Reducing implant-related infections: active release strategies

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Despite sterilization and aseptic procedures, bacterial infection remains a major impediment to the utility of medical implants including catheters, artificial prosthetics, and subcutaneous sensors. Indwelling devices are responsible for over half of all nosocomial infections, with an estimate of 1 million cases per year (2004) in the United States alone. Device-associated infections are the result of bacterial adhesion and subsequent biofilm formation at the implantation site. Although useful for relieving associated systemic infections, conventional antibiotic therapies remain ineffective against biofilms. Unfortunately, the lack of a suitable treatment often leaves extraction of the contaminated device as the only viable option for eliminating the biofilm. Much research has focused on developing polymers that resist bacterial adhesion for use as medical device coatings. This *tutorial review* focuses on coatings that release antimicrobial agents (*i.e.*, active release strategies) for reducing the incidence of implant-associated infection. Following a brief introduction to bacteria, biofilms, and infection, the development and study of coatings that slowly release antimicrobial agents such as antibiotics, silver ions, antibodies, and nitric oxide are covered. The success and limitations of these strategies are highlighted.

Introduction

Bacterial infection at the site of implanted medical devices such as catheters and artificial prosthetics presents a serious ongoing problem in the biomedical arena. Of the 2.6 million orthopedic implants inserted into humans annually in the United States, approximately 112,000 (4.3%) become infected.[1] The annual infection rate for cardiovascular implants is even higher (7.4%). When considering all indwelling devices, the number of implant-associated infections approaches approximately 1 million per year.[1] Perhaps equally concerning, antibiotics administered systemically are showing lesser efficacy against implant-associated infections.[2] As a result, implant removal and/or amputation are increasingly more prevalent. In addition to human pain and suffering, implant-associated infections present a significant economic burden to society. Estimates of the direct medical costs associated with such infections exceed $3 billion annually in the U.S. alone.[1] The number of device-associated infections will continue to rise as more patients receive biomedical implants. From 1996 to 2001, the number of hip and knee joint replacements increased by 14%.[3] The majority of these implant procedures were performed on those patients 65 years of age and older.[4] The worldwide increase in life expectancy and advances in medical technology will lead to greater demand for medical implants and a rising number of implant-associated infections.

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At the cellular level, implant-associated infections are the result of bacterial adhesion to a biomaterial surface.[5] Upon implantation, a competition exists between integration of the material into the surrounding tissue and adhesion of bacteria to the implant surface.[6] For a successful implant, tissue integration occurs prior to appreciable bacterial adhesion, thereby preventing colonization at the implant. However, host defences are often not capable of preventing further colonization if bacterial adhesion occurs before tissue integration.[6] A 6 h post-implantation ''decisive period'' has been identified during which prevention of bacterial adhesion is critical to the long-term success of an implant.[7] Over this period, an implant is particularly susceptible to surface colonization. At extended periods, certain species of adhered bacteria are capable of forming a biofilm at the implant-tissue interface. Biofilms are remarkably resistant to both the immune response and systemic antibiotic therapies, and thus their development is the primary cause of implant-associated infection. The formation of a pathogenic biofilm ensues from the initial adhesion of bacteria to an implant surface. Thus, inhibiting bacterial adhesion is often regarded as the most critical step to preventing implant-associated infection.

Infectious bacteria can be traced to several sources including the ambient atmosphere of the operating room, surgical equipment, clothing worn by medical professionals, resident bacteria on the patient's skin, and bacteria already in the body.[8] Although sterilization and the use of aseptic techniques greatly reduces the levels of bacteria found in hospital settings, pathogenic microorganisms are still found at the site of approximately 90% of all implants.[9] The most common pathogens that cause implant infections include Gram-positive *Staphylococcus aureus* and *Staphylococcus epidermidis*, which are responsible for up to 60% of all prosthetic hip implant infections since 1980.[8] *S. aureus* infections proceed rapidly and are generally more severe than *S. epidermidis* infections. However, *S. epidermidis* has more accessibility as an opportunistic pathogen since it is found ubiquitously on the skin. Other bacteria that have been implicated in implant-associated infections include Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa*, and those from the *Proteus* group (*e.g.*, *P. mirabilis* and *P. vulgaris*).[8]

Due to the fundamental role that bacterial adhesion plays in the development of implant infections, such biofouling has been well documented.[5] The adhesion of bacteria is preceded by the surface adsorption of a conditioning film of small organic compounds and macromolecules including proteins. Subsequently, the physicochemical forces that mediate bacterial adhesion can be divided into two time-dependent phases (Fig. 1).[5] Phase I involves reversible cellular association with the surface over the first 1–2 h post-implantation. This non-specific association is mediated through long (*e.g.*, gravitational, van der Waals, and electrostatic interactions) and short (*e.g.*, hydrogen bonding, dipole–dipole, ionic, and hydrophobic interactions) range forces. Phase II begins approximately 2–3 h later and is characterized by stronger adhesion between the bacteria and the foreign material. Specific chemical reactions between compounds on the cell and substrate surfaces result in irreversible molecular bridging.[10] Both polysaccharides on and adhesin proteins within the bacterial membrane facilitate attachment to substrate surfaces. Beyond Phase II, certain bacterial strains are capable of forming a biofilm if provided with an appropriate supply of nutrients. During biofilm formation, bacteria secrete an exopolysaccharide layer that retains nutrients and protects the microorganisms from the immune response.[5] With the protective polysaccharide coating and sequestered nutrients, bacteria in biofilms exhibit extreme resistance to antibiotics. In some cases, it has been found that killing bacteria in a biofilm requires roughly 1000 times the antibiotic dose necessary to achieve the same results in a suspension of cells.[11]

The tremendous resistance of biofilms to conventional antibiotic therapy has prompted a great deal of research on synthetic surfaces and coatings that resist bacterial colonization. Coatings have been developed that reduce bacterial adhesion by altering the physicochemical properties of the substrate so that conditioning films do not form and/or bacteria-substrate interactions are not favorable. These coatings are referred to as ''passive'' and include surfaces modified with poly(ethylene glycol),[12] poly(ethylene oxide) brushes,[13] and hydrophilic polyurethanes,[14] for example. Unfortunately, the effectiveness of passive coatings for reducing bacterial adhesion is limited and varies greatly depending on bacterial species. The physicochemical properties of the surface (coating) can be masked by an adsorbed conditioning film, thereby diminishing their effectiveness. A recent alternative approach to reducing bacterial adhesion is based on coatings that actively release antibacterial agents. Such ''active'' coatings have been designed to release high initial fluxes of antibacterial agents during the critical short term post-implantation period (several hours) to inhibit the initial adhesion of bacteria. Continued release beyond this short term period is desirable because protective fibrous capsule formation and tissue

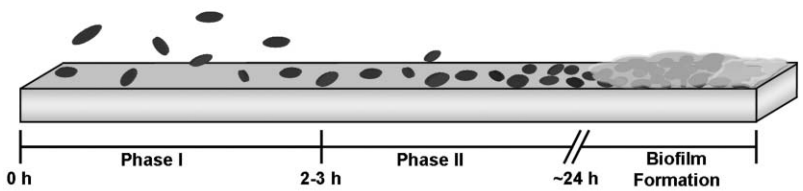


Fig. 1 Representation of bacterial adhesion to a biomaterial substrate. Phase I adhesion involves reversible cellular association with the surface. During Phase II, bacteria undergo irreversible molecular bridging with the substrate through cell surface adhesin compounds. After approximately 1 d, certain bacterial species are capable of secreting a protective exopolysaccharide matrix (biofilm) that protects the adhered bacteria from host defences and systemically-administered antibiotics.

integration occur over a longer time period (weeks to months).[15] Active strategies have been shown to both reduce surface colonization *in vitro* and prevent the formation of biofilms *in vivo*. The preparation and efficacy of several active coatings that show promise for mitigating implant-associated infection are thus the focus of this review.

Controlled release of antibiotics

Perhaps the most direct approach for improving the efficacy of conventional antibiotics against implant-associated biofilms is to deliver the antibiotics in a controlled manner at the implant from a surface coating. The topic of controlled drug release from implanted medical devices was reviewed recently by Wu and Grainger.[16] The primary advantage of delivering antibiotics directly at the site of implantation is that high local doses can be administered without exceeding the systemic toxicity level of the drug. In this fashion, enhanced efficacy can be achieved at the implant site. Localized administration also allows for the tailored selection of antibiotics toward specific pathogens associated with implant infections, circumventing potentially harmful side reactions in other parts of the body. Such delivery also enables long-term antibiotic administration and presumably avoids fostering resistance.[16] Antibiotics that have been used in controlled-release systems include vancomycin, tobramycin, cefamandol, cephalothin, carbenicillin, amoxicillin, and gentamicin.[17,18] The effectiveness of an antibiotic-releasing coating is strongly dependent on the rate and manner in which the drug is released. These properties are determined in part by the matrix into which the antibiotic is doped or loaded. Antibiotic release has been achieved using a wide variety of coatings. Release strategies from polyurethane, biodegradable polymers, and carbonated hydroxyapatite are presented as representative examples.

Controlled antibiotic delivery from polyurethane. The use of biocompatible polymer coatings (*e.g.*, polyurethane, silicone rubber, polyhydroxyalkanoates, *etc.*) that actively release antibiotics represents the first class of local antibiotic delivery strategies.[19,20] The effectiveness of such coatings is strongly dependent on the antibiotic release profile from the polymer, a function of the chemical similarity between the drug and the polymer matrix.[19] Schierholz and colleagues found that using polymers and antibiotics with similar lipophilicity resulted in homogenous drug distribution within the polymer.[19] The distribution of antibiotics (*i.e.*, homogeneity) dictated the antibiotic release rates and the effectiveness of the coatings. Hydrophilic drugs incorporated into hydrophobic polymers leached *via* an initial "burst," followed by substantially lower levels of release at extended periods. For example, ciprofloxacin and fosfomycin (hydrophilic molecules), were released rapidly ($\sim$100 μg cm^{-2} day^{-1}) over the first 24 h from hydrophobic polyurethane, but at significantly lower fluxes at >48 h ($\sim$1 μg cm^{-2} day^{-1}). Conversely, the antibiotic release from drug/polymer coatings that were more similar (*e.g.*, both hydrophobic), was characterized by a less significant burst followed by sustained release for extended periods. For example, lipophilic flucloxacillin was homogenously dispersed in a polyurethane matrix and exhibited a less dramatic initial burst of release ($\sim$25 μg cm^{-2} day^{-1}) and greater overall fluxes over 5 d (10–20 μg cm^{-2} day^{-1}). In terms of efficacy, ciprofloxacin and fosfomycin-loaded coatings exhibited *S. epidermidis* adhesion levels similar to that of control coatings (bare polyurethane), while flucloxacillin release coatings demonstrated near 100% adhesion inhibition of *S. epidermidis* after 72 h in phosphate buffered saline (PBS).[19]

Kwok and co-workers prolonged the antibiotic release from polyurethane coatings by applying an additional thin polymer layer on top of the antibiotic-loaded polymer.[21] The secondary layer served as a barrier to drug diffusion, thereby reducing the initial burst of antibiotic released and extending the overall release duration. The extent of polymeric crosslinking within the barrier layer dictated the kinetics with which the antibiotic was released. A common method of polymer deposition is radio-frequency glow discharge plasma deposition (RF-GDPD). Briefly, RF-GDPD allows the effective deposition of species that polymerize at a substrate surface to form covalently-bound barrier membranes. (The degree of crosslinking is dependent on the operating power of the RF-GDPD apparatus.[21]) Kwok *et al.* employed the RF-GDPD process to deposit a barrier coating of *n*-butyl methacrylate (BMA) to slow the release of ciprofloxacin from poly(ethylene glycol)-doped polyurethane. Uncoated polyurethane films (control) and those coated with BMA at a RF-GDPD power of 5 W both demonstrated an initial burst of antibiotic release ($\sim$0.05 μg cm^{-2} s^{-1}) in the first 24 h followed by a rapid decrease to sub-bactericidal fluxes ($<5.7 \times 10^{-3}$ μg cm^{-2} s^{-1}) at 56 h. Increasing the RF-GDPD power to >5 W increased the extent of crosslinking within the BMA barrier membrane, further reducing the burst effect and lengthening the duration of antibiotic release. In total, BMA membranes deposited at RF-GDPD powers of up to 60 W demonstrated limited initial burst-release ($<$0.03 μg cm^{-2} s^{-1}) followed by sustained delivery of ciprofloxacin at bactericidal fluxes for over 5 d. Deposition at 40 W resulted in the most consistent release rates over the experimental period (Fig. 2).[21]

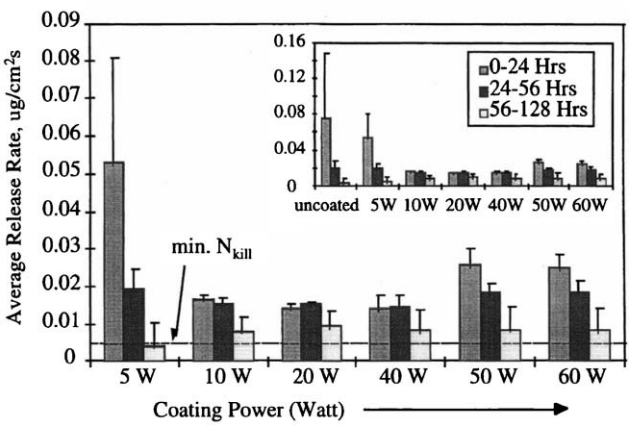


Fig. 2 Average release rates of ciprofloxacin from BMA-coated poly(ethylene oxide)-doped polyurethane as a function of RF-GDPD deposition power. The minimum release rate required to kill adhered *P. aeruginosa* (N_{kill}) is indicated. The inset includes release rates from an uncoated polymer. Reprinted with permission from Kwok *et al.*,[21] *J. Controlled Release*, 1999, **62**, 301. Copyright (1999) Elsevier.

Antibiotic release from biodegradable polymers. While controlled drug release has been demonstrated from non-biodegradable polymers such as polyurethane, silicone rubber, and polymethylmethacrylate (PMMA), certain limitations exist due to their chemical and physical properties. For example, the delivery of gentamicin from PMMA beads was limited to only 7.5% of the loaded amount since most of the drug was not able to diffuse through the pores of the polymer.[18] Non-biodegradable polymer matrices for drug delivery also necessitate an additional surgical procedure for removal. Alternatively, biodegradable coatings have been developed to deliver controlled doses of antibiotics. Such delivery systems include poly(propylenefumarate/methylmethacrylate), collagen, cancellous bone grafts, polyanhydrides, polyorthoesters, and polylactide-*co*-glycolide (PLGA). In general, biodegradable polymers allow for the delivery of higher doses of antibiotic and in the case of PLGA, the degradation products are common metabolites and thus toxicity is less of a concern.[18]

Price and coworkers reported the development of a biodegradable PLGA coating that actively released gentamicin to reduce implant-associated infection.[18] Gentamicin sulfate and PLGA were dissolved in methylene chloride and the resulting solution was deposited on stainless steel fracture plates by a coating/evaporation procedure. The antibiotic release was evaluated *in vitro* for coatings loaded with 10, 20, and 30 wt% gentamicin. The 20 and 30% coatings were both characterized by an initial burst of antibiotic ($\sim$250 μg mL^{-1} and $\sim$2000 μg mL^{-1}, respectively). A burst was not observed with the 10% formulation. The average daily release was determined by initial antibiotic loading levels (10, 20, and 30% mixtures exhibited average daily release of 21 μg mL^{-1}, 133 μg mL^{-1}, and 374 μg mL^{-1}, respectively, over 20 d). For each coating, antibiotic release remained above the minimum inhibitory concentration (1–4 μg mL^{-1}) for common pathogens up to 20 d. An *in vitro* assay demonstrated that 20 wt% gentamicin release coatings reduced bacterial adhesion by >99% over 24 d compared to uncoated controls.[18]

Antibiotic release from hydroxyapatite coatings. The gentamicin-loaded PLGA coatings represent a system capable of maintaining bactericidal antibiotic fluxes for sustained periods despite an initial burst of release, a desirable characteristic because the high dose initially released serves to protect against bacterial adhesion during the most critical period following implantation. Likewise, sustained release, albeit at a lower flux, inhibits bacterial adhesion while the implant is integrated into surrounding tissue. Similar release profiles have also been obtained from carbonated hydroxyapatite (CHA) coatings.[17] Hydroxyapatite (HA) surfaces form strong chemical bonds with bone and have been applied to titanium prostheses as a method for improving long-term implant fixation. These coatings have previously been modified with surface-adsorbed antibiotics by immersion in antibiotic solutions. Although simple, this approach led to rapid antibiotic release, excluding it as a method for preventing implant fouling over long periods. To extend release, Stigter and coworkers loaded antibiotics into a CHA coating *via* a biomimetic co-precipitation process.[17] Titanium alloy plates were immersed in supersaturated solutions of calcium and phosphate containing antibiotics. As HA crystals formed on the implant, antibiotic was deposited as a co-precipitate, resulting in CHA films that ranged in thickness from 8–73 μm. Both acidic (amoxicillin, cefamandol, carbenicillin, and cephalothin) and basic (vancomycin, gentamicin, and tobramycin) antibiotics were evaluated. The acidic antibiotics were incorporated into CHA coatings with greater efficiency than the basic antibiotics because of the calcium-chelating properties of the carboxylate groups.

The chemical structure of the antibiotics also dictated the kinetic release profiles from the antibiotic-loaded CHA coatings. Through calcium–carboxylate chelating interactions, the acidic antibiotics (*e.g.*, cephalothin, Fig. 3A) were retained in the CHA coating to a greater extent relative to the basic antibiotics (*e.g.*, gentamicin, Fig. 3B). While all the antibiotics studied exhibited an initial burst of release, cephalothin release continued from the CHA coating after 16 h. Comparatively, gentamycin exhibited complete release after only 1 h, suggesting that the antibiotic dose and kinetics of release are easily varied as a function of both the chemical structure of the drug and the coating into which it is doped. All antibiotic-loaded CHA coatings demonstrated antibacterial efficacy against two strains of *S. aureus* in agar plate inhibition studies.[17]

Anti-infective silver release coatings

Many antibiotics operate specifically and show limited efficacy against certain bacterial strains. Thus, the controlled release of other antibacterial agents that act more broadly against a wide range of bacteria has been pursued as an alternative strategy for reducing bacterial adhesion. The antibacterial properties of silver have been known for centuries,[22] and while nontoxic to mammalian tissue,[23] the chemical nature of silver affords antibacterial activity in multiple ways. Biomolecules such as proteins, enzymes, and cell-membrane components generally contain nucleophilic sulfhydryl, hydroxyl, and amine functionalities that are capable of coordinating silver cations (Ag^{+}).

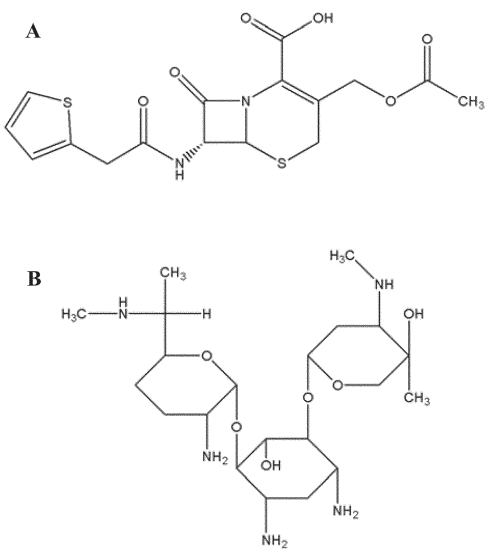


Fig. 3 Structures of A) cephalothin and B) gentamicin.

As a result, Ag^+ reacts with and disrupts the function of bacterial cell membranes and crucial metabolic proteins and enzymes, ultimately leading to cell death. Silver cations also displace other metal ions that are essential to cell survival, including Zn^+ and Ca^{2+}. The bactericidal activity of Ag^+ is thus general and to date, Ag^+ has demonstrated antibacterial efficacy against a broad spectrum of pathogens found at implant sites including *P. aeruginosa*, *E. coli*, *S. aureus*, and *S. epidermidis*. It is also generally accepted that the antibacterial mechanisms of Ag^+ are such that bacteria will not develop resistance.[22] Combined, these characteristics make silver-releasing materials a potential strategy for reducing bacterial adhesion to implanted devices.

Initial studies focused on coating catheters[23] and orthopedic fixation pins[24] with metallic silver. While colonization was significantly reduced for silver-coated catheters *in vitro*,[23] *in vivo* studies with silver-coated fixation pins failed to demonstrate decreased bacterial adhesion.[24] Likewise, Sheehan *et al.* reported that silver coatings applied to model orthopedic implants did not result in decreased *S. epidermidis* and *S. aureus* adhesion.[25] An explanation for the limited antibacterial efficacy of silver coatings is that they do not actively release silver ions. Notably, the antibacterial properties of silver have been attributed to its oxidized form (*i.e.*, Ag^+), a form of silver that is not necessarily present at a surface coated with metallic silver. Polymers that actively release silver in the oxidized state however have exhibited strong antibacterial activity. Such coatings act as reservoirs of silver and are capable of releasing bactericidal levels of Ag^+ for extended periods (>3 months).[26]

Kumar and Munstedt reported on polyamide polymers capable of actively releasing tunable levels of Ag^+.[26] Hygroscopic polyamide matrices were chosen because silver ionization and release are strongly dependent on water uptake. Silver powder was incorporated into the matrix at different weight percentages through a melt-mix process. After solidification upon cooling, Ag^+ release was measured in 0.1 M KNO_3 by anodic stripping voltammetry. Water uptake by the polyamide matrix occurred rapidly over the first three days of immersion. As a direct result, Ag^+ was released in an initial burst over the same period, followed by much slower release from 5–6 d (Fig. 4). An interesting characteristic exhibited by these coatings was that after 6 d, silver ion release again increased to levels equal to or higher than the initial release burst. The coatings continued to release significant levels of Ag^+ after 3 month soak periods.[27] The release longevity was attributed to the interruption of intermolecular hydrogen bonding within the existing polyamide matrix after extended water absorption (>3 d), resulting in increased mobility of the Ag^+ through the plasticized matrix medium.[28] The triphasic release profile of Ag^+ was unique and distinct from the profiles exhibited by other anti-infective coatings. The increasing flux of Ag^+ at longer durations (*i.e.*, $\geqslant$6 d) may allow for increased long-term biocompatibility for implants coated with such polymers.[26]

The flux of Ag^+ from the polyamide polymer was shown to be highly tunable based on polymer crystallinity[28] and the inclusion of various fillers.[27] Decreased matrix crystallinity resulted in greater levels of Ag^+ release, presumably the result

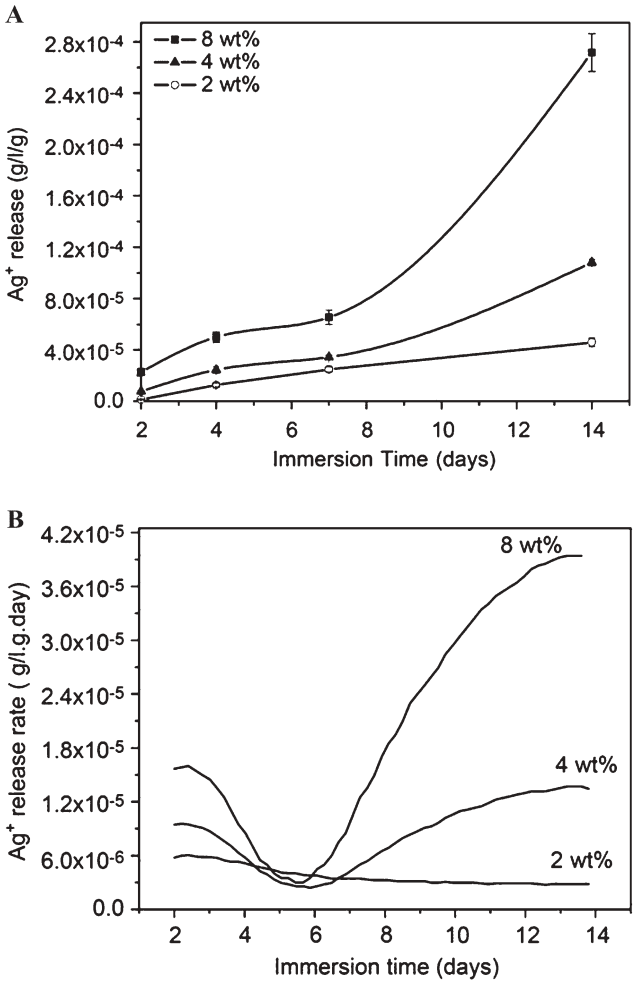


Fig. 4 A) Total Ag^+ release and B) Ag^+ release rate from hygroscopic polyamide matrices at different levels of silver loading. Reprinted with permission from Kumar *et al.*,[26] *Biomaterials*, 2005, **26**, 2081. Copyright (2005) Elsevier.

of increased water uptake. At longer soak durations, this effect was more pronounced. Likewise, silver-based filler composites were shown to release Ag^+ in a controlled manner *via* water uptake by the polymer (polyamide).[27] Hygroscopic fillers resulted in increased water uptake and greater initial levels of Ag^+ release. However, the release of Ag^+ from filler-modified coatings decreased substantially over 3 months. Conversely, Ag^+ release from polymers without filler increased over the same period. The possibility of using a multi-layer coating to maximize the antibacterial efficacy of silver-releasing coatings thus exists. An initial layer incorporating a release-enhancing filler could produce large initial fluxes of Ag^+, while a secondary layer without filler might extend the release of Ag^+ for extended durations (*i.e.*, >3 months).[27]

Recently, Furno and coworkers created antibacterial polymer coatings that released silver particles ranging from 10–100 nm in diameter.[29] Organic silver complexes were dissolved in supercritical carbon dioxide and permeated into medical grade silicone rubber at high pressure. Silver release, as measured by inductively coupled plasma mass spectrometry, was greatest over the first three days, with lower levels of

release following for the next two days. The initial burst of silver was found to be critical with respect to the antibacterial properties of the coatings. To explore if such release originated from silver particles existing at or near the polymer surface, sample coatings were washed copiously after doping to remove surface-bound silver. Indeed, the washed coatings showed no inhibitory zones against *S. epidermidis*, analogous to control polymers that did not contain silver nanoparticles. The antibacterial activity of these coatings was also tested in the presence of a human plasma-derived protein conditioning film at the substrate surface to better mimic *in vivo* conditions. Although Ag^+ generally reacts rapidly with proteins, the bactericidal activity of released Ag nanoparticles was not eliminated in the presence of the *in vitro* protein coating, suggesting that such surfaces would maintain their antibacterial activity upon protein adsorption *in vivo*.[29]

Dowling and coworkers focused on methods to increase the level of Ag^+ delivered from silver surface coatings by also incorporating platinum into the coating.[30] Since platinum is more cathodic than silver in the galvanic series, silver oxidation is enhanced when the two metals are in contact. The authors demonstrated that the addition of 3 wt% Pt to a metallic silver coating increased Ag^+ formation by a factor of two compared to standard silver coatings. As expected, the Pt-doped silver coatings resulted in enhanced antibacterial efficacy due to increased Ag^+ release. *In vitro* experiments demonstrated that inclusion of 1 wt% Pt in a silver coating on polyurethane reduced *S. epidermidis* adhesion by almost two orders of magnitude compared to silver coatings without Pt. Notably, the toxicity of these silver coatings to healthy fibroblast cells was not altered by the inclusion of platinum.[30]

Controlled release of antibodies to prevent bacterial adhesion

The general therapeutic benefits of bioactive antibodies and strategies for their controlled release were recently reviewed.[31] Immunotherapy (*i.e.*, clinical delivery of externally-derived antibodies) has been an effective treatment since the 1800s, exploiting the high specificity of antibody–antigen interactions to achieve therapeutic efficacy for patients with various medical conditions. Additional advantages of immunotherapy include protein stability *in vivo* and the wide range of antigens that pooled antibodies acquired from human donors are able to recognize. For example, polyclonal antibodies purified from serum have been administered to patients suffering from AIDS and other immune deficiency disorders. Other applications of immunotherapy in clinical trials include the treatment of osteoporosis, stroke, arthritis, transplant rejection, and bacterial infections.[31] Of the five major classes of human antibodies (*i.e.*, IgG, IgA, IgD, IgE, and IgM), IgG antibodies have proven to be the most useful as immunotherapeutics. The advantages of IgG include affordable antibody expression and purification, high antigen specificity, and relatively rapid aqueous diffusion.[31] IgG is also directly involved in the natural immune response to infection through opsonization and phagocytosis. Pooled IgG antibodies possess specificity for a wide variety of epitopes expressed on bacteria cell surfaces. Through opsonization (*i.e.*, antibody recognition of and binding to bacterial cell-surface antigens), IgG binds to invading bacteria cells, targeting them for phagocytic destruction by immune system components including neutrophils, monocytes, and macrophages (Fig. 5). IgG opsonization inhibits bacterial adhesion by blocking cell-surface attachment factors and altering the surface hydrophobicity of the cell.[32] The motility of flagellar bacteria such as *E. coli* is also reduced upon opsonization, thereby inhibiting their mechanism of transport.[33] Due to these characteristics, treatment with exogenously-supplied IgG has been shown to diminish the severity of infections and reduce bacterial adhesion to model surfaces.[31]

IgG-mediated reduction of *P. aeruginosa* adhesion to glass substrates has been demonstrated *in vitro* in a parallel-plate flow chamber.[32] After culturing in nutrient broth, *P. aeruginosa* was resuspended in phosphate buffered saline (PBS) with

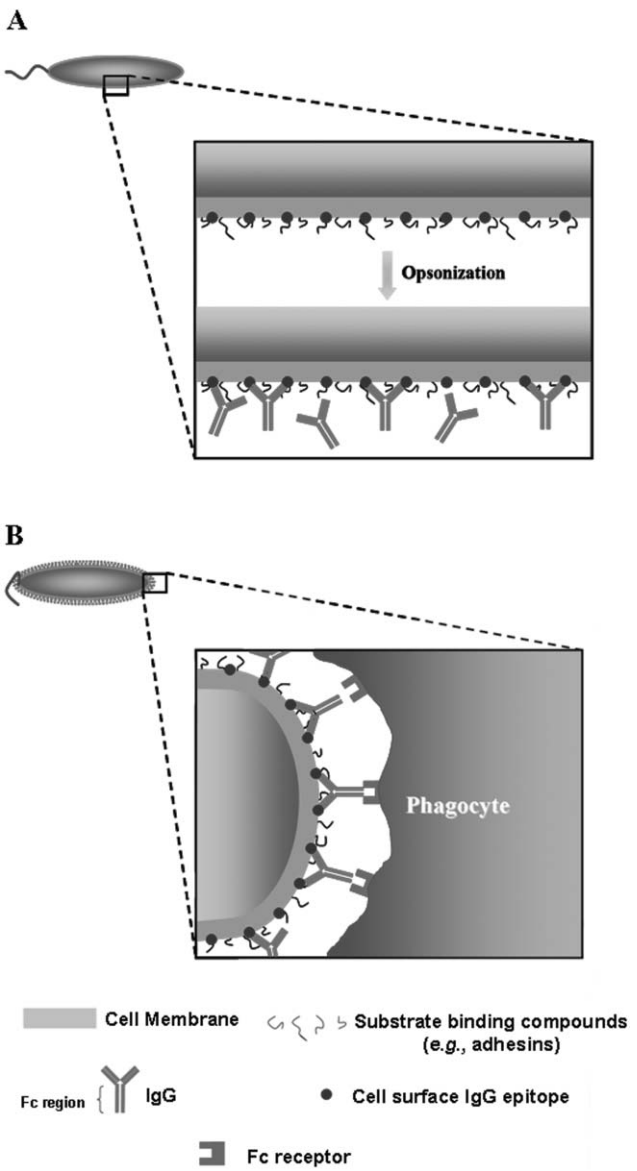


Fig. 5 A) Opsonization of bacteria *via* IgG binding to specific cell-surface epitopes, thereby blocking adhesin compounds. B) Opsonized bacteria are targeted to phagocytes for destruction.

0.2 wt% pooled polyclonal IgG. Bacterial adhesion was monitored in real time with a phase-contrast microscope to determine the percent surface coverage of bacteria. After 2 h, controls (bacteria not exposed to IgG) were characterized by 33% bacterial coverage on the glass substrate in comparison to only 15% coverage for the preopsonized bacteria. Thus, polyclonal IgG was shown to reduce the initial adhesion of *P. aeruginosa* to a model surface (glass) by half.[32] Similar results were observed for soft contact lenses immersed in a suspension of *P. aeruginosa* and pooled polyclonal IgG.[34] Reduced bacterial adhesion was not observed when the bacteria were incubated with serum albumin instead of IgG, indicating that general protein–protein interactions were not responsible for the observed decrease. Combined, the *in vitro* studies suggest that IgG opsonization reduces bacterial adhesion to surfaces and that a similar therapy may mitigate implant-associated infection *in vivo*. To study this hypothesis, Poelstra *et al.* applied pooled human IgG at the site of a spinal implant in a rabbit surgical model.[35] The implant site was challenged with exogenously administered methicillin-resistant *S. aureus*, with blind application of either 1 wt% IgG solution or sterile saline as a control. The wound sites exposed to IgG lavage showed significantly reduced bacteria levels. After 7 d, countable bacteria were found at 75% of saline-lavaged sites (controls) while only 54% of IgG-lavaged sites exhibited bacteria. At longer times, however, no significant difference was observed in infection rate between IgG application and controls. After 28 d, 89% and 85% of wound sites showed no bacterial infection after IgG and saline lavages, respectively.[35]

While the above experiments demonstrated that IgG treatment reduced bacterial adhesion, the antibody solutions were applied externally to the bacterial suspensions and exogenously introduced at the implant site. An alternative strategy involves the spontaneous release of antibodies at the implant site. Rojas *et al.* developed a polyurethane coating that controllably released bioactive IgG for up to 25 h.[33] Lyophilized-pooled human IgG was homogenously dispersed in biomedical grade polyurethane solution (Hypol G-50™ polymer in anhydrous isopropanol) and applied to latex tubing substrates through a dip-coating process. Following curing at 40 °C, antibody release from the coatings was measured in PBS *via* an enzyme-linked immunosorbent assay (ELISA). As with other controlled release coatings, IgG was released in an initial burst over the first 7 h, with lower levels of release through 25 h. The total antibody flux was largely dependent on the initial loading levels. For example, IgG loaded at 15% (w/w in polyurethane) released roughly 50 μg cm^{-2}, while coatings loaded with 10% IgG released approximately 17 μg cm^{-2}.[33] The anti-bacterial adhesion efficacy of the IgG release coatings was then evaluated *in vitro*. *E. coli* adhesion to IgG-releasing polyurethane coatings was reduced by two orders of magnitude compared to blanks after incubation in bacteria suspensions for 4, 8, and 24 h. The authors attributed the reduction in adhesion to steric blockage of bacterial attachment factors and decreased flagellar motility as a result of IgG opsonization. Of note, coatings that released IgG had lower levels of bacterial colonization than blank polyurethane coatings exposed to suspensions of *E. coli* pre-treated with IgG. Polymer-released IgG also maintained its ability to direct phagocytic killing of bacteria. An *in vitro* assay using blood neutrophils showed that IgG released from polyurethane coatings killed 70–90% of *E. coli* cells, compared to <10% for control surfaces. These results were attributed to the IgG-initiated targeting of bacteria to neutrophils (*via* opsonization) for destruction. Antibody released from the polyurethane coatings resulted in greater bacterial killing than aqueous phase IgG added to a bacterial suspension with blank polyurethane. This further substantiates the suggestion that IgG released from a polymer coating is more successful at mitigating implant-associated infection than exogenously-administered antibody treatments.[33]

While examples of the *in vivo* efficacy of antibody-releasing implant coatings are limited, the controlled release of IgG from a hydrogel matrix was shown to reduce implant infection in a mouse model.[7] Carboxymethylcellulose (CMC) was chosen as the delivery matrix (hydrogel) for pooled polyclonal IgG. A formulation of 2 wt% IgG in the hydrogel showed burst-release, with 90% of the IgG released over the first 9 h. Lower levels of IgG were released over the following 21 h, at which time nearly all of the loaded IgG had been released. When applied at the site of a polypropylene mesh implant in the abdominal cavity of female mice, the hydrogel significantly increased the survival rate of mice challenged with *P. aeruginosa*. For example, mice that received a blank CMC hydrogel (*i.e.*, without antibody) that were inoculated with *P. aeruginosa* (strain IFO 3455) had a 0% survival rate after 10 d. However, 70% of mice provided with IgG-releasing CMC hydrogel survived over the same period. With a different strain of *P. aeruginosa* (M-2), mice with blank CMC had a 20% survival rate, while those receiving the IgG-releasing hydrogel had a 100% survival rate. The effect against Gram-positive methicillin-resistant *S. aureus* (MRSA) infection was less dramatic. The survival rate of mice provided with IgG-loaded CMC hydrogel was 0% after 10 d for MRSA, the same rate observed for mice given blanks. When the IgG-releasing CMC hydrogel was combined with 400 μg of the conventional antibiotic cefazolin administered systemically, the survival rate increased to 25% (400 μg of cefazolin alone resulted in 0% survival). The enhanced survival rate indicates that while local antibody delivery may not be entirely effective at eliminating certain implant-associated infections, combination treatments involving locally delivered antibodies and systemically administered antibiotics may reduce the severity of infection and increase patient survival rate. Such effect was found to be antibiotic-specific, however, as joint treatment with systemic vancomycin and local IgG delivery resulted in the same 10 d survival rate as vancomycin and blank CMC hydrogels.[7]

Nitric oxide release coatings

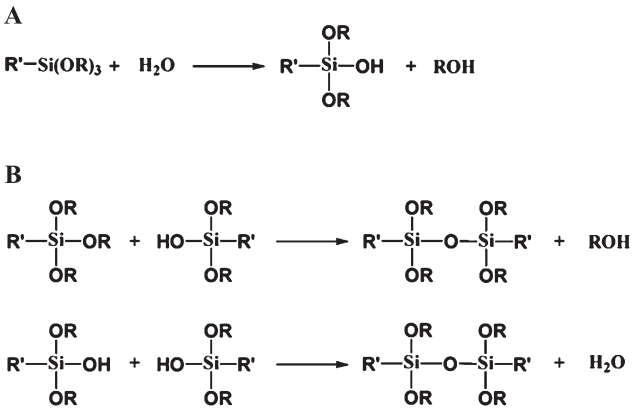
Nitric oxide (NO), a diatomic free radical, serves multiple bioregulatory functions in the cardiovascular, respiratory, gastrointestinal, and nervous systems.[36] Nitric oxide is produced naturally in the body by several sources, including endothelial cells, which generate NO at a flux of 1.67 pmol s^{-1} cm^{-2} as an anti-thrombogenic mediator to regulate vasodilation and platelet activation.[37] Since NO is endogenously produced and has a short half life in biological milieu (on the

order of seconds), NO-releasing polymers have been synthesized as coatings to improve the biocompatibility of implanted devices such as intravascular sensors[38] and extracorporeal tubing.[39] Advances in the development of thromboresistant coatings for the improved biocompatibility of blood-contacting devices *via* NO release have been reviewed recently.[40]

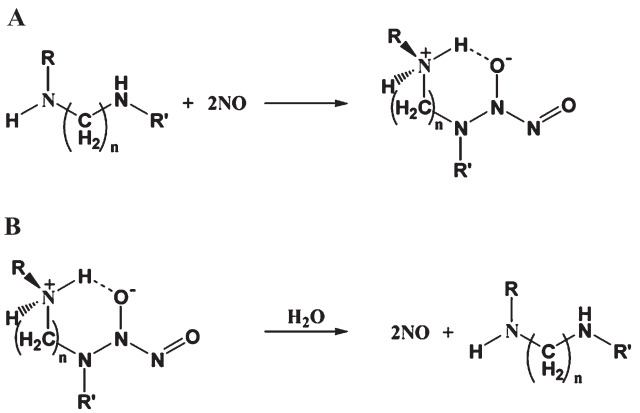
Nitric oxide also plays an important role in the immune response as a potent antimicrobial agent.[41] Bacterial infection and the presence of cytokines stimulate macrophages to produce NO, which is a strong oxidizing agent and likely induces oxidative stress both directly and indirectly through a multitude of reactive intermediates. The most lethal of these reactive species is peroxynitrite ($^{-}$OONO), which is formed *via* the reaction between NO and superoxide (also produced by macrophages). Peroxynitrite has been implicated in cell membrane damage through lipid peroxidation.[42] Nitric oxide is also capable of targeting important structures within bacteria cells, including DNA and proteins, after diffusion across their cell membranes. Oxidation of DNA by NO directly or by reactive intermediates is capable of irreparable damage by breaking the DNA strand. Nitric oxide can also nitrosate tyrosine and cysteine residues of proteins, primarily through its reaction products (*e.g.*, $N_2O_3^{-}$ and $N_2O_4^{-}$). Nitrosation of even one amino acid residue is capable of altering protein function.[43]

Raulli *et al.* demonstrated the bactericidal effects of NO *via* solution-based antibacterial assays.[44] Diethylenetriamine was modified to a diazeniumdiolate NO donor form (DETA/NO). In general, diazeniumdiolate NO donors are readily synthesized and release NO in a predictable fashion. The authors reported the efficacy of DETA/NO against a range of Gram-positive and Gram-negative species including those relevant to implant infections. The minimum inhibitory concentration (MIC) of DETA/NO was found to be lower for Gram-positive species compared to Gram-negative species.

The solution-based antibacterial activity of diazeniumdiolates suggests that NO releasing surfaces would be effective at actively resisting bacterial adhesion. Initially, NO release from surface coatings was established by doping diazeniumdiolate small molecules into hydrophobic polymers.[38] However, the NO-donor was found to leach from the polymer,[45] leading to concerns over the potential toxicity of such coatings. As a solution, Marxer *et al.* covalently attached diazeniumdiolates to the backbone of sol–gel derived (*i.e.*, xerogel) polymers[36] to limit the leaching of amine decomposition products. The NO-releasing xerogel coatings were synthesized by reacting alkoxysilanes (*e.g.*, *iso*-butyltrimethoxysilane [BTMOS]) with aminosilanes (*e.g.*, (3-trimethoxysilylpropyl)-ethylenetriamine [DET3] and *N*-(6-aminohexyl)-aminopropyltrimethoxysilane [AHAP3]) through the sol–gel process (Scheme 1), thereby creating a crosslinked glass-like polymer with covalently linked NO donor precursors (amines) throughout the matrix.[36] Exposure to high pressures of NO ($\sim$5 atm) facilitated the synthesis of diazeniumdiolate NO-donors at secondary amines throughout the xerogel (Scheme 2A). In the presence of a proton source such as water or buffer, diazeniumdiolates spontaneously decompose to yield two equivalents of NO and the parent amine precursor (Scheme 2B). The flux of NO from the xerogel coatings was found to be highly tunable from



Scheme 1 A) Hydrolysis of silane precursors and B) subsequent condensation to form a xerogel polymer where R is typically a methyl or ethyl group and R′ is the amine-containing NO-donor precursor.



Scheme 2 A) Reaction of NO with amines to produce diazeniumdiolate NO-donors and B) subsequent diazeniumdiolate decomposition and NO release in the presence of water.

1–60 pmol s^{-1} cm^{-2} based on the identity (structure) and amount of aminosilane precursor in the xerogel formulation. The NO release profiles for most formulations exhibited trends similar to other controlled-release coatings discussed herein, with the maximal flux occurring soon after immersion in buffer, followed by a gradual decrease in NO release over time. Of note, the NO release from certain xerogel formulations was maintained at relatively consistent levels through 24 h. For example, a 40 v/v% DET3/BTMOS coating initially released $\sim$60 pmol s^{-1} cm^{-2}, a flux that diminished to only $\sim$50 pmol s^{-1} cm^{-2} after 24 h. The duration of NO release was also found to be dependent on the identity and amount of the aminosilane: 40% AHAP3/BTMOS coatings released detectable quantities of NO up to 20 d.[36] Unfortunately, the maximum aminosilane concentration for the xerogels was limited by xerogel stability. As measured by direct current plasma optical emission spectroscopy, aminosilane concentrations above 40% (v/v) led to polymer fragmentation at extended soak periods.[36]

The effectiveness of NO release from xerogel films to bacterial adhesion was also investigated.[9,46] Nitric

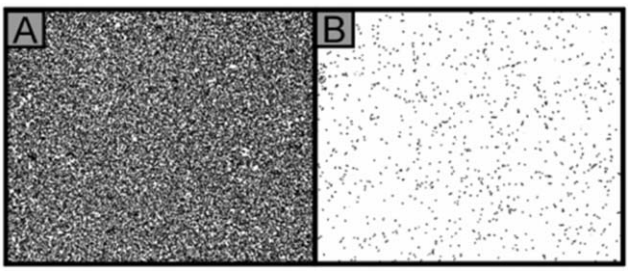


Fig. 6 Phase-contrast optical micrographs (870 × 430 μm) of bacterial adhesion to control (A) and NO-releasing (B) 40% AHAP3/BTMOS xerogels. Bacteria appear black. Reprinted with permission from Marxer *et al.*,[36] *Chem. Mater.*, 2003, **15**, 4193. Copyright (2003) American Chemical Society.

oxide-releasing xerogels were cast onto glass microscope slides and immersed in suspensions of *P. aeruginosa*.[46] Phase-contrast optical microscopy analysis revealed extensive bacterial adhesion at control surfaces (*i.e.*, those that did not release NO) after a 30 minute incubation period. In some instances, clusters of bacteria (cells) were observed, representing possible nucleation sites for biofilm formation. In contrast, NO-releasing surfaces (1–20 pmol s^{-1} cm^{-2}) exhibited significantly reduced levels of *P. aeruginosa* adhesion (Fig. 6). Bacteria present at the surface of NO release coatings were generally dispersed with minimal indication of biofilm nucleation sites.

Nablo *et al.* modified stainless-steel substrates with xerogels to assess their efficacy as antibacterial coatings for orthopedic implant applications.[9] Nitric oxide releasing xerogel-coated stainless steel was shown to significantly reduce *P. aeruginosa* adhesion compared to control (xerogel) and blank (bare steel) surfaces. A similar significant NO-mediated reduction was observed for *S. aureus* and *S. epidermidis* (both Gram-positive), although both adhered to controls at lower levels than *P. aeruginosa*. The results from these studies suggest that NO release polymers represents a new strategy for reducing the surface adhesion of medically-relevant Gram-negative and Gram-positive opportunistic pathogens.

To understand the level of NO necessary to reduce bacterial adhesion, Nablo *et al.* synthesized xerogels of variable aminosilane compositions and NO release characteristics.[47] Poly(vinyl chloride) coatings were applied over the xerogels to ensure consistent surface properties across samples. As shown in Fig. 7, *P. aeruginosa* adhesion was reduced in a linear manner with increasing NO release to ~20 pmol s^{-1} cm^{-2} (75% adhesion reduction). At a flux >20 pmol s^{-1} cm^{-2}, NO had diminished effectiveness at further reducing adhesion.[47] The authors thus concluded that xerogels releasing NO at fluxes >20 pmol s^{-1} cm^{-2} exhibited the greatest anti-adhesion efficacy against *P. aeruginosa*. Of note, the *in vivo* response may be different since the diffusion of NO and its by-products is faster in tissue, thereby reducing the resident concentrations of NO.

The *in vivo* efficacy of NO-releasing coatings against implant-associated infection was subsequently studied.[48] Xerogel coatings (40% AHAP3/BTMOS) were applied to medical grade silicone rubber *via* a dip coating process. Following sterilization, control and NO-releasing implants

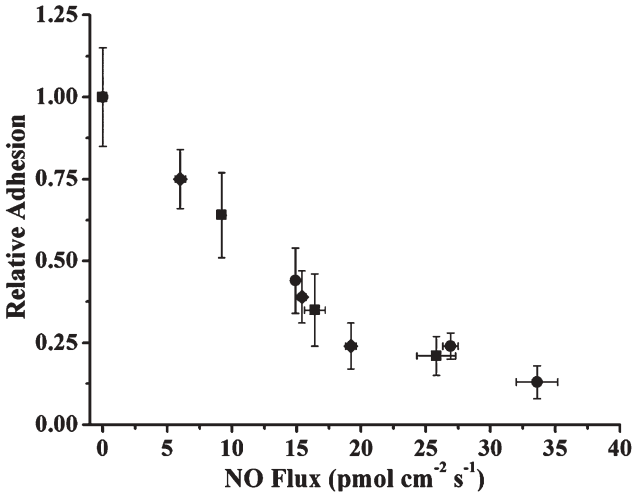


Fig. 7 The influence of NO flux on *P. aeruginosa* adhesion to PVC-coated surfaces. Reprinted with permission from Nablo *et al.*,[47] *Biomacromolecules*, 2004, **5**, 2034. Copyright (2004) American Chemical Society.

were placed subcutaneously in adult male rats and the implant sites were challenged with *S. aureus*. After 8 d, the tissue surrounding each implant was analyzed for infection. Bacteria were present in 11 of the 15 tissue samples surrounding the control implants. In contrast, bacteria were found in only 2 of the 15 samples surrounding the NO-releasing implants, indicating that sustained NO release aids natural defence mechanisms to help clear bacteria and prevent implant-associated infection. Indeed, histological analysis revealed the formation of *S. aureus* biofilms at the sites of uncoated control implants. Such biofilms were not observed at the NO-releasing implants. The tissue surrounding NO-releasing implants appeared similar to that adjacent to uninfected controls.

Conclusions

With an aging population that is increasingly active, biomedical implant use will continue to rise. Consequently, the threat posed by implant-associated infections will affect a larger percentage of the populace. To combat this threat, research must focus on strategies that actively reduce bacterial adhesion and prevent biofilm formation. Coatings that incorporate agents with direct antibacterial activity (*i.e.*, antibiotics, silver ions, and nitric oxide), as well as those with indirect anti-adhesion and antibacterial effects (*i.e.*, antibodies), have proven effective at reducing bacterial adhesion *in vitro* and, in some cases, lessening the effects of implant associated infection *in vivo*. Future research should aim at increasing the antibacterial efficacy of such coatings, possibly through the combination of two or more active strategies, or by coupling suitable active and passive approaches. A major concern involving these coatings that remains uncertain is their effect on healthy tissue and cells in addition to bacteria. Such studies are necessary for the development of clinically useful coatings for reducing implant-associated infection.

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