

Antibacterial surfaces for biomedical devices

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Despite considerable research and development efforts, the problem of infections related to biomedical devices and implants persists. Bacteria evidently can readily colonize surfaces of synthetic materials, such as those used for the fabrication of catheters, hip and knee implants, and many other devices. As the growing colony encapsulates itself with a protective exocellular bacterial polysaccharide layer, the biofilm becomes much harder to combat than circulating bacteria. Thus, there is a strong need to mitigate bacterial colonization by equipping the surfaces of biomedical devices and implants with features such as surface chemistry and surface roughness that are unfavorable for bacterial attachment. Here we review a number of strategies used for the design of antibacterial coatings. We also discuss specific issues that arise from using various types of coatings.

KEYWORDS: antibacterial coating • controlled release • furanone • infection • medical devices • polymer film • quaternary ammonium compound • silver • surface grafting

Biomedical devices have become an essential aspect of the human healthcare system. Over the past three decades, the number of artificial hip and knee implants has increased markedly, and stents, heart valves, vascular grafts and other implanted devices have been used widely to save lives and to restore quality of life for many people. There is also a clinical need for nonimplanted, shorter term-usage biomedical devices, such as various catheters and orthopedic fixation screws, among others. As the lifespan increases in industrialized countries, the demand for such biomedical devices continues to increase, and clinicians express their desire for better or longer lasting biomedical devices.

Device-related infections (DRIs), from bacteria attaching and proliferating on surfaces of biomedical devices and implants, are a significant issue in implant surgery as well as with short-term biomedical devices [1–3]. DRIs often are not detected at an early stage; they pose a substantial health risk to patients, often requiring reoperation and replacement of the infected device, and incur considerable costs to the healthcare system. The severity of DRIs varies greatly among devices and patients but can be serious or even fatal in some instances [1]. With contact lenses, for example, lens-related infections are rare in the hygienic environment of modern societies, and most users have the common sense to remove contact lenses when the eye becomes sore as an

early sign of bacterial infection. There are, however, no analogous early warning signs of bacterial infection for many implants and devices; the outbreak of an infection can be masked by the ongoing soreness of tissue inflammation after surgery, and DRI diagnosis often occurs when a full-blown infection has already caused damage to tissue and host organism. Reoperation of infected implants has at times led to the death of elderly patients already weakened by the previous operation or other conditions.

Today, healthcare systems try to minimize the risk of infection on short-term biomedical devices by prophylactic measures. Catheters are replaced at frequent intervals, but such preventative replacement schedules impose considerable costs to the healthcare system. With implants, the problem cannot be addressed as readily. Improved procedures in sterilization and operating theatres have significantly reduced the frequency of early-stage infections of implants, but delayed infections, occurring many weeks or months after surgery, have barely decreased and continue to pose a serious problem. It is believed that such late-stage infections are not caused by the act of surgery but by planktonic bacteria circulating in the vascular system. Spores landing at an incompletely healed wound site may attach on to the implant surface, multiply and form a biofilm that eventually leads to infection.

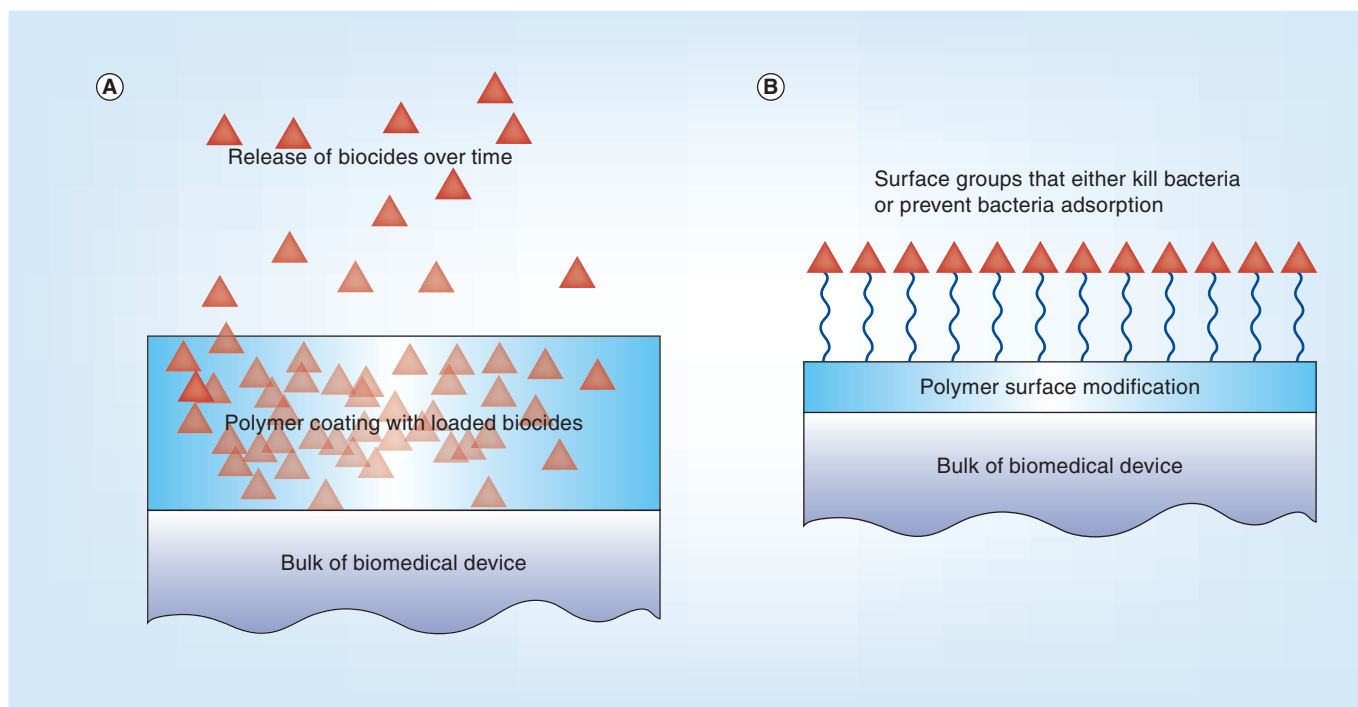


Figure 1. Strategies for antibacterial coatings. (A) Coating the surface of a device with a polymer film containing biocides that are released with time. **(B)** Attachment to the surface of a biomedical device of compounds that either kill bacteria or prevent bacterial adsorption.

Attached and growing bacterial colonies soon produce an extracellular polysaccharide matrix, which protects them against antibiotics and the host body's innate defense system [2,4]. Thus, bacterial biofilms on biomedical device surfaces are much more difficult to eradicate by antibiotics than circulating bacteria; hence the need for surgical removal of a substantial proportion of infected implants. Accordingly, a promising strategy for reducing the occurrence of DRIs is to prevent the initial attachment of bacteria to implant and device surfaces. This has spurred research efforts on the development of thin coatings that can be applied to biomedical devices to confer resistance to bacterial colonization. Such antibacterial coatings should deter DRIs while not affecting other properties, such as the visual clarity of contact lenses or the flexibility of vascular grafts. Obviously nor should such coatings have adverse effects on cells or fluids (e.g., blood or tear fluid) of the human host.

Here we review recent reports on the development of antibacterial surfaces for biomedical devices, concentrating on polymeric materials and polymer coatings owing to their widespread usage. Given the variety of devices and implants, as well as causative bacteria, one single approach may not be universally successful, and antibacterial strategies may need to be tailored to specific product needs. For some products, such as contact lenses, resistance to the attachment of bacteria is required along with biofouling resistance, whereas for hip and knee implants one wishes to deter bacteria while encouraging the close apposition of host cells for close integration of the implant with human tissue. These device-specific requirements have to be tackled using different designs of antibacterial bio-interfaces.

Approaches to antibacterial device surfaces

Polymeric materials offer great flexibility for the design of biomedical devices. However, most polymers are readily colonized by bacteria. A few polymers are known to kill bacteria [5–8] or prevent bacteria from attaching [9–12], but those polymers are often not suitable as bulk materials for the fabrication of devices due to other considerations, such as strength or flexibility. Thus, the emphasis has been on using bactericidal or attachment-resistant polymers as surface coatings applied onto existing devices.

In contrast to the relatively small number of antibacterial polymers, a considerable number of low-molecular-weight molecules and some inorganic ions are known to possess antibacterial effectiveness in solution. Unlike bactericidal polymers, such antibiotics cannot be applied directly, for example, by solvent coating onto device surfaces, as they adhere too weakly and are displaced rapidly. Low-molecular-weight antibiotics can be used in two ways to combat DRIs. One approach is to use a controlled-release approach (FIGURE 1A), in which the antibiotic is released from the biomedical device and intercepts bacteria in the vicinity. This has been studied with some organic compounds, but by far the most common antibiotic used is silver; several silver-based approaches are well advanced. The extensive literature on the development of antibiotic-releasing devices has been the subject of several reviews [13–15]. Various antimicrobial compounds have been loaded into polymers or polymer composite films, including organic antibiotics [16–18], silver [19–21] and nitric oxide [22] for various intended biomedical applications. Other metal ions have also been tested, but adverse effects on human tissue present a

concern. The disadvantage of the release approach is that the duration and effectiveness of antibacterial action is limited by loading and release kinetics.

The second approach consists of the application of a molecular surface layer of covalently immobilized ('grafted') antibiotic molecules that can prevent bacterial attachment to materials surfaces (FIGURE 1B). In addition to potentially much longer, perhaps indefinite, effectiveness, this approach is also favorable when seeking regulatory approval for new devices; if it can be ascertained that the antibiotics are durably grafted such as to remain on the device surface, one can eliminate concerns regarding possible adverse effects due to accumulation of antibiotics in body tissues, such as the brain, liver and spleen.

Polymers that by themselves are not resistant to bacterial colonization are of great value as vehicles for the controlled out-diffusion of dispersed antibiotics or for the covalent grafting of antibacterial small molecules. In some cases antibiotics may be delivered from 'bulk' polymers used for device manufacture, but in many cases this may be incompatible with manufacturing processes, such as extrusion, which would destroy imbibed antibiotics. Often it may be advantageous to first manufacture a device and then apply a polymeric coating that can deliver or surface-graft an antibiotic. For metallic and ceramic devices in particular, such as hip and knee implants, antibiotic molecules can usually be neither imbibed nor grafted onto the inorganic surface; although rare exceptions exist, such as the use of carbonated hydroxyapatite coatings that were precipitated onto titanium implants in a manner suitable for incorporation of antibiotics containing carboxylic groups, such as cephalothin, carbenicillin and cefamandol [23], and hydroxyapatite loaded with gentamycin for cementless joint prostheses [17] and post-traumatic osteomyelitis [24]. However, in most cases, a thin polymeric coating serves as an essential vehicle. Thus, much of the literature on antibacterial surfaces for implants and biomedical devices focuses on polymeric coatings of various design variants.

Biocidal polymers

Both synthetically designed and natural polymers offer significant promise for use as antibacterial coatings. Polymer coatings can be placed onto device surfaces via various techniques, such as dip coating, spin coating, layer-by-layer plasma polymerization or Langmuir–Blodgett extrusion. This provides enormous flexibility for applying various polymers onto surfaces of biomedical devices and implants for achieving antibacterial action. According to Kenawy *et al.* the ideal antimicrobial polymer should possess the following characteristics [25]:

- Easily and inexpensively synthesized
- Stable in long-term usage and storage at the temperature of its intended application
- Not soluble in water for a water disinfection application
- Does not decompose to and/or emit toxic products
- Should not be toxic or irritating to those who are handling it

- Can be regenerated upon loss of activity
- Active against a broad spectrum of pathogenic microorganisms upon brief contact.

Most synthetic polymers that have been reported to be bactericidal [5–8], as well as the natural polymer chitosan [26], are cationic. For example, a coated layer of poly(dimethylaminomethyl styrene) has been deposited onto nylon fabric [7]. It appears reasonable to assume that their mode of action is membrane lysis, analogous to that of the better known and older bactericidal approaches of using cationic peptides and quaternary amine compounds (discussed later). The main issue with such cationic polymers is that they cause adverse effects on human cells and therefore are limited in their use as coatings for implants and biomedical devices. By contrast, Salick *et al.* designed a hydrogel scaffold from the self-assembling peptide MAX1 for tissue regeneration applications; its surface exhibited inherent antibacterial activity [27]. The MAX1 gel was tested with bacterial solutions ranging in concentrations from 2×10^3 to 2×10^9 colony-forming units (CFUs)/dm² and was found to exhibit broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria. Interestingly this hydrogel surface was nonlytic for human erythrocytes, which maintained a healthy morphology when in contact with the gel surface.

A number of studies have reported the attachment of antibacterial moieties onto polymer chains (thus producing derivatized polymer chains, as opposed to surface-derivatized solid polymer materials, discussed in a later section). Biocidal *N*-halamines were synthesized from 2-(perfluorooctyl) ethyl acrylate in a two-step procedure using free radical polymerization and microwave irradiation [28]. Copolymers incorporating *N*-halamine siloxane and quaternary ammonium salt siloxane units were prepared for application as water-based coatings [29,30]. Vanillin, *p*-hydroxybenzaldehyde, *p*-chlorobenzaldehyde, anisaldehyde, methyl-4-hydroxy-benzoate, methyl-2,4-dihydroxy-benzoate, propyl-3,4,5-trihydroxy-benzoate and 2-hydroxy-methylbenzoate were attached to amine groups of chitosan to yield antimicrobial polymers [31]. The same compounds were also attached to polyacrylamide that had been equipped with amine groups by reaction with ethylenediamine [32]. Polyurethanes (PUs) with soft blocks containing end-fluorinated ($-\text{CH}_2\text{OCH}_2\text{CF}_3$) and 5,5-dimethylhydantoin pendent groups were prepared and used (at 2 wt%) as biocidal polymeric surface modifiers [33]. Polysiloxanes with pendant biocidal *N,N'*-dialkylimidazolium salt (ImS) groups were synthesized and compared with polysiloxanes bearing conventional biocidal quaternary ammonium salt (QAS) groups [34]. The ImS-containing polymers were found to have high antibacterial potency against all bacteria studied, similar to polymers substituted with QAS groups but with better thermal stability.

Such derivatized polymers can then be used either as materials *per se* for device fabrication or, more commonly, as coatings on existing devices, although the absence of covalent bonding to the device material may eventually lead to delamination of such coatings; unfortunately, few studies exist that address the question of longer term stability and performance of solvent-coated protective

antibacterial polymer layers. An instructive study by Kingshott *et al.* showed that physisorbed polyethylene oxide polymers were not effective in reducing bacterial adhesion, whereas polyethylene oxide chains covalently attached to a bulk polymer material were effective [11]. A likely explanation is that bacteria can act as very large surfactants with interfacial affinity for the material surface, displacing physically adsorbed polymer chains from the bulk material surface, whereas covalently attached (surface-grafted) polymer chains resist such displacement. It would seem advisable to use the pathway of covalent attachment of antibacterial polymer coatings onto devices in order to prevent possible loss in biological milieu. For low-molecular-weight antibacterial compounds, discussed in a later section, covalent attachment is essential for long-term performance, as they are more readily detached from a surface than polymeric coatings. The main point to note here is that even polymeric coatings may not remain on the device surface when in contact with biological media, in which many surface-active proteins and cellular entities may have sufficient affinity for the surface of biomedical devices to displace physisorbed coatings.

Release of antibiotics & germicides from polymers & polymeric coatings

The release of antimicrobial compounds from biomedical devices has attracted considerable attention. Compared with systemic drug delivery, a key advantage of local delivery of antibiotics at a specific site is that high local doses can be administered without exceeding the systemic toxicity level of the drug and risking, for example, renal and liver complications. Thus, increased doses could be applied at the site of a medical implant. One also needs to bear in mind whether the active compound is an antibiotic or a germicide. For example, chlorhexidine and triclosan are germicides while gentamicin and norfloxacin are antibiotic drugs. The latter can be used for both internal and external uses, while germicides should be used only for external applications.

An important issue concerning releasing devices is the kinetics of release of the antimicrobial compound. Fast release provides relatively high doses but short-term action. Slow release may not reach the required therapeutic level, and might also lead to bacterial resistance at the release site due to survival of strains that adapt. The released antibiotics must act before a protective extracellular matrix layer protects the colony. Bacteria protected by a biofilm can require 1000-times the antibiotic dose necessary to combat bacteria in suspension. An ideal release coating should provide fast initial release in the first 6 h after intervention to protect the site while the immune system is weakened, as well as continuous 'prophylactic' slow release.

Many polymers suitable for biomedical devices, such as silicones, can be blended, or loaded by in-diffusion, with antimicrobial compounds that are then released when in contact with a fluid environment. For example, Gaonkar *et al.* impregnated silicone catheters with various antibiotic and antiseptic compounds and investigated the performance in delaying the onset of urinary tract infection [35]. Catheters loaded with both chlorhexidine and triclosan showed the best performance, delaying infections for up

to 31 days. A similar approach is exemplified by the study of Park *et al.*, who incorporated norfloxacin into polymer coatings that were applied onto catheters [36]. While in this case the release of the antibiotic occurs by diffusion of the low-molecular-weight compound out of the polymer or polymer coating, the use of a polymer matrix that degrades in the body environment provides delivery of the antibiotic by a combination of diffusion and polymer matrix erosion. Loading of gentamicin into poly(hydroxybutyric-co-hydroxyvalerate) and its release was studied by Rossi *et al.*, who were able to control the release of the antibiotic by varying the content of hydroxyvalerate in the formulations [37]. Biodegradable polymeric nanofibers of poly(lactide-co-glycolide) (PLGA) and nanofibers of PLGA fabricated by electrospinning were used for delivery of cefazolin [38]. Biodegradable, injectable, gelling polymeric devices for the controlled release of gentamicin sulfate for the treatment of invasive bacterial infections were developed using poly(sebacic-co-ricinoleic-esteranhydride); the *in vitro* activity of the formulations was tested against *Staphylococcus aureus* [39]. Biodegradable poly(L-lactic acid) and poly(D,L-lactic-co-glycolic acid) films containing gentamicin, suitable for application on the surface of metallic or polymeric fracture fixation devices, were reported by Aviv *et al.* [40]. The release from biodegradable polymeric scaffolds containing amikacin and gentamycin was studied by Prabu *et al.* [41].

Other systems in which specific combinations proved advantageous have been reported. For example, Blanchemain *et al.* developed an antibacterial delivery system by coating Dacron® (polyethyleneterephthalat; PET) vascular grafts with cyclodextrins and subsequent loading with vancomycin [42,43]. Linear release of vancomycin was observed over 50 days.

Mixing biodegradable poly(lactic-co-glycolic acid) (PLGA) microspheres into setting bone cement achieved controlled local release of antibiotics [16]. The microspheres did not compromise the mechanical properties of the cements. The prophylactic use of antibiotic bone cement is, however, controversial [44,45].

The release of nitric oxide as an antibacterial agent has been the subject of several studies. Nitric oxide-releasing sol-gel polymer coatings for orthopedic devices showed a significant reduction of adhesion of *Pseudomonas aeruginosa*, *S. aureus* and *Staphylococcus epidermidis* [22]. Similar results were also reported by Charville *et al.* [46]. Polymers incorporating nitric oxide-releasing/generating moieties were shown not only to be effective against bacterial adhesion but also to improve the biocompatibility of blood-contacting medical devices [47].

Silver-releasing coatings

Considerable attention has focused for several decades on the use of silver as an antibacterial agent. The antibacterial properties of silver have been known since the time of the ancient Greeks. Other ancient civilizations, such as the Egyptians, Romans and Mesopotamians, were also aware of the benefits of silver for tableware and storage containers.

Silver, silver compounds and silver nanoparticles have enjoyed a great deal of attention in recent years [48]. A number of commercial products including wound dressings, bandages for burns and

chronic wounds, silver- and silver nanoparticles-coated catheters, and other medical devices have emerged. The efficiency of some of the coatings has, however, been subject to controversy, and clinical concerns have also surfaced, as discussed later. A number of factors, both from the coating and the environment, come into play in determining the efficiency of silver-based coating. It was found that coatings based on metallic silver are least efficient. This is because silver needs to be in its oxidized form (Ag^+) in order to exhibit antibacterial action. Ag^+ ions can be complexed by chorine ions to produce AgCl_2 , which precipitates, and is detrimental for antibacterial efficiency. Another important problem is that in many cases most of the loaded silver is released very quickly from the coating, thus limiting the time of protection.

There have been substantial research efforts towards engineering thin polymer films loaded with silver and silver nanoparticles in order to achieve a more prolonged release. Many studies have shown effectiveness *in vitro*, but these new approaches are yet to be verified *in vivo*. While some studies have applied contiguous silver film coatings onto substrates such as fibers [49], most studies focus on silver (nano)particles incorporated within polymeric matrices. For biomedical devices it may be advantageous to use thin polymer films containing silver nanoparticles fully encapsulated within the polymer matrix, as opposed to the situation where larger silver particles partly protrude from the polymer film layer. Ho *et al.* presented an interesting approach, first forming silver nanoparticles within a thin film of polyethylene imine [50]. The polymer was copolymerized with 2-hydroxyethyl acrylate, which allowed further surface grafting of polyethylene glycol (PEG). In this manner, coatings with two functions were generated, with the PEG layer preventing bacterial adhesion and the silver ions released from the coating toxic against *S. aureus*. Vimala *et al.* prepared hydrogel networks based on cross-linked poly(acrylamide) and three different carbohydrate polymers; gum acacia, carboxymethylcellulose and starch [51]. Silver nanoparticles were loaded into the hydrogel networks via *in situ* reduction of silver nitrate using sodium borohydride as the reducing agent. Lee *et al.* generated multilayer films, by layer-by-layer assembly, rich in catechol groups and used these groups to reduce silver ions to metallic silver when immersed in an aqueous metal salt solution [52]. This resulted in the generation of silver nanoparticles within the films. In another study, de Santa Maria *et al.* synthesized silver nanoparticles in commercial cross-linked Amberlite resins. Sulfhydryl and $-\text{N}(\text{CH}_2\text{COOH})_2$ groups present on these resins were used for Ag^+ chelation. Reductants were used to reduce the Ag^+ ions under an alkaline pH. The coatings were bactericidal against strain AB1157 *Escherichia coli* within a few minutes [53].

An interesting approach comprises the simultaneous deposition of silver and the polymer matrix coating. Poly(tetrafluoroethylene) film can be sputter-deposited with concurrent incorporation of nanosilver [54] or the use of plasma polymerization to form a polymeric thin film while simultaneously adding silver by sputtering [55]. The advantages of vapor deposition of polymeric matrices for nanosilver are that vacuum deposition often creates better interfacial adhesion and coating uniformity at lower, controllable thicknesses, compared with

solvent coating processes. However, the issue of even more limited loading and thus limited duration of antibacterial action needs to be considered.

Eksik *et al.* used electron transfer reaction and free radical polymerization processes to prepare triglyceride oil-based polymer-silver nanocomposites [56]. The antibacterial effect of the polymer nanocomposite was examined against Gram-positive, Gram-negative and spore-forming bacteria. Furno *et al.* impregnated silicone with nanoparticulate silver metal using supercritical carbon dioxide and examined bacterial adherence, the rate of killing of planktonic bacteria by chemiluminescence and viable counts [57]. The release rate of silver ions from the polymers in the presence and absence of plasma was measured using inductively coupled plasma mass spectrometry (ICP-MS). It was found that the antibacterial efficiency of the coatings was dependent on the rate of release of silver ions. Kelly *et al.* prepared polypyrrole and polyaniline conducting-polymer composites with individual fibers of cellulose by direct polymerization of the respective monomers, and used the redox active surface of the polymers to reduce silver ions to silver metal, thus producing cellulose/conducting-polymer-silver composites [58]. Kong *et al.* synthesized poly(methyl methacrylate) nanofibers containing silver nanoparticles by radical-mediated dispersion polymerization and tested antibacterial properties against Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria; minimum inhibitory concentration results showed that the silver-loaded nanofibers had superior antimicrobial efficacy compared with that of silver sulfadiazine and silver nitrate at the same silver concentration [59]. Lui *et al.* developed a procedure for the synthesis of silver nanoparticles in transparent polyvinyl films via a two-step procedure consisting of ion incorporation in the matrix followed by thermal reduction [60]. These composite films containing silver nanoparticles showed excellent antimicrobial performance toward bacteria, such as *E. coli*. Silver nanoparticles were also synthesized in covalently linked, layer-by-layer polymeric assemblies from methoxysilane polymers [61], in methoxy polyethylene glycol [62], epoxy polymer matrix [63], poly(ether urethanes) [64], polyethylene [65], poly(vinyl alcohol) [66], diamond-like carbon [67], polyacrylate film [68], polytetrafluoroethylene [54] and polyrhodanine nanofibers [69]. Vachon *et al.* loaded silver ions into sulphonated polymer hydrogels [70]. Kumar *et al.* published an environmentally friendly chemistry approach to synthesize silver nanoparticle-embedded paint in a single step from common household paint, taking advantage of the naturally occurring oxidative drying process in oils, involving free radical exchange, as a mechanism for reducing silver salts and dispersing silver nanoparticles in the oil media without the use of any external reducing or stabilizing agents [71]. Surfaces coated with silver nanoparticle paint showed excellent antimicrobial properties by killing both Gram-positive human pathogens (*S. aureus*) and Gram-negative bacteria (*E. coli*).

For potential application in bone tissue regeneration, Loher *et al.* described a general approach to increase the antimicrobial activity of a silver-containing surface by two-to-three orders of magnitude [72]. The authors used 1–2 nm silver particles

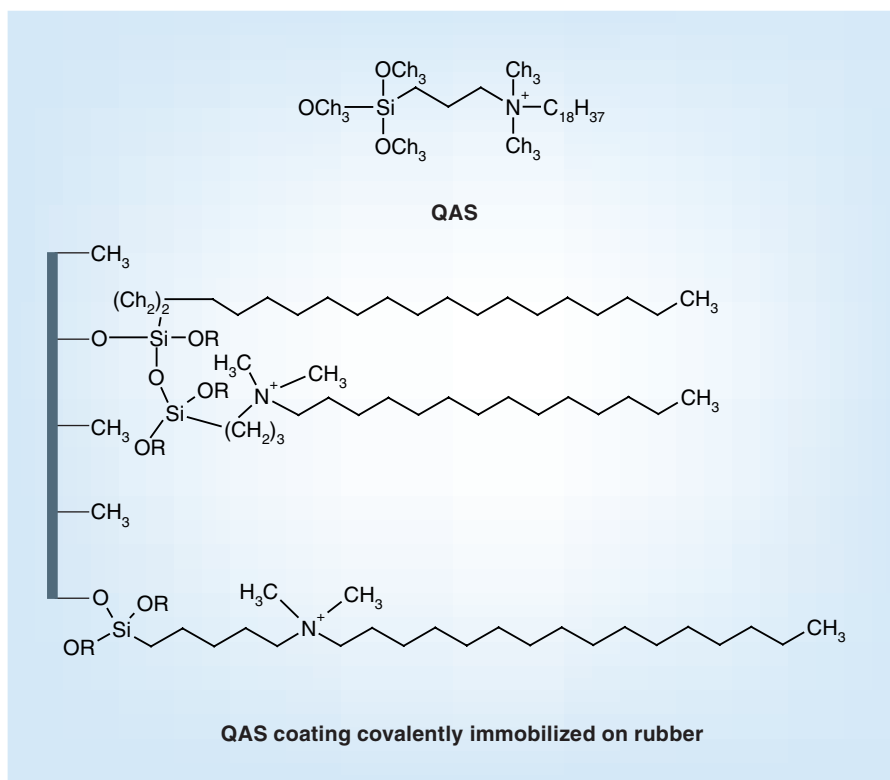


Figure 2. An example of a quaternary ammonium compound and a method for its immobilization onto ceramic device surfaces.

QAS: 3-(trimethoxysilyl)-propyldimethyloctadecylammonium chloride. Modified from [89].

decorating the surface of 20–50 nm carrier particles consisting of a phosphate-based, biodegradable ceramic, which allowed triggered release of silver as growing microorganisms dissolved the carrier and thus released the silver nanoparticles. The authors also described the rapid self-sterilization of polymer surfaces containing silver on calcium phosphate nanoparticles using several human pathogens. Schneider *et al.* described the use of silver on amorphous tricalcium phosphate (TCP) nanoparticles for electrospun, highly porous PLGA fibrous composites [73]. *In vitro* bioactivity tests of a PLGA/Ag-TCP composite containing 0.5 wt% silver showed hydroxyapatite deposition on the nanocomposite within 2 days. Antibacterial tests using *E. coli* demonstrated a prolonged antibacterial effect of the scaffolds containing finely dispersed silver on TCP compared with current clinically used methods based on soaking the scaffolds with a tetracycline solution prior to implantation.

The scientific consensus is that silver ions, released from silver metal coatings, or polymer materials or coatings doped with silver nanoparticles, enter bacteria and affect their biological functions. Silver appears to be the broadest spectrum antibiotic available, and it does not appear to induce resistance. The antibacterial action of silver for biomedical devices has been the subject of a number of studies and while opinions differ, the most probable scenario seems to be that silver ions bind to the bacterial cell membrane and damage it by interfering with membrane receptors and interfering with bacterial electron transport (impeding the

production of ATP and the cell's energy production). Another theme is that silver binds to bacterial DNA and thus damages cell replication, causing the intracellular formation of insoluble compounds with certain nucleotides, proteins and the amino acid histidine (making them unavailable as intracellular 'building blocks').

Given the similar structural elements of human cells, the question arises as to whether silver ions will also interfere with human cell and tissue functions in analogous ways. Indeed, Schrand *et al.* reported that induction of reactive oxygen species (ROS), degradation of mitochondrial membrane integrity, disruption of the actin cytoskeleton and reduction in proliferation after stimulation with nerve growth factor were observed upon exposing neuroblastoma cells to silver nanoparticles [74]. Concerns have also been raised by clinicians regarding the release of silver ions from biomedical products. For example, it was found that a person with burns who received local treatment with a commercial silver wound dressing showed hepatotoxicity and argyria-like symptoms, and the silver levels in his plasma and urine were clearly elevated, as well were his liver enzymes, during the treatment

period, raising concerns of potential silver toxicity. Monitoring silver levels in plasma and/or urine during treatment has been suggested [75]. Yet, despite such concerns regarding toxicity to human cells and strong evidence of an absence of antibacterial effectiveness *in vivo* [76,77], a number of companies are building their business on silver-releasing coatings, and research continues on variations of this theme. Perhaps more thought needs to be given to where and when such coatings may be suitable or unsuitable; for example, does it matter if a layer of cells next to an implant is killed by antibacterial silver? For some, it may, whereas for others, it may not, for example when fibrous encapsulation occurs anyway.

In summary, silver ions are clearly effective against various bacteria *in vitro*. However, the questions of clinical effectiveness and damage to human cells are the subject of continuing controversy. Silver-releasing coatings may find some applications but given the concern of potential side effects from silver complexing with proteins and altering their functions, we are of the opinion that application of silver-release coatings for biomedical devices must be approached with considerable caution, except where, we conjecture, much of the silver is likely to be removed from the body, such as with urinary catheters.

Surface-grafted antibiotics

The covalent grafting of antibacterial compounds onto polymer surfaces has been the subject of considerable research in the search for antibacterial surfaces with longer lasting effectiveness than is

possible via release approaches. The polymer surfaces onto which grafting was performed were either bulk materials or coatings themselves; in the latter approach, the polymeric coating serves as an adhesive interlayer and provides the chemical surface groups, for covalent grafting that are not available on the underlying bulk material/device. Antimicrobial agents that contain chemically reactive groups, such as hydroxyl, carboxyl or amino groups, can be covalently linked to a wide variety of polymer surfaces. A review by Kenawy *et al.* outlined various chemical routes for attaching antimicrobial agents [25]. An example of surface modification of a bulk polymer is attaching penicillin to poly(tetrafluoroethylene) via a PEG spacer [78].

While covalent grafting typically is preferred for its, in principle, better adhesive stability, noncovalent attachment modes can also provide useful coatings in some circumstances. For example, Statz *et al.* synthesized antimicrobial peptoid oligomers (ampeptoids) with an adhesive peptide moiety that enabled anchoring onto TiO₂ substrata [79].

Surface-bound quaternary ammonium compounds

Surfaces modified with quaternary ammonium compounds (QACs) have attracted considerable interest over the past several decades, since the pioneering work of Tiller *et al.* [8]. QACs are antiseptics that act against both Gram-positive and Gram-negative bacteria. The mechanism of antibacterial action of QACs is still not fully resolved; however, two hypotheses appear to be lead contenders. The first and most-cited hypothesis is that sufficiently long cationic polymer chains penetrate the cell membrane [1,8]. The second hypothesis, proposed by Kugler *et al.*, states that a highly charged surface can induce ion exchange between the positive charges on the surface and structurally essential mobile cations within the membrane [80]. Being in close contact with a cationic surface, vital divalent cations cannot perform their normal role in charge neutralization of the head groups of membrane lipids, which results in a loss of membrane integrity. In an elegant experiment Murata *et al.* attempted to weigh both hypotheses [81]. The authors varied the chain length containing the quaternary ammonium group and the surface density of quaternary ammonium groups in a gradient manner. It was concluded that the density of surface quaternary ammonium groups, and thus the density of cationic surface charges, is a key parameter; however, the authors did not exclude that membrane insertion of alkyl chains may also be a possible mechanism of action. The relative contributions of these modes may well differ with the molecular nature of the QAC.

An extensive body of literature exists on the development of various QACs and methods for their application; here we discuss a limited number of representative studies. Silane-based QACs have received substantial attention because of the possibility of immobilization onto hydroxylated surfaces, such as metal oxides on orthopedic implant surfaces [81–88]. The incorporation of a silyl group, for example, in the structure of 3-(trimethoxysilyl)-propoyldimethyloctadecylammonium chloride (FIGURE 2) enables covalent attachment of the QAC to a variety of substrates including glass, cotton and silicon rubber (upon plasma activation) [89], demonstrating the capability of QACs as antibacterial coatings for biomedical devices [23,41].

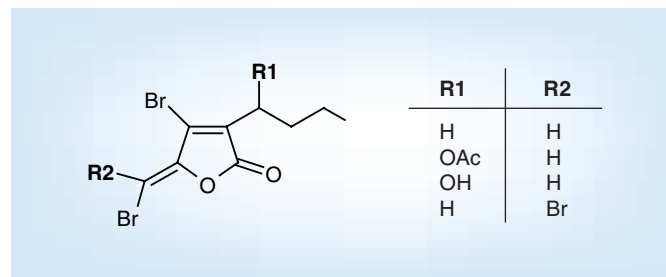


Figure 3. Furanones investigated as surface-grafted antibacterials.

Reproduced from [112].

The covalent bonding of QACs to the substrate may lead to long-term antibacterial activity as it avoids leaching of the antibacterial compound over time. The self assembly of silanes on hydrophilic surfaces is well known; the silane group anchors covalently to the substrate and the quaternary ammonium group remains available for bioactivity. Alternatively, quaternary ammonium thiol derivatives have been grafted onto metal surfaces [90]. Relevant to medical devices, QACs were also immobilized on polyethylene by a sol-gel process [91] and via a covalent hydrolyzable ester linkage [92] on stainless steel [93]. Other methods for depositing a coating containing QACs on surfaces include photopolymers [94], atom transfer radical polymerization [95,96], plasma polymerization [97] and layer-by-layer deposition [98].

Several studies have investigated QACs embedded in bone or dental cements. Shi *et al.* investigated the use of chitosan nanoparticles (CS NP) and quaternary ammonium chitosan derivative nanoparticles (QCS NP) as bactericidal agents in poly(methyl methacrylate) (PMMA) bone cement used to fix the implant, and observed significant inhibition of *S. aureus* and *S. epidermidis* growth [99]. Beyth *et al.* developed quaternary ammonium polyethylenimine (PEI) nanoparticles that were embedded at 1% w/w with clinically used bonding, flowable and hybrid dental composite resins [100]. The coatings were tested against *Streptococcus* mutans. The results indicated that the quaternary ammonium PEI nanoparticles immobilized in resin-based materials were strongly antibacterial without leaching of the nanoparticles and without compromise in mechanical properties. Li *et al.* incorporated an antibacterial monomer, methacryloxyethyl cetyl dimethyl ammonium chloride (DMAE-CB) in a dental adhesive and investigated the effect of the composite on the adherence, growth and membrane integrity of *Streptococcus* mutans [101].

Thus, as for silver, there is no doubt that QACs can be strongly antibacterial *in vitro*. There are, however, concerns related to the use of QAC on medical devices [84]. The main concern remains the cytotoxicity of QACs. It stands to reason that membrane disruption by QACs, whether by insertion or an ion disruption mechanism (or both), would equally apply to human cell membranes. As mentioned earlier for silver, however, in some clinical implant applications the disruption and death of a layer of cells may be tolerable, whereas, for example, for contact lenses the irritation caused by QACs rupturing cells on the eye and the eyelid would probably not be tolerable.

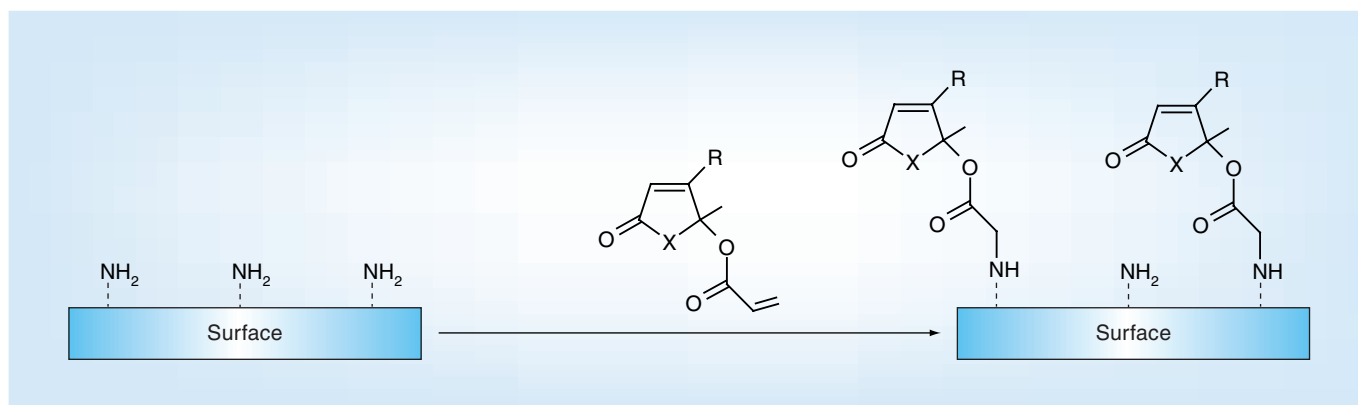


Figure 4. The attachment strategy for furanone-coated contact lenses using amine groups attached by grafting poly(allylamine) to an aldehyde plasma functionalized contact lens surface.

Modified from [113].

Low-molecular-weight antibacterial compounds

The covalent attachment of low-molecular-weight antibacterial compounds onto medical device surfaces may provide a route to achieving permanent protection against biofilm formation. There are, however, a number of issues that need to be addressed. First, the covalent grafting may cause side reactions that may convert the molecules to a different molecular structure; for example, β -lactams and furanones readily undergo ring opening in alkaline and acid media, thus, grafting must be performed in close to neutral pH conditions. Second, covalently grafted molecules may have geometrically restricted ability to perform their function, such as integrin docking or membrane lysis. Third, some antibiotics are thought to have an intracellular action, such as the gyrase inhibition of novobiocin and the *lux* gene deactivation by furanones; can such compounds be active when covalently attached onto a medical implant and thus unable to diffuse into bacterial cells?

Many existing antibiotics are derived from leads provided by compounds extracted from natural sources, and plants and marine organisms continue to provide interesting lead compounds. Antibiotics extracted from natural sources form the basis of many novel therapeutic agents as their chemical diversity provides limitless opportunities for new drug leads. Defence mechanisms against microbial colonization, commonly through the biosynthesis of antimicrobial compounds, are observed in a variety of organisms, from marine life to terrestrial plants [102–104]. For example, the antimycobacterial agent erogorgiaene, extracted from West Indian sea whips, has great potential as a new anti-tubercular drug [201]. Antibacterially active natural products offer structural variety and complexity, including distinctive ring architectures (e.g., vancomycin) or multifaceted supramolecular pharmacophores (e.g., erythromycin). The antibacterial penicillin-binding proteins (PCBs) and β -lactams are examples of naturally derived compounds with novel and even multiple modes of action [104]. The following sections review some antibacterial coatings that utilize compounds from plant and marine life origins, which may be able to provide durable biofilm protection to biomaterial and biomedical devices.

Furanones

Halogenated furanones (also known as fimbrolides), extracted from the Australian marine algae *Delisea pulchra*, were found to have strong antibacterial activity against a number of bacteria strains, including *S. aureus* [105], *E. coli* [106], *Bacillus subtilis* [107] and *S. epidermidis* [108,109]. The antibacterial nature of furanones is attributed to their ability to interfere with two bacterial cell communication strategies: quorum sensing [103,106] and swarming [110]. The use of these furanones as antibacterial coatings to prevent biofouling has several advantages; for instance, there is no toxicity to fibroblasts [109] and furanones are unlikely to lead to resistant strains of bacteria since the bacteria are not killed by the furanone, rather, biofilm formation is inhibited [111,112] by interference with quorum sensing, which is an essential step in the phenotype transformation of surface-adhered bacteria progressing to biofilm formation.

Highly active furanone compounds are based on 4-bromo-5-methylene-2(5H)-furanone (FIGURE 3). The furanone ring structure and the R2 position appear to be essential for antibacterial function, and the molecule thus needs to be covalently attached to surfaces via a reactive functional group remote from the furanone ring, such as R1 or the end of the alkyl side chain. From a synthesis perspective, incorporation of a suitable group at R1 is the easier option.

Al-Bataineh *et al.* reported a nitrene-based method for the covalent immobilization of furanones onto surfaces of model biomedical device materials [103,113], and Zhu *et al.* immobilized furanones onto contact lenses using a Michael-type addition reaction [112]. Surface spectroscopic evidence showed that both strategies are effective in achieving a high density of surface-bound furanone molecules, and it was also shown that the furanone molecules fully resisted attempts at their removal by extended washing, thus indicating that covalent surface attachment had indeed occurred, as opposed to physical adsorption or diffusion into the material. The nitrene radical approach, using a surface-bound azide group that reacts under light activation with adsorbed furanone molecules to form interfacial bonds, has little chemical specificity and will thus attach an unknown percentage of the molecules

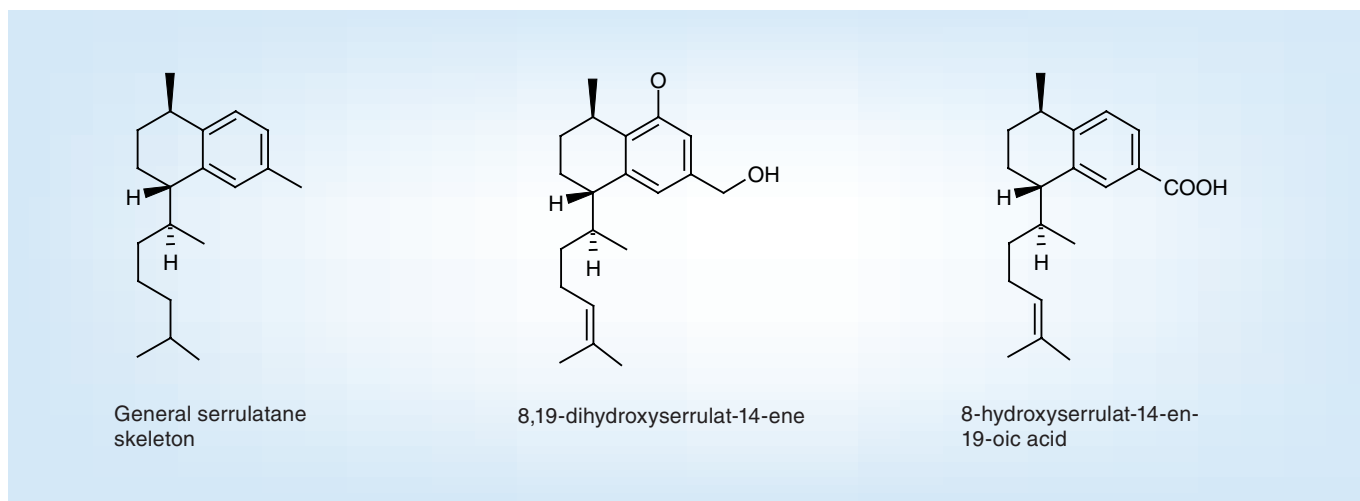


Figure 5. Some antibacterial constituents of the leaf resins of *Eucalyptus neglecta*.
Reproduced from [118].

in ways that interfere with activity (e.g., by linking into R2), although the alkyl tail provides a sufficient number of linking sites based on statistical distribution. Coated surfaces were extensively characterized by x-ray photoelectron spectroscopy (XPS) [103] and time-of-flight secondary ion mass spectroscopy (TOF-SIMS) [113] to probe for molecules attached to the surface and to check that the functional groups important for antibacterial activity (the lactone ring and the bromines) were still present. Experimentally it was found that nitrene-grafted furanones showed considerable antibacterial activity, with a 90% reduction in biofilm formation by *S. aureus* [111]; thus a sufficient percentage of the molecules must have been attached in ways that did not interfere with their biological function [111].

The other attachment strategy comprised reaction between an amine group on the contact lens surface and a pendent acrylic acid group attached to position R1 (FIGURE 4). As contact lenses do not contain amine surface groups, the lenses were first equipped with a layer of covalently grafted (poly)allylamine prior to furanone attachment. Other options exist for functionalizing contact lens surfaces with amine groups, such as ammonia plasma treatment, but a polyallylamine layer offers the best hydrophilicity, which is essential for contact lenses. This strategy is advantageous in terms of the linking reaction being specific to the acrylic acid side group and, thus, prevention of destruction of the activity of some of the immobilized furanone molecules. However, the reaction is much slower and the antibacterial activity of the furanone molecules is decreased upon fictionalization with the acrylate ester side group. Contact lenses coated with furanones were studied in both guinea-pigs, where the lenses were worn for 1 month, and human volunteers during 22-h wear. A reduction in bacterial adhesion of up to 92% was observed when comparing furanone-coated lenses with control lenses [112]. While these results are promising, cytotoxicity and mutagenic properties of these compounds have not been fully established, and other natural antibacterial compounds have shown even greater antibacterial activity [114].

Serrulatanes

The Australian plant genus *Eremophila* has attracted attention as it was used extensively in traditional Australian Aboriginal medicine. For example, leaves of the resin-producing *Eremophila duttonii* plant were applied to treat minor dermal wounds and infected lesions, while decoctions were used as a sore throat gargle and for treatment of eye and ear pain [115,116]. Similarly, *Eremophila neglecta* was used to maintain general well-being through ingesting infusions of the leaves [117]. The symptoms treated with *Eremophila* plant extracts suggest antibacterial activity, and this was indeed found to be the case [118–120]. Ndi *et al.* screened leaf extracts of over 70 *Eremophila* plant species, many of which were found to have excellent antibacterial activity against several Gram-positive bacterial strains, including methicillin-resistant *S. aureus* [117]. Isolation and structural elucidation of the antimicrobially active compounds in *E. neglecta* [117], *Eremophila serrulata* [121], *Eremophila sturtii* [122] and *E. duttonii* [115] has led to the identification of novel diterpene compounds of the serrulatane class that possess activity against bacteria associated with biomedical device infections. FIGURE 5 shows the general serrulatane skeleton and the structures of some serrulatanes isolated from *Eremophila* plants. In all cases of activity, the serrulatane diterpene hydrocarbon skeleton is modified with hydrophilic substituents such as -COOH, -OH, -OMe or -OAc in one or both rings.

Covalent attachment of 8-hydroyserrulat-14-en-19-oic acid to polymer and ceramic substrates via thin polymeric interlayers with amine groups demonstrated the potential of these natural antibacterials as biomedical device coatings. Covalently grafted serrulatane coatings have been achieved, for example, via aldehyde plasma polymer deposition onto a substrate followed by grafting of a thin, hydrophilic layer of polyallylamine, to which serrulatane attachment can be achieved by either reacting the 'tail' carbon double bond with surface amines or carbodiimide-mediated reaction between surface amines and the carboxyl group on the compound. XPS and TOF-SIMS have been employed to characterize the surface attachment of the serrulatanes. Effectiveness of the coatings

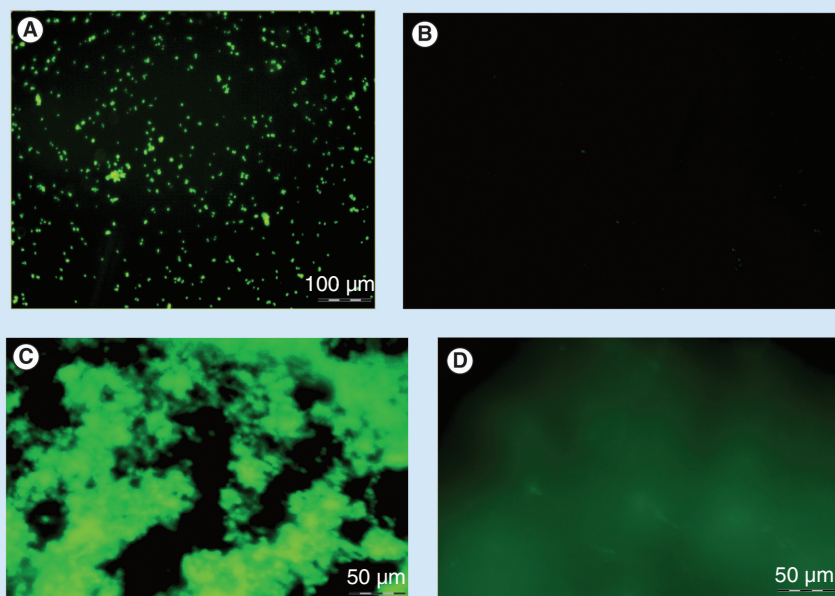


Figure 6. Optical micrographs of (A & B) amine plasma polymer control surfaces showing bacterial adherence and biofilm formation; and (C & D) serrulatane-coated surfaces. The diffuse color in (D) is due to diffusion of dye into the polymer material, not biofilm staining as in (B). Reproduced from [115].

has been tested through *in vitro* studies against biofilm-forming *S. epidermidis*. Reduction in bacterial colonization greater than 99% has been observed, as shown in FIGURE 6, where control surfaces became heavily colonized with biofilm while serrulatane-coated surfaces remained free of biofilm. These *in vitro* results suggest the potential of these compounds as antibacterial-coated layers.

Antimicrobial resistance

The ability of microorganisms to develop resistance to antibiotics is the most serious issue for infection control. Bacteria are exposed to repeated cycles of treatment, which eliminates most but causes selection of resistant strains. It has been known for some time that bacterial species such as *Staphylococci*, which are responsible for the majority of DRIs [1], are resistant to β -lactam antibiotics, such as penicillin and methicillin. It is therefore of crucial importance to test antibacterial surfaces against multidrug-resistant *S. aureus* and *S. epidermidis* strains. It is also important to consider whether bacteria might evolve to adapt to antibacterial surface coatings. In orthopedic implant situations, bacteria need to be deterred from colonizing the implant surface until host tissue has integrated with the implant. For catheters, on the other hand, bacterial colonization remains a challenge throughout the period of use.

Expert commentary

A wide variety of strategies have been investigated for the fabrication of antibacterial coatings for biomedical devices. Yet none has gained wide acceptance. One reason is that it is relatively straightforward to perform antibacterial testing for attachment and biofilm formation on biomedical device surfaces *in vitro*,

whereas clinical studies are much more complex and costly. Another reason is that *in vitro* tests utilize simplified environments that may neglect some key environmental factors that the coating needs to tolerate, such as inflammation. It is likely that there is no universal answer to the problem of biomedical device infection; coatings may have to be designed for specific applications taking into account the bacteria involved, their attachment and growth mechanisms, and the specifics of the biological milieu.

Much work has focused on the use of silver, released as silver ions, for combating bacterial infections, and a number of companies, mostly US based, promulgate this technology, usually in the form of nanosilver particles encapsulated within a polymeric matrix that can be coated onto devices. However, as some studies have reported high levels of cell toxicity to mammalian cells, caution is indicated; for example, Heidenau *et al.* found a lethal dose of 3.5×10^{-3} mmol/l for Ag^+ exposure of L929 fibroblasts [123]. The silver-release approach may be suitable for some clinical

applications but for others this high toxicity may cause serious problems. Input by clinicians and tissue biologists needs to be solicited to decide where the presence of killed cells adjacent to a biomedical device may or may not be a problem, or where silver ions may not accumulate to toxic levels, for example, possibly with urethral catheters. In any case, the overly optimistic and general tone of many scientific publications and company literature on silver antibacterial approaches needs to be put in perspective and qualified in terms of possible uses; it is not a panacea.

Other highly effective biocides, such as QACs, exhibit the same disadvantage of rapid cell death of mammalian cells. As for silver, this necessitates careful consideration of possible uses and where use may not be indicated.

Antibiotics that possess biologically specific action against bacteria can offer substantial therapeutic windows in that their toxicity levels to human cells are significantly above the threshold for antibacterial action. A number of studies have incorporated antibiotics into polymer matrices and studied their release and antibacterial effectiveness. A substantial number of reports indicate success *in vitro*; some *in vivo* successes have also been reported.

An issue common to all release approaches is the limited time over which the released concentration exceeds the therapeutic threshold. The effectiveness decays and usually vanishes within a few weeks or days. This is not a problem for some applications, such as many catheters, but for many biomedical implants, much longer durations are desired. For such long-term applications, a suitable strategy may be the covalent anchoring of antibacterial molecules onto biomedical device surfaces. Some studies have shown this approach to be successful *in vitro*, but

very little *in vivo* data exist, and the potential of this approach, and possible problems, cannot be assessed reliably at this point in time.

Five-year view

Several issues need to be addressed in order to arrive at viable solutions for a range of biomedical devices and implants within a 5-year time frame. A key issue is the question of cytotoxicity. There is controversy in the literature regarding cytotoxicity even for the most widely studied system, silver. Why? Is this a reflection of test systems that neglect key biological variables, of the use of different cells, of different ways in which silver was applied or other factors? Firm cytotoxicity data for various cell lines are essential. Such data can then form a basis for informed decisions regarding where silver – and other – strategies may be suitable, and where they should be avoided.

For the design of antibacterial strategies for specific biomedical devices and implants, it is highly desirable to have clinical input into the desired duration of antibacterial action. This may not equal the use time or lifetime of a device or implant; for example, once a full fibrous capsule forms around a soft tissue implant, can bacteria still reach the implant surface?

From a materials science perspective, another issue is that many of the studies performed by clinicians contain little or no reliable information on the materials used. For example, to what concentrations and depth distributions within the materials were antibacterial

additives incorporated? In the absence of such information, it can be difficult to arrive at secured conclusions, let alone repeat such studies. It is highly recommended that materials scientists be included in such teams in order to ensure that the materials/devices used are well characterized, free of surface contaminants such as processing aids and reproducible. In turn, materials scientists need to include biologists to ensure that their studies are not biologically naive.

Over a 5-year time frame, possibly the most exciting prospect is the development and design of smart coatings that offer triggered release of antibiotics when demand is indicated by a signal, such as a bacterial metabolite or enzyme. Such on-demand release, with no release unless bacteria are present, may circumvent the limited duration offered by current release approaches. Advances in the understanding of bacterial biochemistry and advances in nanotechnology now offer the potential for the rational design of such triggered systems, and we expect that in the next few years there will be significant progress in this area.

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Key issues

- Cytotoxicity of antibacterial coatings: many of the compounds used for antibacterial coatings are toxic to mammalian cells, and this needs to be investigated more thoroughly as it might confine the range of applications for some coatings to specific medical devices.
- Duration over which antibiotic release is needed: there have been various opinions regarding how long protection against bacterial adsorption and colonization is necessary. Opinions range from several hours to years and vary among devices. The exact answer (if there is one) to this question will be useful for designing more efficient antibacterial coatings.
- Close collaboration between clinicians and material scientists in the development and evaluation of the performance of antibacterial surfaces is needed to successfully combat infections related to medical devices.
- Design of smart coatings for triggered release: coatings that are capable of releasing antibacterial compounds only when bacteria adhere to the surface, by responding to some bacterial metabolic signal, will benefit many medical devices and may be a solution for the problems of limited release times and cell cytotoxicity.
- Antimicrobial resistance of pathogens is one of the most serious issues in modern infection control. It is also important to consider whether bacteria might evolve to adapt to antibacterial surface coatings.

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