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Antibiofilm Approaches: Prevention of Catheter Colonization

Review

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Bacteria frequently attach to medical devices such as intravascular catheters by forming sessile multicellular communities known as biofilms, which can be the source of persistent infections that are recalcitrant to systemic antibiotic therapy. As a result of this persistence, a number of technologies have been developed to prevent catheter-associated biofilm formation. Whereas the most straightforward approaches focus on impregnating catheter material with classical antimicrobial agents, these approaches are not universally effective, thereby underscoring the need for more potent and more sophisticated approaches to the prevention of catheter-related biofilm infections.

Introduction

Medical devices such as intravascular and urinary catheters are routinely employed in healthcare settings for a number of purposes, including the infusion of chemotherapeutic agents, hemodialysis, and the treatment of urinary incontinence [1–3]. Although these devices are essential components of the modern-day medical armament, they are also highly susceptible to microbial contamination. Microbial pathogens attach to catheter surfaces, forming sessile multicellular biofilm communities that will often persist in the presence of large doses of traditional antimicrobial agents [4].

A number of factors conspire to render catheter implants especially susceptible to microbial contamination. First, catheter implantation often compromises the skin's protective barrier, providing a direct route to bypass the body's first line of immunity. In addition, upon insertion into the host, the outer surface of the catheter is quickly covered with host proteins that facilitate microbial attachment [5, 6]. There is also evidence that implanted abiotic material itself causes local attenuation of antimicrobial immune responses, thereby providing a fertile breeding ground for microbial biofilm formation [7]. Finally, patients who possess the greatest need for implanted medical devices are often immunocompromised and are therefore more susceptible to bacterial infection [8].

The catheters themselves are infected via one of two general routes, typically by organisms that comprise the natural flora surrounding the site of catheter insertion (Table 1). First, microbes may contaminate the catheter along its outer surface, and it is believed that this type of infection often occurs during initial insertion of the catheter as microbes track along with the catheter as it tunnels to its appropriate destination [9]. Catheters can also be contaminated in their lumenal compartments where fluid flow from contaminated infusate solutions can provide microbial pathogens rapid access to the vasculature [10]. In either of these scenarios, establishment of a catheter-associated biofilm is a natural progressive step after initial colonization. The contaminating biofilm then serves as a growing, often antibiotic-resistant reservoir that seeds infection throughout the host.

Consequently, catheter-related bloodstream infections are notoriously difficult to treat via conventional antibiotic therapy, with associated mortality rates ranging from 12% to 25% [11, 12]. Indeed, the removal of microbially contaminated catheters is often the only viable remedy. These unsatisfying treatment regimens extend hospital stays, necessitate active intervention on the part of healthcare personnel [13], and have driven the estimated annual domestic healthcare cost associated with complications arising from these catheter-related biofilm infections to more than nine billion dollars [11, 12, 14]. To address this problem, various technologies have been developed, or are being developed, to prevent biofilm formation on medical devices, with each effort possessing its own particular constellation of potential pitfalls and advantages. These efforts can be broadly classified into two areas: (1) prevention of biofilm formation with bactericidal or bacteriostatic agents, and (2) prevention of biofilm formation with nonbactericidal antibiofilm agents that inhibit the microbial attachment process.

Current Therapies: Bactericidal and Bacteriostatic Approaches

Conceptually, the simplest method for preventing bacterial colonization and eventual biofilm formation on catheters is to impregnate the catheter itself with a broad-spectrum antimicrobial agent that elutes from the device and impairs bacterial growth through traditional bactericidal or bacteriostatic mechanisms. Here, the antimicrobials are used prophylactically, preventing biofilm formation by eradicating even the first microbial pathogens to contaminate the device. This general approach is also the one that has progressed furthest in clinical development, with some antimicrobial-impregnated devices currently used in clinical settings [15–20] and others in various stages of development [21–27].

This approach, however, is not without its technical hurdles. Care must be taken to ensure that impregnation of the medical device does not alter its desired physicochemical properties. Catheters have desired degrees of lubriciousness, persistence length, and compatibility with host tissue that cannot be radically altered without impairing their utility. In addition, each device must be loaded with enough of the antimicrobial agent in question such that the catheter releases its antimicrobial payload at bactericidal or bacteriostatic concentrations for the lifetime of the device.

This is a nontrivial demand that has been addressed through different approaches with varying degrees of

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Table 1. Microbial Spectrum and Infection Rates of Implanted Catheters

	Microbial Spectrum of Specific Catheter-Related Infections	Range of Device Infection Rate (Percentage Likelihood of Infection over Lifetime of Device)
Catheter-associated urinary tract infections (urethral catheters)	*Candida spp*. (31%) *Escherichia coli* (19%) *Enterococcus spp*. (14%) *Pseudomonas aeruginosa* (10%) other gram-negative bacilli (10%) *Klebsiella spp*. (9%) coagulase-negative Staphylococci (3%) *Staphylococcus aureus* (1%)	10%–50%
Peritoneal dialysis-related peritonitis (peritoneal dialysis catheters)	coagulase-negative Staphylococci (CNS) (30%–40%) *S. aureus* (10%–20%) *Streptococcus spp*. (10%–15%) *E. coli* (5%–10%) other gram-negative bacilli (7%–16%) *Pseudomonas spp*. (5%–10%) fungi (primarily *Candida spp*.) (2%–10%) *Enterococcus spp*. (3%–6%) anaerobes (2%–5%) other (3%–7%) negative culture (10%-20%)	20%–50%
Intravascular device-related bloodstream infections (peripheral venous catheters, arterial catheters, central venous catheters, hemodialysis catheters)	CNS (31%) *S. aureus* (18%) *P. aeruginosa* (18%) enteric gram-negative bacilli (14%) *Candida spp*. (6%) *Corynebacterium spp*. (5%) *Enterococcus spp*. (4%) other (14%)	0.2%–0.4% (for peripheral venous catheters) 18%–22% (for long-term central-venous catheters)

This table is adapted from [10].

success. For example, the polyurethane walls of one commercially available central-venous catheter (CVC) are impregnated with minocycline and rifampin in an effort to ward off microbial contamination [20]. Although the device is clearly effective when compared to uncoated catheters, reducing the frequency of catheter colonization in one study from 26% to 8% and significantly reducing the frequency of catheter-related bloodstream infections [28], the device is still not universally effective at preventing catheter contamination. One additional concern is that the prophylactic use of antibiotics in this setting provides a potential mechanism for increasing the overall proportion of antibiotic-resistant microbes in the nosocomial environment. While there has been little examination of the spread of antibiotic-resistant microbes as a result of the commercially available antibiotic-impregnated catheters, the phenomenon has been observed when other topical antibiotics are employed to prevent bacterial contamination of CVCs and other implants [26, 29].

In a departure from the impregnation approach, DiTizio and colleagues have described a method by which ciprofloxacin was loaded into a liposomal hydrogel, which was then crosslinked to the external (nonlumenal) surface of Foley catheters [23]. These antibiotic-loaded hydrogels were capable of releasing bactericidal doses over 7 days in vitro, but were less effective in a rabbit animal model, delaying the average onset of bacteriuria by only 1.8 days [25].

Other studies have described silicone catheters designed with distinct inner and outer surfaces that sandwich a minocycline/rifampin reservoir capable of releasing an effective antimicrobial dose for almost one year [27]. This particular catheter design, however, has only been examined in vitro.

Although a number of other antibiotics, including ramoplanin, dicloxacillin, clindamycin, and triclosan [21, 22, 30], have been examined for their effectiveness at preventing catheter colonization, the general approach of antimicrobial impregnation of catheters is not solely restricted classical antibiotics. There are many examples of nonspecific antiseptics used for this purpose as well, including silver sulfadiazine, nitrofurazone, chlorhexidine, and the quaternary ammonium species benzalkonium chloride [31–34]. Like the antibiotic-based approaches, the general goal is to harness the broad-spectrum antimicrobial effects of these antiseptics to prevent the colonization of and eventual biofilm formation on catheters. One of the theoretical advantages of these approaches is that the nonspecific antiseptics reach beyond the prokaryotic realm and may help to prevent fungal biofilm contamination of medical devices as well [35].

In general, these approaches are similar to the antibiotic-based approaches as far as their development is concerned, with versions of nitrofurazone-, silver-, chlorhexidine-, and benzalkonium-impregnated catheters commercially available [31–34]. However, whereas nonspecific antiseptic approaches are conceptually promising, the empirical evidence of their efficacy is mixed, with even the most promising results [36–38] highlighting limitations associated with antiseptics used as antibiofilm agents on catheters. For example, in a study by Darouiche and colleagues, CVCs impregnated with a combination of silver-sulfadiazine and chlorhexidine were less effective at preventing catheter-related bloodstream infections than similar antibiotic-impregnated devices [20]. In addition, although some small clinical studies have shown that silver-oxide-coated catheters are associated with a reduction in catheter-associated urinary tract infections (CAUTI) in certain patient subgroups [38], a larger trial [16] was unable to document a statistically significant reduction in the frequency of CAUTI when comparing silver-impregnated versus unimpregnated urinary catheters.

One explanation for the lack of consistent demonstration of efficacy is that the current designs of antiseptic-coated devices such as the silver-sulfadiazine/chlorhexidine CVC do not deliver an appropriate sustained antimicrobial dose for the lifetime of the device. At least two approaches have been devised to address this concern.

In the first approach, Raad and colleagues [39, 40] have described an electrochemical method whereby a silver iontophoretic catheter releases silver ions near the proximal region of a vascular catheter when connected to a low-current power source. This electrochemical approach should provide a more sustainable source of silver ions, preventing microbes that are attached to the distal catheter surface from contaminating the proximal catheter region and the vasculature for longer periods. This device has been studied in vitro and in vivo and appears to be broad spectrum in its efficacy. Although these initial studies are promising, the device has not yet been examined in the clinic.

The second approach focuses on the use of covalent surface modification in an attempt to render catheter surfaces inhospitable to bacterial colonization. Many of these covalent-modification technologies can be viewed as an attempt to permanently affix an antimicrobial agent to the catheter surface, thereby circumventing the drawbacks associated with the transient efficacy of antimicrobial compounds that elute from other catheters [41]. For example, silicone rubber surfaces have been functionalized with 3-(trimethoxysilyl)-propyldimethyloctadecylammonium chloride (QAS), whose antimicrobial activity is similar to the membrane-disruptive function of soluble quaternary ammonium species [41]. Whereas biofilm-inhibitory effects are observed in vitro, the antimicrobial effects of QAS-coated silicone is not broad spectrum, displaying only a modest reduction in the viability of attached gram-negative organisms and showing limited efficacy when rigorously examined in vivo [41]. These observations are not unexpected in light of the surface alterations that take place on all implanted medical devices in vivo. Once implanted in human tissue, medical devices are quickly coated with extracellular matrix proteins and other host-derived biopolymers [42]. As such, it is reasonable to propose that covalently bound quaternary ammonium functional groups will quickly become coated and masked in vivo, neutralizing their antimicrobial function and leaving a fresh surface that is amenable to bacterial colonization and biofilm formation. Thus, unless technologies are developed to enable covalently attached antimicrobial agents to extend physically beyond the outer layers of the host's matrix coating, the elution-based antimicrobial technologies will likely prove superior.

Although antimicrobial-impregnated catheters can reduce catheter-related infection rates in some instances, their potency and ability to ward off microbial biofilm formation as they are currently configured is somewhat limited. Consequently, future efforts will likely aim to increase the local concentrations and sustained delivery of the currently employed antimicrobial agents. While these efforts should have some degree of success, alternative technologies are being developed that should complement the traditional bactericidal and bacteriostatic approaches. These approaches are nonbactericidal in spirit, focusing rather on methods of preventing microbial attachment and preventing the phenotypic hyperresistance changes that accompany biofilm formation.

Potential Future Therapies: Nonbactericidal Antibiofilm Approaches

There are a number of studies indicating that microbial biofilms are able to withstand host immune responses as well as massive doses of a wide spectrum of antimicrobial agents, often persisting in the presence of antimicrobials at concentrations that are 1000-fold more than would be necessary to eradicate an equivalent free-floating, or planktonic, population [43–47]. There appear to be a number of factors that contribute to these hyperresistance phenotypes, not the least of which is that biofilm communities are typically encased in extracellular biopolymeric "slime" that consists of polysaccharide, protein, and in some cases, nucleic acid [48–50]. This extracellular polymeric material can impede both the penetration of antimicrobial agents as well as the function of phagocytic immune cells [51–58]. In addition, biofilm communities are often slow growing and are thus inherently less susceptible to antibiotics that require rapid cell division for efficacy [59, 60]. Finally, a number of proteomic- and genomic-based studies comparing biofilm and planktonic cells have highlighted wholesale alterations in the prokaryotic physiological program when bacteria enter a biofilm mode of growth [61–69]. It is likely that some of these alterations also contribute to the hyper-resistance phenotypes described above.

Given this information, one plausible approach to preventing catheter colonization and catheter-related systemic infections is to develop diffusible catheter-impregnated antibiofilm compounds that render pathogenic microbes incapable of attaching to the catheter surface. Medical devices impregnated with such compounds would abolish the biofilm reservoir that normally seeds persistent systemic infections, rendering invading bacteria susceptible both to the host's immune system and to traditional antimicrobial therapies. While efforts to develop these technologies are still in their early stages, a number of approaches hold promise.

Genetic studies aimed at identifying the molecular components critical to biofilm formation indicate that biofilm formation is a regulated process, with specific adhesins mediating cellular attachment to abiotic surfaces [70–76] and other genetic elements controlling the overall microscopic architecture of the biofilm [77, 78]. Accordingly, one general approach for generating antibiofilm agents is to identify compounds that impair the production or proper assembly of these adhesins.

For gram-positive pathogens like *Staphylococcus aureus* and *Staphylococcus epidermidis*, which are the predominant contaminants of vascular catheters, a critical component of the attachment process is the polysaccharide intercellular adhesin (PIA), whose synthesis is directed by the *ica* gene cluster [70, 72]. This polysaccharide, which consists of repeated N-succinyl-β-1-6-glucosamine subunits, is critical to biofilm formation both in vitro as well as in an infected-CVC animal model [70, 79, 80]. Although previously described anti-infective efforts have focused on PIA as an antigen for vaccine-based therapies [81], the PIA biosynthetic enzymes could serve as useful targets for small molecule antibiofilm agents whose aim is to curb Staphylococcus-derived catheter infections.

In the case of many gram-negative pathogens, the adhesins that mediate attachment-related virulence functions, including biofilm formation, are the surface-exposed multimeric protein appendages termed Type I and Pap pili [74, 82, 83]. Whereas the specific pilin subunits that comprise these pili differ slightly among gram-negative species, the quaternary assembly process appears to be conserved, involving a periplasmic chaperone, PapD, which is essential for proper pilus assembly [84]. As such, one possible method for obtaining antibiofilm agents would be to target this conserved assembly step. This notion is bolstered by the fact that the carboxyl terminus of all Type I and Pap pilin subunits bind to an invariant domain found in all known PapD homologs [85]. Using this information, Svensson et al. have synthesized a series of pyridinone derivatives, termed pilicides, which disrupt the interaction between PapD and pilin subunits by functioning as mimetics of the pilin carboxyl terminus [85]. Although the pilicide mimetics have not yet been examined in vivo, they may ultimately prove useful as targeted antibiofilm agents to prevent gram-negative biofilm formation.

In a similar fashion, a recent study has indicated that lactoferrin, a mammalian protein involved in native immunity, is able to prevent biofilm formation of *Pseudomonas aeruginosa* through an iron-chelation mechanism [86]. The chelation of iron appears to derange the process of pilus-mediated twitching motility, which itself is required for proper biofilm formation in *P. aeruginosa* [73]. Although the effects of lactoferrin on biofilm formation in other species have not been described, this protein or other synthetic iron chelators may also hold promise as general antibiofilm agents.

The adhesion process can also be impaired by compounds that generally perturb the physico-chemical adhesive forces needed for biofilm attachment. With this sentiment in mind, some recent studies have described biosurfactants, including surfactin from *Bacillus subtilis* [87] and surfactants from two Lactobacillus species [88, 89], that inhibit biofilm formation in vitro. While surfactin has only been examined for antibiofilm effects against gram-negative organisms in vitro [87], one surfactant from *Lactobacillus fermentum* was effective at inhibiting Staphylococcal colonization of silicone catheter material in a rat model [89]. Although the lability of these biosurfactants may interfere with their efficacy in applied settings, these compounds may ultimately help in the design of synthetic surfactants that are less prone to

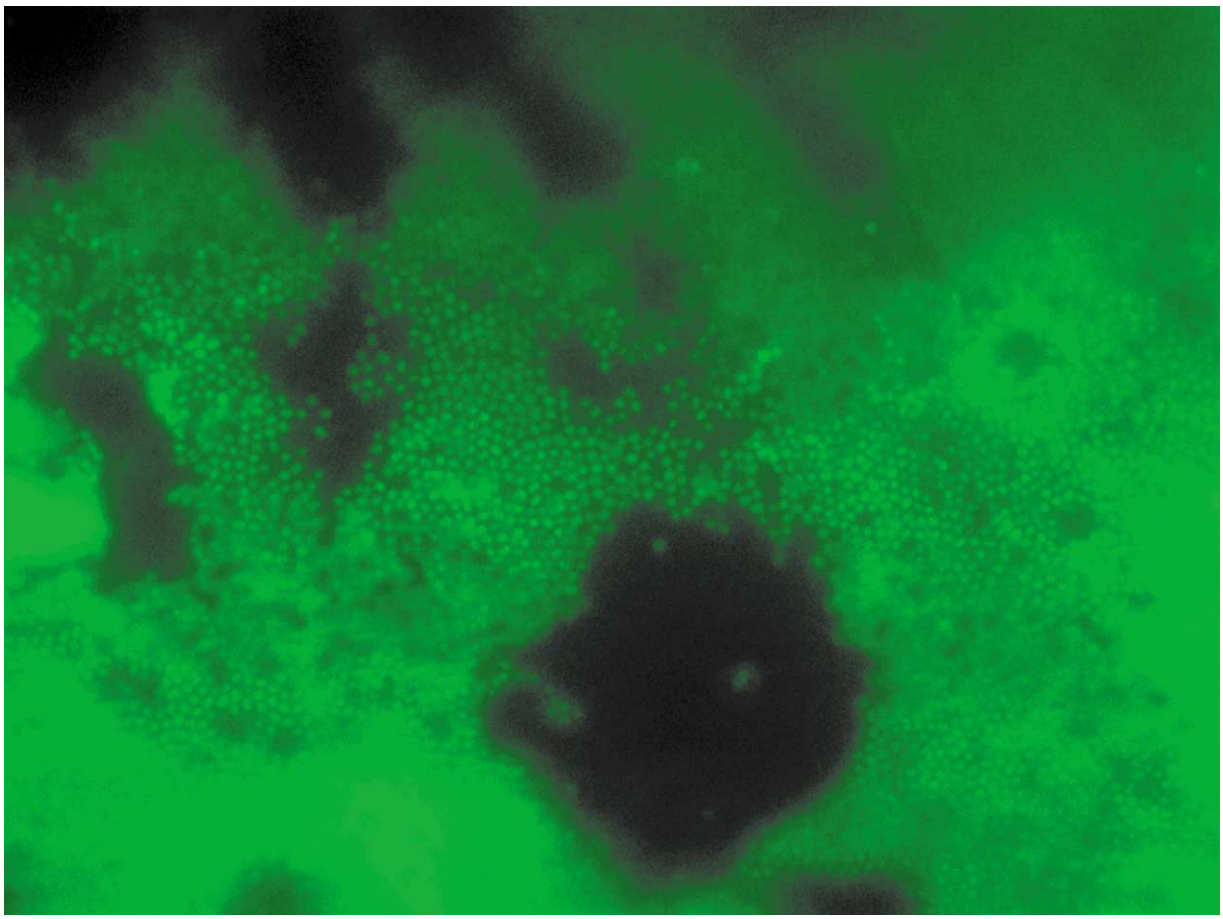

Figure 1. Fluorescence Micrograph of a *Staphylococcus aureus* Biofilm Formed on an Implanted Silicone Catheter after 4 Days of Growth In Vivo

biological turnover and are therefore capable of serving as durable antibiofilm agents.

Traditional definitions of bacterial biofilms are based largely on the microscopic structural features of these communities, which indicate that biofilms often consist of biopolymer-encased microcolonies of cells punctuated by aqueous channels that likely serve to transport nutrients and waste to their appropriate destinations (Figure 1) [90]. Given this high degree of microscopic structure, it is not surprising that a number of studies have documented a role for intercellular signaling molecules in the control of the biofilm formation process [77, 91–97]. For example, *P. aeruginosa* biofilms require the *las* quorum-sensing signaling system, which controls the synthesis of the diffusible intercellular signaling molecule *N*-(3-oxododecanoyl)-L-homoserine lactone to establish appropriate biofilm architecture [77], leading to a suggestion that similar mechanisms are employed in other gram-negative pathogens [98]. The *las* system is one of many bacterial quorum-sensing systems whose roles are to control a variety of physiological functions in response to cell density, including, in some instances, virulence gene expression [99]. Consequently, a number of research efforts have been devoted to identifying and examining compounds that interfere with these signaling systems, with the intention of interfering with the biofilm formation process.

One class of compounds that appears to antagonize certain quorum-sensing systems is the group of natural halogenated furanones produced by *Delisea pulchra* [100–102], a marine alga renowned for its ability to ward off microbial colonization [103]. These naturally produced furanones are at least partly accountable for this phenomenon, as purified synthetic versions of the furanones display antibiofilm effects against *B. subtilis*, *Escherichia coli*, and *P. aeruginosa* in vitro [100, 102, 104]. If these compounds are able to exert their antibiofilm effects broadly across the prokaryotic spectrum, they could ultimately be used to antagonize the biofilm formation process on implanted medical devices.

Conclusions

None of the currently available bactericidal-based technologies is completely effective at preventing microbial colonization of medical catheters. It is, of course, possible and prudent to improve upon the existing bactericidal technologies by combining them in an effort to increase the frequency in which catheter-related bacterial infections are prevented. For example, combinatorial approaches using antiseptic- and antibiotic-impregnated

catheters should provide better protection against microbial contamination than either approach alone. However, this approach does not directly or completely address the central limitations associated with current technologies. The first issue is the rise in acquired, genetic-based resistance of bacteria (in both the planktonic and biofilm modes of growth) to traditional antibiotic treatments, which underscores the need for nonantibiotic catheter-related anti-infective technologies. The second issue also centers on resistance to current antimicrobial therapies—namely, the increased epigenetic antibiotic resistance of biofilm bacteria relative to their planktonic counterparts. Indeed, this second issue may be the central obstacle in effectively treating device-related infections.

One method for addressing both of these issues is to examine more thoroughly the untapped physiology of biofilm microbes. An understanding of biofilm biology should reveal important themes about the mechanisms that bacteria employ for microbial adhesion as well as the mechanisms that sessile communities use to survive the toxic vicissitudes of the external environment. These types of studies should ultimately highlight new screens that are capable of identifying compounds with novel mechanisms of action to antagonize biofilm bacteria in a wide variety of settings. By employing such antibiofilm compounds, microbial pathogens would be rendered more susceptible to both the host's antimicrobial immune responses and to traditional antibiotic therapies.

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