Biofilms: A Clinical Perspective

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Biofilms play an increasingly recognized role in many aspects of human disease. Most of our understanding of infections is based on research that has examined free-living organisms. The results do not necessarily apply to biofilm organisms, since metabolic and synthetic characteristics of free-living organisms can change when they assume the biofilm mode of growth. Biofilms reduce our ability to eradicate infections, causing relapses after seemingly appropriate therapy. Awareness of biofilms, prevention of contamination of implanted or invasive devices, and use of appropriate antimicrobial dosing and treatment durations can limit the negative impact of biofilms while we strive for new technological solutions.

Introduction

The slippery coating of microbial growth that forms on wet environments is not the simple entity once envisioned by clinicians and microbiologists. Once considered to be equivalent to free-living (*ie*, planktonic) organisms with the addition of a simple polysaccharide "slime-layer," we now know that microorganisms, when growing as biofilms, display remarkably different synthetic and metabolic characteristics compared with their planktonic counterparts [1]. As our understanding of microbial biofilms increases, we are discovering levels of microscopic complexity and interdependence that belie the simple macroscopic appearance of these structures. Conventional approaches to microbiologic diagnosis and treatment used for planktonic organisms are not reliable when applied to organisms in the biofilm mode of growth [2•]. The range and prevalence of biofilm-related infections in clinical medicine make awareness of this mode of growth and how it affects our approach to patient management necessary for the delivery of optimal care.

A biofilm is a community of microorganisms adherent to a surface in an aqueous environment. It may be comprised of one or more species of organisms, including bacteria, yeast, and protozoa, that generate an extracellular polymer matrix composed of polysaccharides and proteins [2•] (Fig. 1). Formation of a biofilm begins when a surface in an aqueous environment is colonized with discrete organisms that first adhere, then divide, forming microcolonies [3]. Model systems using *Pseudomonas aeruginosa* have demonstrated that these adherent organisms produce intercellular signaling molecules that, when present in adequate concentration, trigger the formation of complex, mushroom-like structures [4••]. This cell-to-cell signaling is referred to as "quorum-sensing" and includes signals to release planktonic organisms back into the surrounding fluid environment, as well as to build and control the structure of the biofilm. Ultimately, a mature biofilm is a thick sheet of channels and pillars made of organisms and extracellular matrix [3] (Fig. 2).

Research on biofilms was originally directed toward sessile organisms in the environment, where attachment to surfaces enhanced the organisms' exposure to nutrients [1]. Thereafter, the presence of biofilms was demonstrated in the airways of patients with cystic fibrosis, helping to explain the chronicity of lung infections in this population. There has since been a growing realization that similar biofilms are a factor in almost every aspect of health care [5].

Medical Biofilms

Biofilm formation requires two things: an aqueous environment with a constant flow of nutrients, and a surface to which organisms can adhere [2•]. These criteria are not as limiting as might be imagined. Considered on a microscopic scale, the requirement for a "constant flow" is satisfied in the human body by the movement of saliva across a gingival crevice caused by normal mouth movement, or the movement of extracellular fluid against a sternal wire suture caused by respiratory movements of the chest wall. Microenvironments such as these are ubiquitous.

As technology allows us to extend lives and solve diagnostic and therapeutic challenges, it has also created myriad new opportunities for microorganisms to form biofilms. Vascular access devices, synthetic tissue replacements, water handling systems in health care, and sterilization devices are all examples of technologies that have created new niches for biofilms that, in turn, can pose a threat to human health [3,6–10]. The potential impact is amplified by demographic trends that include growing populations of older individuals and patients with chronic diseases, such as diabetes. Both are examples of

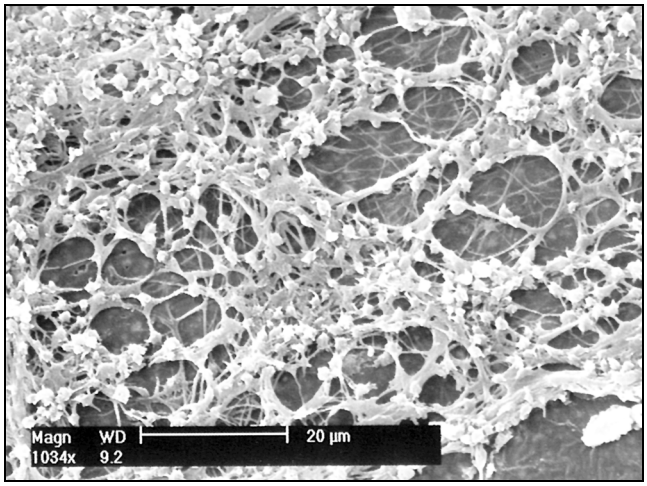

Figure 1. Scanning electron micrograph of staphylococcal biofilm on catheter lumen. (*Courtesy of* Janice Carr, Division of Healthcare Quality Promotion, National Center for Infectious Diseases, Centers for Disease Control and Prevention.)

populations more likely to require medical care and invasive (*eg*, intravascular) devices, and to be more susceptible to infections [11].

The familiar clinical vignette of the patient who receives a protracted course of antimicrobial therapy, *eg*, for osteomyelitis, defervesces and demonstrates clinical improvement, only to relapse a few days after completing treatment, is a classic example of an infection that involves biofilms that confound otherwise appropriate therapy. Devitalized bone provides surfaces to which organisms can adhere in an aqueous microenvironment. An example of device-associated infection related to biofilms is ventilator-associated pneumonia, where organisms from the oropharynx attach to and colonize an endotracheal tube. The biofilm on the endotracheal tube then can serve as a source of organisms that cause lower respiratory tract disease.

Native tissue infections

Biofilm-associated infections of native tissues (Table 1) share the characteristics of having aqueous microenvironments with surfaces including devitalized bone (*eg*, mastoiditis and osteomyelitis), accumulated inflammatory tissue (*eg*, sinusitis, cystic fibrosis-related bronchitis), or mineral deposits, such as with cholelithiasis and renal stones [2•,5,6,8,9,12–14]. These surfaces provide protected sites across which nutrients flow. Eradication of the infection almost always is dependent on removal of the colonized surface [2•].

Non-native tissue infections

Biofilm-associated infections in the setting of non-native tissues and prostheses (Table 2) behave similarly to the native tissue infections described above, with the inserted or implanted material serving as the surface for attachment. While some of these surfaces are amenable to easy

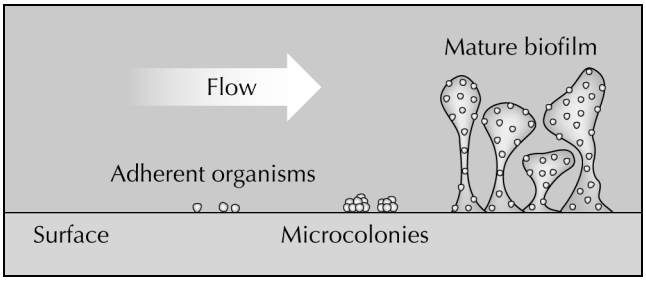

Figure 2. Illustration of a mature biofilm.

removal (*eg*, contact lenses or sutures), and thus eradication of the source of infection, many are implanted and require nontrivial surgical removal if infected [2•,3,6–8,10,15–18]. Among the most challenging sites are those that are endovascular, *eg*, prosthetic heart valves and synthetic vascular grafts. The risk of removal may be so great that true eradication of the infection may not be possible without risking the patient's life. In such cases, clinicians may choose to suppress the infection with chronic antimicrobial therapy [19,20]. Though useful for relatively short-term management, relapse is highly probable should antimicrobial suppression be discontinued for any reason.

Environmental Biofilms

Human health can also be affected by biofilms unrelated to the body. Environmental biofilms are sources of pathogens that can cause infections in humans, including large outbreaks of illness. Aerosols from contaminated water systems have caused large outbreaks of pneumonia due to *Legionella* species. Coexisting with bacterial and protozoal saprophytes, *Legionella* inhabit polymicrobial biofilms found in water heaters and other segments of water handling systems, including hospital water systems [21]. A very different example of an environmental biofilm related to human disease is that of *Vibrio cholera*, which forms biofilms in estuaries, providing a source for outbreaks of foodborne disease [22]. Ventilator-associated pneumonia can be caused by organisms forming biofilms, not only on the surfaces of endotracheal tubes, but throughout the wet environment of the ventilator circuit [23]. Examples of medically significant biofilms can be found in our homes, *eg*, contact lens storage cases, which have been shown to harbor biofilms with organisms linked to corneal infections [24]. Finally, machinery and equipment used for sterilization or manufacturing can support biofilms that lead to introduction of organisms that cause infection. For example, if an automated sterilization device becomes colonized with a biofilm, this may serve as a source of organisms that can contaminate endoscopes, leading to either pseudo-outbreaks or true procedure-related infections [25]. If similar colonization of manufacturing devices occurs, there can be systematic contamination of solutions intended to be sterile, leading to outbreaks of infection among product recipients.

Table 1. Native tissue infections involving biofilms

Superficial	Deep
Dental plaque	Otitis media
Dental caries	Mastoiditis
Hidradenitis	Sinusitis
Otitis externa	Osteomyelitis
	Peptic ulcer disease
	Cholecystitis
	Pyelonephritis, associated with nephrolithiasis
	Prostatitis
	Endocarditis
	Chronic bronchitis/pneumonia, associated with cystic fibrosis

Table 2. Non-native tissue sites prone to biofilm-associated infections

Easily removed for treatment	Difficult to remove/may need antibiotic suppression
Contact lenses	Prosthetic joints
Sutures	Spinal stabilization rods
Dental implants/pins	Internal orthopedic fixation devices
Urinary catheters, indwelling	Penile prostheses
Vascular catheters	Vascular grafts
Gastrostomy tubes	Prosthetic heart valves
Percutaneous drainage tubes	
Vocal cord prostheses	
Ventriculoperitoneal shunts	
Pacemakers	

Antimicrobial Susceptibility

Organisms growing as a biofilm are problematic because they are not reliably killed by standard antimicrobial therapy [3]. Current antimicrobial agents alone almost never succeed in eradicating infections involving biofilms unless the biofilm itself is removed or excised [26•]. Our understanding of this problem is hampered by the fact that evaluation of new antimicrobial agents, measurements of antimicrobial susceptibility, and assessment of growth characteristics and metabolic or structural target sites have all been done using pure cultures of planktonic organisms [27]. Thus, the data on which we base much of our antimicrobial strategies may not apply to many of the infections we attempt to treat.

The ability of these organisms to withstand treatment appears to be multifactorial.

Charge effects: The polymer matrix of biofilms does not appear to function as a simple physical barrier to diffusion. Antimicrobials have been shown to penetrate efficiently. There may, however, be electrostatic effects of the net-negatively charged matrix. Negatively charged molecules of antimicrobials, *eg*, aminoglycosides, can be repelled, and positively charged molecules can be attracted, causing them to be caught in the matrix rather than arriving at their cellular target site [3,26•,28].

pH and oxidative gradients: Micropipette and microelectrode studies have demonstrated that biofilms contain steep gradients of pH and oxygen concentration, creating many microenvironments where intact antimicrobials, despite arriving at their target, are unable to bind at the active site [26•]. This can be due to alterations of charge or conformation at the binding site for the molecule caused by the local pH or oxidative state.

Metabolic alterations: Antimicrobials requiring metabolic activity of target organisms to be effective, *eg*, β-lactam drugs, may not function in biofilms due to the decreased metabolic rates of organisms growing within microcolonies and stalks of a biofilm [3,26•].

Structural alterations: Biofilms may contain a mixed population of organisms, with most cells in a growth state in which they remain somewhat susceptible to antimicrobial agents, and other cells in a metabolically inert, spore-like state that is much more resistant to killing. The latter cells may act as a source from which a renewed population of organisms can arise, even after most of the biofilm has been sterilized [26•].

In cases of polymicrobial colonization, resistance features may be shared, *eg*, one species may express a β-lactamase that diffuses through the biofilm, conferring resistance to other species.

These factors strongly suggest that standard antimicrobial susceptibility testing performed on planktonic cells is unlikely to yield meaningful information about how biofilm organisms might respond in vivo to a prescribed antimicrobial agent. Research in progress is exploring the potential utility of antimicrobial susceptibility testing on model biofilms [27]. Testing may even be extended to polymicrobial biofilms.

Therapeutic implications

Current antimicrobial therapies do not allow us to reliably eradicate infections involving biofilms [26•]. In the presence of a biofilm, there will always be microenvironments where antimicrobials have diminished effectiveness. Viable organisms that persist form a nidus for renewed infection after treatment has ended. As clinicians, we must be quick to consider the presence of biofilms when faced with treatment failures and relapsing symptoms despite seemingly appropriate antimicrobial treatment. In such cases, whenever possible, identifying and removing biofilms (or the device on which they form) is the key to successful care. When removal is not possible, chronic antimicrobial suppression may be the only safe option. In the rare instances when it must be used, suppressive antimicrobial treatment should utilize an agent with as narrow a spectrum of activity as possible to which the pathogen is confirmed to be susceptible in routine (*ie*, planktonic) testing. Although the primary site of infection will not

be eradicated, appropriate blood levels of a targeted antimicrobial are used to kill any viable organisms released from the biofilm, and to prevent systemic infection and seeding of secondary sites. Methods for measuring antimicrobial susceptibility of organisms in the biofilm mode of growth are being developed, but these techniques are not yet widely available. Other areas of investigation include identification of synthetic molecular signals to trigger conversion from biofilm to planktonic growth (*eg*, for existing infections not amenable to removal); or, to prevent biofilm formation, inhibiting quorum-sensing by blocking the appropriate receptors (*eg*, on implantable devices) [4••]. Antimicrobial flushes or locks that are used for vascular access devices may have an impact on biofilms within the device. Research in this area is ongoing as well.

Conclusions

A fundamental shift is needed in our approach to patient care and infectious diseases if we are to prevent device-associated infections, treatment failures, and undesired consequences (*eg*, selection for antimicrobial-resistant bacterial strains) due to biofilm-related infections. Until research leads us to better ways to eradicate clinically significant biofilms, prevention of biofilm-related complications is the most meaningful goal. Awareness of the role of biofilms and how they limit our current ability to treat infections is the first step. We must reduce opportunities for biofilm formation by avoiding unnecessary use of invasive devices, and using the best available methods for insertion or implantation to prevent the introduction of organisms whenever possible. Treatment plans should ensure appropriate debridement when indicated, and adequate durations of treatment for infections likely to be associated with biofilms. Suppressive therapy, when unavoidable, should be as focused as possible.

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