Biofilm Elimination on Intravascular Catheters: Important Considerations for the Infectious Disease Practitioner

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The presence of biofilms on intravascular catheters and their role in catheter-related bloodstream infections is well accepted. The tolerance of catheter-associated biofilm organisms toward systemic antimicrobial treatments and the potential for development of antimicrobial resistance in the health care environment underscores the importance of alternative treatment strategies. Biofilms are microbial communities that exhibit unique characteristics that must be considered when evaluating the potential of biofilm prevention or control strategies. Because biofilm-associated infections do not respond consistently to therapeutically achievable concentrations of many antimicrobial agents, treatments that are more effective against slowly growing biofilm cells or combination treatments that can penetrate the biofilm matrix may be more effective. Alternative strategies that do not incorporate antimicrobial drugs have also been investigated. These approaches have the potential to prevent or eradicate biofilms on indwelling intravascular catheters and prevent or resolve catheter-related infections.

BIOFILMS AND HEALTH CARE–ASSOCIATED INFECTIONS

Intravascular catheters are used for the administration of fluids, medications, parenteral nutrition, and blood products; to monitor hemodynamic status; and to provide hemodialysis [1]. Use of intravascular catheters for patient care may be associated with increased risk of central line–associated bloodstream infection (CLABSI). Approximately 80,000 CLABSIs occur among patients in US intensive care units each year [2], but the estimate is much higher (250,000 cases per year) when data from the entire hospital is included [3]. These infections result in significant morbidity, mortality, and costs for health care delivery. The occurrence of these infections is associated with formation of a microbial biofilm on the device. Microorganisms introduced from the skin of the patient at the catheter insertion site, from a contaminated catheter hub, or from hematogenous seeding of the device can attach to the external and internal surfaces of indwelling intravascular catheters to form a biofilm. Biofilms are sessile microbial communities in which the organisms produce an extracellular polymeric substance (EPS) matrix. The process of biofilm formation is complex and, in the case of intravascular catheters, depends on multiple factors, such as the characteristics of the catheter material, presence of a conditioning film, hydrodynamics, physical and chemical properties of the liquid in contact with the catheter surface, and properties of the microbial cells [4]. It has been reported that biofilms may form within 3 days after catheter insertion [5]. Studies have also shown that biofilm formation is more predominant on the external surface of catheters in place for <10 days; however, with increasing catheter duration (>30 days), biofilm formation in the catheter lumen tends to predominate [6]. After organisms become established in a biofilm, the individual cells exhibit tolerance to antimicrobial agents.

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Because biofilms are considered to be microbial communities, we need to view the individual cells in this community in the context of their interactions not only with the substratum and the external environment but also with the other biofilm-associated microbial cells. This is illustrated by a mixed culture biofilm shown in Figure 1, in which Escherichia coli (shown as yellow-stained cells) were grown as a biofilm for 24 h before introduction of Klebsiella pneumoniae (shown as red-stained cells) and incubated for an additional 24 h. Single cells and microcolonies (clusters of cells) of each organism can be observed. K. pneumoniae cells have attached to the noncolonized substratum and to E. coli cells in the biofilm, suggestive of coaggregation interactions in biofilm formation [11]. Other intercellular interactions may result from cell-to-cell contact, including conjugal gene transfer [12, 13] and quorum sensing [14] in a biofilm. This ecological perspective may illuminate aspects of susceptibility of biofilm-associated organisms to antimicrobial agents, natural mechanisms of attachment and detachment, and virulence expression that cannot be understood by the examination of planktonic (liquid) cultures of biofilm isolates in the laboratory.

Biofilm formation by Pseudomonas aeruginosa has been characterized by 5 distinct developmental stages, beginning with reversible attachment and progressing to dispersion. Cell motility, production of alginate (the biofilm EPS of P. aeruginosa), and quorum sensing by biofilm-associated cells are influenced by the stage of development of the biofilm [15]. Streptococcus pneumoniae also exhibits a sequential biofilm developmental process in which there is a large increase in the number of proteins associated with microbial attachment, resistance, and virulence [16]. It is likely that other microorganisms also exhibit a biofilm development process associated with distinct phenotypes in each developmental phase. This has implications for the susceptibility of these organisms to antimicrobial agents, the host immune system, and biofilm eradication approaches. For example, during the final stage of P. aeruginosa biofilm development, cells become motile and disperse from the biofilm [15]. The protein expression patterns for cells in this stage are more similar to the patterns observed for planktonic cells than to those observed for cells in the immediately preceding developmental stage in the biofilm (termed maturation-2 stage). If, as could be speculated, these dispersed cells also exhibit greater susceptibility to antimicrobial agents, this suggests a novel treatment strategy, in which a signal for dispersion of the biofilm is combined with administration of an antimicrobial agent for killing the dispersed organisms, could be successful.

An important variable that can influence the susceptibility of biofilm-associated organisms is the age of the biofilm [17–19]. Monzon et al [20] reported that increasing age of Staphylococcus epidermidis biofilms was significantly associated with reduced efficacy of several antimicrobial agents, including cephalothin, clindamycin, erythromycin, vancomycin, and teicoplanin. It is possible that increased amounts of EPS produced as a biofilm ages result in nutrient and oxygen gradients, affecting cell metabolism and growth rates and impacting the activity of antimicrobial agents. This suggests that the characteristics of the biofilm determine whether, to what extent, and which systemic antimicrobial treatments are likely to be effective. If this is the case, after a biofilm forms on an intravascular catheter, it will become increasingly more difficult to eliminate the biofilm as it ages.

Biofilm species composition may also influence susceptibility to antimicrobial agents. For example, β-lactamase–positive Moraxella catarrhalis reduced the susceptibility of S. pneumoniae...
to β-lactam antibiotics when the 2 organisms were grown together in a biofilm [21]. A similar protective effect was demonstrated in a mixed bacterial-fungal biofilm composed of *S. epidermidis* and *Candida albicans*; in this case, the staphylococcal extracellular polymer likely protected the yeast cells from azoles, and the yeast cells appeared to reduce the activity of vancomycin against bacterial cells [22].

Particles of nonmicrobial components from the host, such as erythrocytes and fibrin, may accumulate in catheter-associated biofilms and could potentially affect diffusion of antimicrobial agents into the biofilm structure [23]. Figure 2 shows a biofilm of *Alcaligenes xylosoxidans* in which the cells are associated with a fibrin-like matrix that has developed on the surface of an intravascular catheter.

**IMPROVING THE CHANCES OF SUCCESS**

Elimination of planktonic cells in the bloodstream does not imply that the biofilm has been eliminated, because dispersed cells rapidly exhibit increased susceptibility to antimicrobial agents [7]. Drug levels sufficient to kill dispersed cells may be ineffective against biofilm-associated cells. Therefore, the nondetection of organisms in blood cultures or resolution of patient symptoms after a treatment regimen does not imply that the biofilm has been eliminated from the indwelling catheter. Biofilm growth after cessation of antimicrobial treatment, resulting in re-infection of the patient, is a possible outcome in this situation.

The probability of biofilm eradication could be improved by the use of laboratory protocols designed to screen antimicrobial agents against biofilms of the infectious agent. The susceptibility of clinically relevant bloodstream isolates should be evaluated in research studies using an in vitro model system that reasonably simulates the indwelling catheter biofilm with respect to substrate, properties of the growth medium, biofilm age, cell density, and presence of serum proteins. Goeres et al [24], Curtin and Donlan [25], Pierce et al [26], and Ceri et al [27] describe specific testing approaches. If possible, results from the in vitro model system testing should be evaluated under more rigorous conditions using either explanted biofilms as done by Kite et al [28] or animal model systems. It is also important to establish that a proposed treatment regimen will be tolerated by the patient and compatible with the normal-use regimen of the device in a well-designed clinical trial.

**WHICH CURRENT ANTIMICROBIAL TREATMENTS ARE MOST EFFECTIVE?**

Organisms in a biofilm exhibit tolerance to a wide spectrum of antimicrobial agents, but the degree of tolerance to different agents may vary substantially. For example, antimicrobial agents that inhibit cell wall synthesis (eg, glycopeptides) may be less effective, because biofilm organisms exhibit substantially reduced growth rates [29–32]. Agents that penetrate the biofilm matrix, such as rifampin [33] and the fluoroquinolones [34], have been shown to be effective. The macrolides have also been reported to reduce the biofilm EPS and allow greater penetration of other antimicrobial agents [35–38]. There is also a basis for combining agents with differing mechanisms of action. For example, rifampin substantially enhanced the effectiveness of glycopeptides [30, 35, 39, 40] and linezolid [30] against *Staphylococcus* biofilms. Gentamicin significantly reduced the minimum biofilm inhibitory concentration of ampicillin, vancomycin, and linezolid against *Enterococcus* species [31].

An approach for the treatment of biofilms on intravascular catheters is the antimicrobial lock, in which a high concentration of an antimicrobial agent is instilled in the catheter in situ for a sufficient dwell time to prevent colonization and biofilm formation or to eliminate the biofilm. The antimicrobial lock technique (ALT) was first reported by Messing et al [41]. Antimicrobial locks have been used to treat gram-positive, gram-negative, and fungal catheter-associated infections, and the antimicrobial agents chosen for ALT have been based on results of broth microdilution testing of blood culture isolates [42]. Berrington and Gould [43] suggested that bactericidal rather than bacteriostatic agents be used for ALTs and that the highest practical antimicrobial concentration that will not cause patient toxicity if the agent diffuses into the bloodstream should be used [43]. Mermel et al [1] suggested that antimicrobial locks should contain 1–5 mg/mL in a volume sufficient to fill the catheter lumen. A recent review of published reports evaluating the effect of antimicrobial lock treatments in patients indicated that, when high concentrations of the antimicrobial agent (milligram per milliliter range) were used for dwell times of ≥12

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**Figure 2.** Biofilm of *Alcaligenes xylosoxidans* in a fibrin-like matrix on the surface of an explanted intravascular catheter. (Scanning electron microscopic image by Janice Carr, Centers for Disease Control and Prevention [Atlanta].) Image originally published by author in Emerging Infectious Diseases.
h, most bloodstream infections associated with bacterial biofilms on catheters were treated successfully within 14 days [42]. However, for most of these reports, effectiveness of the treatment was based on negative culture results of blood samples collected through the catheter or the absence of clinical symptoms in the patient after completion of therapy rather than on observed presence or absence of biofilms on the catheter, which is the true measure of elimination. One concern with the use of ALT is the potential for toxicity to the patient resulting from the diffusion or inadvertent flushing of the lock solution into the systemic circulation. For example, Dogra et al [44] reported 4 cases of dizziness without vertigo in a study involving 83 patients undergoing hemodialysis who were treated with a gentamicin and citrate antimicrobial lock. This underlines the importance of further studies to investigate the optimal antimicrobial concentration used in ALT. Another concern is the potential for development of antimicrobial resistance. Yahav et al [45] reviewed 11 randomized control trials of ALT containing gentamicin alone or gentamicin combined with other antimicrobial drugs in patients receiving hemodialysis and detected only 1 case of resistance (to gentamicin). In a meta-review of vancomycin ALT by Safdar and Maki [46], vancomycin-resistant organisms were not detected in device-related BSIs and did not colonize intravascular devices in any of the 7 studies reviewed. However, Yahav et al [45] note that this does not preclude the development of resistance to longer and more extensive use of ALT. In this respect, the Healthcare Infection Control Practices Advisory Committee and the Centers for Disease Control and Prevention recommended that the use of ALTs containing vancomycin should be discouraged or used only in special circumstances, such as treatment of patients with long-term cuffed or tunneled catheters or ports who have a history of multiple catheter-related bloodstream infection [47].

ARE THERE ANY NEW OR NOVEL STRATEGIES TO ELIMINATE BIOFILMS ON MEDICAL DEVICES?

There are numerous novel strategies that have been reported in the published literature to control biofilms. Four classes of antibiofilm agents, each with a specific mode of action against biofilm-associated cells, are discussed here. Of these 4 approaches, 2 (chelating agents and ethanol) can be considered to be translational, in that they have been evaluated in patients for the treatment of catheter-associated bloodstream infection.

Chelating Agents

Metal cations, such as calcium, magnesium, and iron, may be involved in maintaining the biofilm structure matrix [48, 49]. Chelating agents may destabilize the biofilm structure [50], and some chelating agents, such as ethylene diaminetetraacetic acid (EDTA), may also have antimicrobial properties against bacteria and fungi [51, 52]. Tetrasodium EDTA or disodium EDTA used alone or in combination with minocycline have been used effectively against bacterial and fungal biofilms. Percival et al [53] and Kite et al [28] found that 40 mg/mL of tetrasodium EDTA could eradicate biofilms in an in vitro model and on explanted hemodialysis catheters, respectively. Brookstaver et al [54] demonstrated significant reductions in biofilms of Staphylococcus species and P. aeruginosa on Hickman catheter segments in vitro with use of combinations of tigecycline plus disodium EDTA and gentamicin plus disodium EDTA. Raad et al [55] demonstrated efficacy of a combination of minocycline and disodium EDTA against biofilms on explanted catheter tips and in an in vitro model system. This disodium EDTA lock was also effective in the treatment of catheter-related bloodstream infections in 3 different patient studies, as evidenced by remission of symptoms and nondetection of organisms by catheter tip culture [51]. Antimicrobial locks containing a combination of minocycline and EDTA have also been evaluated clinically. Chatzinikolaou et al [56] evaluated a minocycline-EDTA lock solution in patients with cancer in a prospective cohort study. There were no port infections or other adverse events in patients treated with the lock solution, compared with 10 infections in the control group. Other clinical studies have demonstrated a reduction in CRBSI in patients receiving hemodialysis after treatment with a minocycline-EDTA lock solution [57–59].

Sodium citrate, another chelating agent, has also been reported to exhibit antimicrobial activity and inhibit biofilm formation by several strains of Staphylococcus aureus and coagulase-negative staphylococci in vitro at concentrations >.5% in the growth medium [60]. The suggested mechanism of inhibition was depletion of cations from the growth medium or removal of essential cations from the bacterial cells. Takla et al [61] found that a combination of 4% trisodium citrate and 30% ethanol prevented biofilm formation by clinical isolates of S. aureus, S. epidermidis, P. aeruginosa, and E. coli for 72 h in vitro. A combination of 7% trisodium citrate, .05% methylene blue, .15% methyl paraben, and .015% propyl paraben exhibited efficacy against preformed biofilms of S. aureus [62]. This treatment combination resulted in substantial structural changes in the biofilm, suggesting potential to eradicate preformed biofilms of this organism from surfaces.

Ethanol

Metcalfe et al [63] reported the resolution of a catheter-related E. coli bloodstream infection by installation of 70% ethanol in the patient’s Hickman catheter in combination with intravenous amoxicillin. The catheter was locked with ethanol between total parenteral nutrition infusions for a 3 days. Sanders et al [64] also reported a significant reduction in catheter-associated...
bloodstream infection in immunosuppressed hematology patients with tunneled cuffed intravascular catheters who were treated with a 70% ethanol lock, compared with the control group.

Recent laboratory studies have provided further support for the ethanol ALT. Qu et al [65] found that 24-h biofilms of *S. epidermidis*, *S. hominis*, and *S. capitis* were completely eradicated by exposure to 20% ethanol for 24 h. Exposure to 40% ethanol for 1 h or 60%–80% ethanol for 1 min completely eradicated the biofilm cells. The authors suggested that the effectiveness of ethanol is attributable to its hydrophilic nature and the small molecular weight, enabling effective penetration of the hydrated biofilm EPS matrix. Balestrino et al [66] found that a 60% ethanol treatment for 30 min completely eradicated 4- and 24-h biofilms of *P. aeruginosa*, *K. pneumonia*, *S. aureus*, *S. epidermidis*, and *C. albicans*. Venkatesh et al [67] demonstrated a significant reduction in biomass and mean biofilm thickness after a 12.5% ethanol treatment of *S. epidermidis* and *C. albicans* biofilms for 24 hours. Raad et al [68] found that 25% ethanol alone was relatively ineffective against biofilms of methicillin-resistant *S. aureus*, but combination with minocycline (3 mg/mL) and EDTA (30 mg/mL) resulted in complete eradication.

However, Slobbe et al [69] found that a 70% ethanol lock treatment did not significantly reduce the incidence of CRBSI in hematology patients with long-term tunneled catheters, compared with patients receiving a placebo. In another study, a 50% ethanol lock was effective against *C. albicans* but ineffective against *S. epidermidis* and *S. aureus* biofilms in a rabbit catheter

Table 1. Examples of Technologies That Do Not Incorporate Antimicrobial Drugs and Have Potential Application for Prevention or Control of Biofilms on Intravascular Catheters

<table>
<thead>
<tr>
<th>Treatment Approach</th>
<th>Mechanism of action</th>
<th>Potential application</th>
<th>Validated in human studies</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chelating agents</td>
<td>Antimicrobial; destabilizes EPS</td>
<td>Lock treatment to remove established biofilm (bacteria and fungi)</td>
<td>Yes</td>
<td>[48, 53, 62]</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Antimicrobial; penetrates EPS</td>
<td>Lock treatment to remove established biofilm (bacteria)</td>
<td>Yes</td>
<td>[63, 66]</td>
</tr>
<tr>
<td>Taurolidine-Citrate</td>
<td>Antimicrobial</td>
<td>Lock treatment to prevent colonization or to remove established biofilm (bacteria)</td>
<td>Yes</td>
<td>[80–82]</td>
</tr>
<tr>
<td>Biofilm dispersant</td>
<td>Disperses cells from the biofilm</td>
<td>Lock treatment to remove established biofilm (bacteria and fungi)</td>
<td>No</td>
<td>[74]</td>
</tr>
<tr>
<td>Bacteriophage</td>
<td>Antimicrobial; degrades EPS</td>
<td>Pretreatment of catheter surface to prevent colonization or lock treatment</td>
<td>No</td>
<td>[25, 78, 79]</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>Releases NO$^p$ from coated surface to augment immune system</td>
<td>Pretreatment of catheter surface to prevent colonization</td>
<td>No</td>
<td>[83]</td>
</tr>
<tr>
<td>GlmU enzyme inhibitor</td>
<td>Antimicrobial; Anti-adhesin</td>
<td>Pretreatment of catheter surface to prevent colonization</td>
<td>No</td>
<td>[84]</td>
</tr>
<tr>
<td>RIP Quorum-Sensing Inhibitor</td>
<td>Inhibits quorum-sensing required for <em>S. aureus</em> biofilm formation</td>
<td>Parenteral injection of treatment to remove established biofilm</td>
<td>No</td>
<td>[85]</td>
</tr>
</tbody>
</table>

NOTE. EPS, extracellular polymeric substance; GlmU, *N*-acetyl-D-glucosamine-1-phosphate acetyltransferase; NO, nitric oxide; RIP, RNAIII-inhibiting peptide.
Biofilm Dispersants
Microbial cells are dispersed from biofilms by shedding of daughter cells during active growth as a result of change in nutrient levels or quorum sensing or by shearing of biofilm aggregates because of flow effects [4]. Treatment of biofilms with oxidizing biocides, such as chlorine, surfactants, or enzymes, can also disrupt the biofilm and lead to cell detachment [72, 73]. Davies and Marques [74] reported that cis-2-decanoic acid (CDA), an unsaturated fatty acid produced by *P. aeruginosa*, could induce dispersion of several clinically relevant, biofilm-associated bacteria and *C. albicans* in vitro. They suggested that release of cells from the biofilm was the result of degradation of the EPS produced by neighboring cells of the same or other species (in the case of polymicrobial biofilms) in response to the presence of the signaling molecule CDA. This treatment approach is designed to remove cells from the surface; additional treatment with bactericidal agents would be required to prevent these detached cells from reattaching to the surface or colonizing the bloodstream and causing a systemic infection.

Bacteriophage
Phage have been used to treat infectious diseases in animals [75] and plants [76]. Phage therapy has also been performed in humans for the treatment of infections caused by *Staphylococcus* species, *Streptococcus* species, *E. coli*, *P. aeruginosa*, *Shigella* species, and *Salmonella* species [77]. During the phage lytic cycle, infection of a single bacterial cell by a phage particle will result in production of multiple progeny phage. Some phage strains also produce polysaccharide depolymerases that can potentially degrade the biofilm EPS. Two recent studies reported the efficacy of a phage-treated hydrogel catheter in preventing biofilm formation by *S. epidermidis* and *P. aeruginosa* [25, 78]. In the study of *P. aeruginosa* biofilm control [78], use of a combination of phages controlled biofilm formation, demonstrated by a significant reduction in the number of colony-forming units on the phage-treated catheter surface (Figure 3), and reduced the incidence of bacterial resistance to the phage treatment. Lu and Collins [79] demonstrated the efficacy of a genetically engineered phage for killing biofilm cells and reducing the biofilm EPS through the action of the phage-associated depolymerase. These results suggest that phage could potentially provide a multi-pronged approach by reducing bacterial attachment, killing biofilm-associated cells after they have attached, and eradicating the biofilm EPS matrix.

Table 1 provides a listing of diverse technologies that do not incorporate antimicrobial drugs and have the potential to prevent or eradicate biofilms on intravascular catheters. With the exception of ethanol, chelating agents, and taurolidine, none of these technologies have yet been evaluated for effectiveness at controlling biofilms on indwelling intravascular catheters. Translating results from laboratory studies to the bedside for the control of intraluminal biofilms will require evaluations in animal models, followed by clinical trials for safety and efficacy in catheterized patients.

CONCLUSIONS
Elimination of biofilms on intravascular catheters is a challenge for the infectious diseases practitioner. Viewing the biofilm as a microbial community is a first step in designing and evaluating effective treatments. Alternative approaches that avoid the use of antimicrobial drugs or combine alternative treatments with antimicrobial drugs have the potential to totally eliminate biofilm formation on the indwelling device, prevent regrowth of the infectious organisms on the catheter, and resolve patient symptoms.

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