Robert A. Weinstein, Section Editor

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

Rodney M. Donlan

Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, Georgia

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 $<$10 days; however, with increasing catheter duration ($\geqslant$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

Received 20 September 2010; accepted 11 January 2011.

Correspondence: Rodney M. Donlan, MD, Div of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Mail Stop C16, 1600 Clifton Rd NE, Atlanta, GA 30333 (rld8@cdc.gov).

Clinical Infectious Diseases 2011;52(8):1038–1045
Published by Oxford University Press on behalf of the Infectious Diseases Society of America 2011.
1058-4838/2011/528-0014$14.00
DOI: 10.1093/cid/cir077

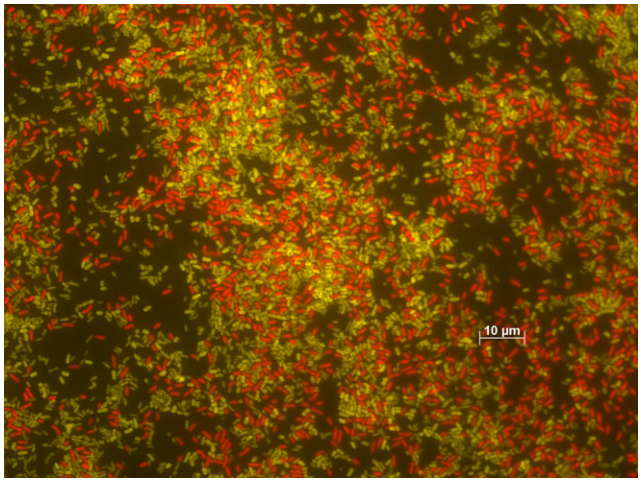

Figure 1. Mixed species biofilm of *Escherichia coli* and *Klebsiella pneumoniae*. Biofilms of *E. coli* 11775 were allowed to form on glass coupons in a CDC Biofilm Reactor containing 10% Trypticase soy broth for 24 h, then inoculated with *K. pneumoniae* 3635 and grown for an additional 24 h. The biofilm was stained with 2 species-specific 16S rRNA fluorescent in situ hybridization probes, each labeled with a different fluorescent dye, visualized using a Zeiss Axioplan epifluorescence microscope with an Axiocam monochrome camera and 63X oil immersion objective, and rendered using Axiovision image analysis software (Carl Zeiss). *E. coli* cells in the image are yellow and *K. pneumoniae* cells are red.

[7] and do not respond consistently to therapeutically achievable concentrations of antimicrobial agents [1, 8]. Biofilm organisms may elicit disease processes by detachment of individual cells or aggregates of cells from the device surface or by production of endotoxins or other pyrogenic substances, and biofilms may provide a niche for the development of antimicrobial-resistant organisms [9]. Biofilm formation on intravascular catheters is best detected by direct examination of the explanted catheter surface with use of a method that uses mechanical forces to recover biofilm-associated microbial cells [10].

CAN WHAT WE KNOW ABOUT THE BIOFILM PROCESS HELP DIRECT CLINICAL DECISION MAKING?

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* cells (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 teichoplanin. 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*

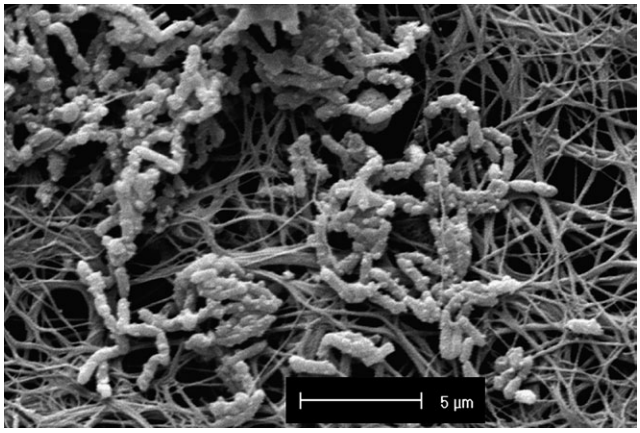

Figure 2. Biofilm of *Alcaligenes xylosoxidans* in a fibrin-like matrix on the surface of an explanted intravascular catheter. (Scanning electron microscopic image by Janice Carr, Centers for Disease Control and Prevention [Atlanta].) Image originally published by author in *Emerging Infectious Diseases*.

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 reinfection 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 substratum, 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 $\geqslant$12

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

Metcalf 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.

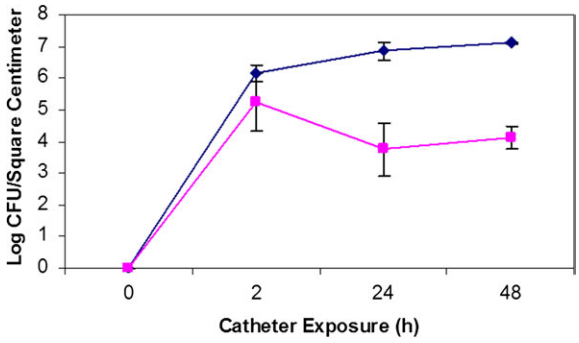

Figure 3. Effect of *Pseudomonas aeruginosa* phage cocktail treatment of hydrogel catheter surface on biofilm formation by *P. aeruginosa* M4 during a 48-hour exposure in a laboratory model system. Closed diamonds, untreated catheter; closed squares, phage-treated catheter. Data are means ± standard deviation ($n = 3$).

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

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

NOTE. EPS, extracellular polymeric substance; GlmU, *N*-acetyl-D-glucosamine-1-phosphate acetyltransferase; NO, nitric oxide; RIP, RNAIII-inhibiting peptide.

model system [70]. Although there is support from clinical studies that the ethanol ALT is effective in reducing CLABSI, Maiefski et al [71] suggest that studies investigating the effect on colonized nonsilicone catheters, using varying dwell times, with or without additional agents are needed. In summary, ethanol can be effective at killing cells in the biofilm and reducing the biofilm structure, but further clinical studies are needed to investigate its effect against biofilms of different organisms on a variety of catheter types using a range of ethanol concentrations, dwell times, and duration.

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 polymicrobic 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.

Acknowledgments

I thank Lisa Hodges and Dr Margaret Williams for assistance with fluorescent in situ hybridization and image acquisition of biofilms shown in Figure 1. The findings and conclusions in this report are those of the author and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Potential conflicts of interest. Author certifies no potential conflicts of interest.

References

1. Mermel LA, Farr BM, Sherertz RJ, Raad II, O'Grady N, Harris JS. Guidelines for the management of intravascular catheter-related infections. Clin Infect Dis **2001**; 32:1249–72.
2. Mermel LA. Prevention of intravascular catheter-related infections (Erratum: Ann Intern Med 2000;133:395). Ann Intern Med **2000**; 132:391–402.
3. Maki DG, Kluger DM, Crnich CJ. The risk of bloodstream infection in adults with different intravascular devices: a systematic review of 200 published prospective studies. Mayo Clin Proc **2006**; 132:391–402.
4. Donlan RM. Biofilms: microbial life on surfaces. Emer Infect Dis **2002**; 8:881–90.
5. Anaissie E, Samonis G, Kontoyiannis D, et al. Role of catheter colonization and infrequent hematogenous seeding in catheter-related infections. Eur J Clin Microbio Infect Dis **1995**; 14:135–7.
6. Raad I, Costerton W, Sabharwal U, Sacilowski M, Anaissie E, Bodey GP. Ultrastructural analysis of indwelling vascular catheters: a quantitative relationship between luminal colonization and duration of placement. J Infect Dis **1993**; 168:400–7.
7. Stewart PS, Mukherjee PK, Ghannoum MA. Biofilm antimicrobial resistance. In: Ghannoum M, O'Toole GA, eds. Microbial biofilms. Washington, DC: ASM Press, 2004;250–68.

8. Marre KA, Sexton DJ, Conlon PJ, Corey GR, Schwab SJ, Kirkland KB. Catheter-related bacteremia and outcome of attempted catheter salvage in patients undergoing hemodialysis. Ann Inter Med **1997**; 127:275–80.
9. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev **2002**; 15:167–93.
10. Donlan RM. Biofilm formation: a clinically relevant microbiological process. Clin Infect Dis **2001**; 33:1387–92.
11. Rickard AH, Gilbert P, High NJ, Kolenbrander PE, Handley PS. Bacterial coaggregation: an integral process in the development of multi-species biofilms. Trends Microbiol **2003**; 11:94–100.
12. Roberts AP, Cheah G, Ready D, Pratten J, Wilson M, Mullany P. Transfer of Tn916-like elements in microcosm dental plaques. Antimicrob Agents Chemother **2001**; 45:2943–6.
13. Ehlers LJ, Bouwer EJ. RP4 plasmid transfer among species of *Pseudomonas* in a biofilm reactor. Wat Sci Tech **1999**; 39:163–71.
14. Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science **1998**; 280:295–8.
15. Sauer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG. *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. J Bacteriol **2002**; 184:1140–54.
16. Allegrucci M, Hu FZ, Shen K, et al. Phenotypic characterization of *Streptococcus pneumoniae* biofilm development. J Bacteriol **2006**; 188:2325–35.
17. Anwar H, Strap JL, Chen K, Costerton JW. Dynamic interactions of biofilms of mucoid *Pseudomonas aeruginosa* with tobramycin and piperacillin. Antimicrob Agents Chemother **1992**; 36:1208–14.
18. Chuard C, Vaudaux P, Waldvogel FA, Lew DP. Susceptibility of *Staphylococcus aureus* growing on fibronectin-coated surfaces to bactericidal antibiotics. Antimicrob Agents Chemother **1993**; 37:1771–6.
19. Amorena B, Gracia E, Monzon M, et al. Antibiotic susceptibility assay for *Staphylococcus aureus* in biofilms developed in vitro. J Antimicrob Chemother **1999**; 44:43–55.
20. Monzon M, Oteiza C, Leiva J, Lamarta M, Amorena B. Biofilm susceptibility testing of *Staphylococcus epidermidis* clinical isolates: low performance of vancomycin in relation to other antibiotics. Diag Microbiol Infect Dis **2002**; 44:319–24.
21. Budhani RK, Struthers JK. Interaction of *Streptococcus pneumoniae* and *Moraxella catarrhalis*: investigation of the indirect pathogenic role of a beta-lactamase-producing Moraxelae by use of a continuous-culture biofilm system. Antimicrob Agents Chemother **1998**; 42:2521–6.
22. Adam B, Baillie GS, Douglas LJ. Mixed species biofilms of *Candida albicans* and *Staphylococcus epidermidis*. J Med Microbiol **2002**; 51:344–9.
23. Srinivasan R, Stewart PS, Griebe T, Chen C-I, Xu X. Biofilm parameters influencing biocide susceptibility. Biotechnol Bioeng **1995**; 46:553–60.
24. Goeres DM, Loetterle LR, Hamilton MA, Murga R, Kirby DW, Donlan RM. Statistical assessment of a novel laboratory method for growing biofilms. Microbiology **2005**; 151:757–62.
25. Curtin JJ, Donlan RM. Using bacteriophages to reduce formation of catheter-associated biofilms by *Staphylococcus epidermidis*. Antimicrob Agents Chemother **2006**; 50:1268–75.
26. Pierce CG, Uppuluri P, Tristan AR, et al. A simple and reproducible 96-well plate-based method for the formation of fungal biofilms and its application to antifungal susceptibility testing. Nat Protoc **2008**; 3:1494–500.
27. Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J Clin Microbiol **1999**; 37:1771–6.
28. Kite P, Eastwood K, Sugden S, Percival SL. Use of in vivo-generated biofilms from hemodialysis catheters to test the efficacy of a novel antimicrobial catheter lock for biofilm eradication in vitro. J Clin Microbiol **2004**; 42:3073–6.
29. Curtin J, Cormican M, Fleming G, Keelehan J, Colleran E. Linezolid compared with eperezolid, vancomycin, and gentamicin in an in vitro model of antimicrobial lock therapy for *Staphylococcus epidermidis* central venous catheter-related biofilm infections. Antimicrob Agents Chemother **2003**; 47:3145–8.
30. Raad I, Hanna H, Jiang Y, et al. Comparative activities of daptomycin, linezolid, and tigecycline against catheter-related methicillin-resistant Staphylococcus bacteremic isolates embedded in a biofilm. Antimicrob Agents Chemother **2007**; 51:1656–60.
31. Sandoe JAT, Wysome J, West AP, Heritage J, Wilcox MH. Measurement of ampicillin, vancomycin, linezolid and gentamicin activity against enterococcal biofilms. J Antimicrob Chemother **2006**; 57:767–70.
32. Giacometti A, Cirioni O, Ghiselli R, et al. Comparative efficacies of quinupristin-dalfopristin, linezolid, vancomycin, and ciprofloxacin in treatment, using antibiotic-lock technique, of experimental catheter-related infection due to *Staphylococcus aureus*. Antimicrob Agents Chemother **2005**; 49:4042–5.
33. Souli M, Giamarellou H. Effects of slime produced by clinical isolates of coagulase-negative staphylococci on activities of various antimicrobial agents. Antimicrob Agents Chemother **1998**; 42:939–41.
34. Abdi-Ali A, Mohammadi-Mehr M, Alaei YA. Bactericidal activity of various antibiotics against biofilm-producing Pseudomonas aeruginosa. Int J Antimicrob Agents **2006**; 27:196–200.
35. Peck KR, Kim SW, Jung S-I, et al. Antimicrobials as potential adjunctive agents in the treatment of biofilm infections with *Staphylococus epidermidis*. Chemother **2003**; 49:189–93.
36. Yasuda H, Ajiki Y, Koga T, Yokata T. Interaction between clarithromycin and biofilms formed by *Staphylococcus epidermidis*. Antimicrob Agents Chemother **1994**; 38:138–41.
37. Kandemir O, Oztuna V, Milcan A, et al. Clarithromycin destroys biofilm and enhances bactericidal agents in the treatment of *Pseudomonas aeruginosa* osteomyelitis. Clin Orthop Rel Res **2005**; 430:171–5.
38. Yamasaki O, Akiyama H, Toi Y, Arata J. A combination of roxithromycin and imipinem as an antimicrobial strategy against biofilm formed by *Staphylococcus aureus*. J Antimicrob Chemother **2001**; 48:573–7.
39. Simon VC, Simon M. Antibacterial activity of teicoplanin and vancomycin in combinations with rifampin, fusidic acid, or fosfomycin against staphylococci on vein catheters. Scan J Infect Dis Suppl **1990**; 72:14–9.
40. Gagnon RF, Richards GK, Wiesenfeld L. *Staphylococcus epidermidis* biofilms: unexpected outcome of double and triple antibiotic combinations with rifampin. ASAIO Trans **1991**; 37:M158–60.
41. Messing B, Peitra-Cohen S, Debure A, Beliah M, Bernier J-J. Antibiotic-lock technique: a new approach to optimal therapy for catheter-related sepsis in home-parenteral nutrition patients. J Paren Enteral Nutrit **1988**; 12:185–9.
42. Donlan RM. Biofilms on central venous catheters: is eradication possible? In: Romeo T, ed. Bacterial biofilms. Current Topics in Microbiology and Immunology 322. Berlin: Spinger-Verlag, 2008;133–61.
43. Berrington A, Gould FK. Use of antibiotic locks to treat colonized central venous catheters. J Antimicrob Chemother **2001**; 48:597–603.
44. Dogra GK, Herson H, Hutchison B, et al. Prevention of tunneled hemodialysis catheter-related infections using catheter-restricted filling with gentamicin and citrate: a randomized controlled study. J Am Soc Nephrol **2003**; 13:2133–9.
45. Yahav D, Rozen-Zvi B, Gafter-Gvili A, Leibovici L, Gafter V, Paul M. Antimicrobial lock solutions for the prevention of infections associates with intravascular catheters in patients undergoing hemodialysis: systematic review and meta-analysis of randomized, controlled trials. Clin Infect Dis **2008**; 47:83–93.
46. Safdar N, Maki DG. Use of vancomycin-containing lock or flush solutions for prevention of bloodstream infection associated with central venous access device: a meta-analysis of prospective, randomized trials. Clin Infect Dis **2006**; 43:474–84.
47. Centers for Disease Control and Prevention. Guidelines for the prevention of intravascular catheter-related infections. MMWR **2002**; 51:1–13.

48. Raad II, Fang X, Keutgen XM, Jiang Y, Sheretz R, Hachem R. The role of chelators in preventing biofilm formation and catheter-related bloodstream infections. Curr Opin Infect Dis **2008**; 21:385–92.
49. Patrauchan MA, Sarkisova S, Sauer K, Franklin MJ. Calcium influences cellular and extracellular product formation during biofilm-associated growth of a marine *Pseudoalteromonas* sp. Microbiology **2005**; 151: 2885–97.
50. Turakhia MH, Cooksey KE, Characklis WG. Influence of a calcium-specific chelant on biofilm removal. Appl Environ Microbiol **1983**; 46:1236–8.
51. Raad I, Buzaid A, Rhyne J, et al. Minocycline and ethylenediaminetetraacetate for the prevention of recurrent vascular catheter infections. Clin Infect Dis **1997**; 25:149–51.
52. Root JL, McIntyre R, Jacobs NJ, Daghlian CP. Inhibitory effect of disodium EDTA upon the growth of *Staphylococcus epidermidis* in vitro: relation to infection prophylaxis of Hickman catheters. Antimicrob Agents Chemother **1988**; 32:1627–31.
53. Percival SL, Kite P, Eastwood K, et al. Tetrasodium EDTA as a novel central venous catheter lock solution against biofilm. Infect Control Hosp Epidemiol **2005**; 26:515–9.
54. Brookstaver PB, Williamson JC, Tucker BK, Raad II, Sherertz RJ. Activity of novel antibiotic lock solutions in a model against isolates of catheter-related bloodstream infections. Ann Pharmacother **2009**; 43:210–9.
55. Raad I, Chatzinikolaou I, Chaiban G, et al. In vitro and ex vivo activities of minocycline and EDTA against microorganisms embedded in biofilm on catheter surfaces. Antimicrob Agents Chemother **2003**; 47:3580–5.
56. Chatzinikolaou I, Zipf TF, Hanna H, et al. Minocycline-ethylenediamine-tetraacetic lock solution for the prevention of implantable port infections in children with cancer. Clin Infect Dis **2003**; 36:116–9.
57. Nori US, Manoharan A, Yee J, Besarab A. Comparison of low-dose gentamicin with minocycline as catheter lock solutions in the prevention of catheter-related bacteremia. Am J Kidney Dis **2006**; 48:596–605.
58. Bleyer AJ, Mason L, Russell G, Raad II, Sherertz RJ. A randomized, controlled trial of a new vascular catheter flush solution (minocycline-EDTA) in temporary hemodialysis access. Infect Control Hosp Epidemiol **2005**; 26:520–4.
59. Feely T, Copley A, Bleyer A. Catheter lock solutions to prevent bloodstream infections in high-risk hemodialysis patients. Am J Nephrol **2007**; 27:24–9.
60. Shanks RMQ, Sargent JL, Martinez RM, Graber ML, O'Toole GA. Catheter lock solutions influence staphylococcal biofilm formation on abiotic surfaces. Nephrol Dial Transplant **2006**; 21:2247–55.
61. Takla TA, Zelenitsky SA, Vercaigne LM. Effectiveness of a 30% ethanol/4% trisodium citrate locking solution in preventing biofilm formation by organisms causing haemodialysis catheter-related infections. J Antimicrob Chemother **2008**; 62:1024–6.
62. Sauer K, Steczko J, Ash SR. Effect of a solution containing citrate/Methylene Blue/parabens on *Staphylococcus aureus* bacteria and biofilm, and comparison with various heparin solutions. J Antimicrob Chemother **2009**; 63:937–45.
63. Metcalf SCL, Chambers ST, Pithie AD. Use of ethanol locks to prevent recurrent central line sepsis. J Infect **2004**; 49:20–2.
64. Sanders J, Pithie A, Ganly P, et al. A prospective double-blind randomized trial comparing intraluminal ethanol with heparinized saline for the prevention of catheter-associated bloodstream infection in immunosuppressed haematology patients. J Antimicrob Chemother **2008**; 62:809–15.
65. Qu Y, Istivan TS, Daley AJ, Rouch DA, Deighton MA. Comparison of various antimicrobial agents as catheter lock solutions: preference for ethanol in eradication of coagulase-negative staphylococcal biofilms. J Med Microbiol **2009**; 58:442–50.
66. Balestrino D, Souweine B, Charbonnel N, et al. Eradication of microorganisms embedded in biofilm by an ethanol-based catheter lock solution. Nephrol Dial Transplant **2009**; 24:3204–9.
67. Venkatesh M, Rong L, Raad I, Versalovic J. Novel synergistic antibiofilm combinations for salvage of infected catheters. J Med Microbiol **2009**; 58:936–44.
68. Raad I, Hanna H, Dvorak T, Chaiban G, Hachem R. Optimal antimicrobial catheter lock solution, using different combinations of minocycline, EDTA, and 25-percent ethanol, rapidly eradicates organisms embedded in biofilm. Antimicrob Agents Chemother **2007**; 51:78–83.
69. Slobbe L, Doorduijn JK, Lugtenburg PJ, et al. Prevention of catheter-related bacteremia with a daily ethanol lock in patients with tunneled catheters: a randomized, placebo-controlled trial. LoS One **2010**; 5:e10840.
70. Mukherjee PK, Mohamed S, Chandra J, et al. Alcohol dehydrogenase restricts the ability of the pathogen *Candida albicans* to form a biofilm on catheter surfaces through an ethanol-based mechanism. Infect Immun **2006**; 74:3804–16.
71. Maiefski M, Rupp ME, Hermsen ED. Ethanol lock technique: review of the literature. Infect Control Hosp Epidemiol **2009**; 30:1096–108.
72. Chen X, Stewart PS. Biofilm removal caused by chemical treatments. Water Res **2000**; 34:4229–33.
73. Johansen C, Falholt P, Gram L. Enzymatic removal and disinfection of bacterial biofilm. Appl Environ Microbiol **1997**; 63:3724–8.
74. Davies DG, Marques CNH. A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. J Bacteriol **2009**; 191:1393–403.
75. Barrow P, Lovell M, Berchieri Jr. Use of lytic bacteriophage for control of experimental *Escherichia coli* septicemia and meningitis in chickens and calves. Clin Diag Lab Immunol **1998**; 5:294–8.
76. Fox J. Phage treatments yield healthier tomato, pepper plants. SM News **2000**; 66:455–6.
77. Sulakvelidze A, Alavidze Z, Morris JG Jr. Bacteriophage therapy. Antimicrob Agents Chemother **2001**; 45:649–59.
78. Fu W, Forster T, Mayer O, Curtin JJ, Lehman LM, Donlan RM. Bacteriophage cocktail for the prevention of biofilm formation by *Pseudomonas aeruginosa* on catheters in an in vitro model system. Antimicrob Agents Chemother **2010**; 54:397–404.
79. Lu TK, Collins JJ. Dispersing biofilms with engineered enzymatic bacteriophage. Proc Nat Acad Sci USA **2007**; 104:11197–202.
80. Sha CB, Mittelman MW, Costerton JW, et al. Antimicrobial activity of a novel catheter lock solution. Antimicrob Agents Chemother **2002**; 46:1674–9.
81. Betjes MGH, van Agteren M. Prevention of dialysis catheter-related sepsis with a citrate-taurolidine-containing lock solution. Nephrol Dial Transplant **2004**; 19:1546–51.
82. Simon A, Ammann RA, Wiszniewsky G, Bode U, Fleischhack G, Besuden MM. Taurolidine-citrate lock solution (TauroLock) significantly reduces CVAD-associated gram positive infections in pediatric cancer patients. BMC Infect Dis **2008**; 8:102.
83. Nablo BJ, Prichard HL, Butler RD, Klitzman B, Schoenfisch MH. Inhibition of implant-associated infections via nitric oxide release. Biomaterials **2005**; 26:6984–90.
84. Burton E, Gawande PV, Yakandawala N, et al. Antibiofilm activity of GlmU enzyme inhibitors against catheter-associated uropathogens. Antimicrob Agents Chemother **2006**; 50:1835–40.
85. Balaban N, Cirioni O, Giacometti A, et al. Treatment of *Staphylococcus aureus* biofilm infection by the quorum-sensing inhibitor RIP. Antimicrob Agents Chemother **2007**; 51:2226–9.