400,000 patients per year in the USA).[89] Therefore primary prophylaxis for stent placement is not routinely advocated due to the low risk.[89]

Antimicrobial surfaces or materials can also be used for the prophylaxis of prosthetic joint infections in orthopaedic surgery. In the United States more than 1.3 million people have an artificial joint.[90] The infection rates of total joint hip arthroplasties range between 0.5% and 3.0% in primary total hip arthroplasty, despite strict antiseptic operative procedures and systemic antibiotic prophylaxis.[90–92] According to a recent study, one of the most common causes of revision in the hip arthroplasty in USA was infection (14.8%).[93] The average length of hospital stay was 6.2 days, and the average total charges were $54,553.[93] In order to reduce the number of infections, the implants can be impregnated with antibiotics.[94] Unfortunately, sub-inhibitory concentrations of antibiotics can lead to the emergence of multiresistant microorganisms.[94] The use of permanent antimicrobial surfaces on hip replacement devices might be an alternative to prevent microbe colonization and consequent biofilm formation.

5 Future prospects

Further research work is needed to elucidate the antimicrobial mechanism of the different agents immobilized onto surfaces or materials. It is unclear whether the antimicrobial agent mechanism changes after being immobilized onto a surface and why some antimicrobial agents kill microorganisms (bacteria, fungi, virus) with variable membrane composition and have little cytotoxic effect against human cells.[26] This information will be essential to design more effective synthetic agents against microorganisms and yet preserving the viability of human cells.

Another important aspect to be considered is that killed microbes will accumulate on the coated surface, and therefore might reduce its antimicrobial activity over time.[27] New platforms should be developed in the near future in order to remove

the microbes. A recent study reported a switchable polymer surface that integrates antimicrobial and nonfouling properties and is biocompatible[95] (Table 1). The cationic precursor of poly-(sulfobetaine methacrylate) is able to kill bacterial cells effectively and, through ester hydrolysis, switches to a zwitterionic nonfouling surface that releases dead bacterial cells upon hydrolysis. Moreover, the resulting nonfouling zwitterionic surface can further prevent the attachment of proteins and microorganisms and reduce the formation of a biofilm on the surface.

Given that most of these coatings or materials will be used in contact with biological fluids and human cells, it will be of utmost importance to evaluate the effect of the antimicrobial surfaces against blood[96] and human cells[26] and ultimately to evaluate their *in vivo* biocompatibility and antimicrobial efficacy.[54]

Overall, we have the feeling that the field of antimicrobial surfaces is advancing fast but further research is needed for an efficient design of a new arsenal of medical devices with antimicrobial properties that will benefit patients all over the world.

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