Recurring and Antimicrobial-Resistant Infections: Considering the Potential Role of Biofilms in Clinical Practice

Donald E. Saye, DPM

Micro-organisms commonly produce biofilm, a polymeric matrix that is adherent to inert or living substances and frequently forms on environmental surfaces, medical devices, and traumatized or compromised living and nonviable necrotic tissues such as wounds. The micro-organisms in a biofilm interact with each other and their environment. They are refractory to conventional therapy and resist conventional methods for culturing; their coordinated activities can lessen the effect of antimicrobials and the host’s defenses. The multifactorial mechanism of resistance varies and depends, in part, on the strain of the micro-organism. A biofilm is dynamic and may shed bacteria or bacteria may be released by trauma, resulting in local or systemic infectious disease. Released bacteria lose their protection — they become responsive to appropriate levels of antimicrobials and may be cultured using conventional culturing methods. Micro-organisms in biofilms may remain dormant for weeks or years before causing local or systemic signs and symptoms of infection and are commonly responsible for recurring infections after repeated trials of antibiotics. Most biofilm infection-related research findings have not reached clinical practice yet. However, clinician knowledge about the development of and difficulties culturing micro-organisms in biofilms and their resistance to antibiotics and biocides may lead to improved clinical outcomes in soft tissue and bone infections and the treatment of wounds.

KEYWORDS: infection, biofilms, wounds, micro-organisms


A biofilm colony is a complex, structured, interdependent community of micro-organisms enclosed in a self-produced polymeric matrix (the biofilm, frequently referred to as glycocalyx or slime). Biofilm is adherent to inert and living surfaces that have sufficient moisture and/or nutrients to sustain its survival. Like other infections, the biofilm colony may be a single species or a mixture of species of bacteria and/or fungi and may be different strains of the same species.

Biofilm formation is not uncommon or limited to a small number of micro-organisms or tissues. The Centers for Disease Control and Prevention (CDC) has suggested that biofilms account for 80% of human infections. Alam et al screened 111 cultures of pus, exudate, joint aspirate, and blood obtained aseptically

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from cases of osteomyelitis and septic arthritis. Glycocalyx was found in 76.3% of isolates of *Staphylococcus aureus*, 57.1% of *S. epidermidis*, 50% of *Pseudomonas aeruginosa*, and 75% of *Escherichia coli*. Gristina et al. examined tissues from biomaterials and prosthesis-related infection in 25 surgical patients in a general hospital setting and found that 76% of the causative bacteria grew in biofilms; 17 of these infections were associated with orthopaedic prostheses, 59% of which were in biofilms. However, organisms in biofilms do not always produce an infectious disease and are not always harmful. Current knowledge of biofilm development, resistance of micro-organisms to antibiotics and biocides, and issues related to culturing micro-organisms in biofilms is summarized to help clinicians improve clinical outcomes in soft tissue and bone infections and the treatment of wounds. A glossary of relevant terms (see “Glossary of Terms”) has been provided.

**Biofilm Development**

Biofilms form on wet or moist surfaces. Biofilm formation begins immediately upon micro-organism contact with biologic tissue or a medical device (see Figure 1).

**Biologic tissue.** Initially, organic molecules in tissue fluids form a layer on the tissues or medical device called a *conditioning film*. The different strains or species of micro-organisms in the immediate vicinity co-aggregate; subsequently, cell-to-cell adhesion to the conditioning film occurs. With continued adhesion of micro-organisms, a multilayered colony of cells is formed. The colony of micro-organisms anchors firmly and is surrounded with a self-produced, glue-like slime matrix, comprised primarily of exopolysaccharide and some lipids, proteins, and nucleic acids. Once the colony is anchored, the process becomes irreversible.

The biofilm has a rough, irregular surface that contains many individual colonies of non-uniform, mushroom-shaped or finger-like columns surrounded by fluid-filled channels in which nutrients, enzymes, and waste products circulate. Biofilms produced under different conditions differ in their cellular morphology and matrix content. The biofilm's strength of attachment depends on its adhesion to the conditioning film. Human blood has been shown to enhance development of Gram-positive and Gram-negative bacterial biofilms; heparin has been shown to promote *S. aureus* biofilm formation.

A mature biofilm may take a few hours or several weeks to fully develop. In one study, a methicillin-resistant *S. aureus* biofilm was found to be six cells thick and covered 10% of the surface of a silastic catheter after 2 hours. The biofilm may entrap minerals — mineral build-up is associated with catheter blockage.

Biofilm colonies form on traumatized or compromised living tissues and nonviable necrotic tissues such as burns, wounds and skin ulcers, and exposed or damaged tendon. Impetigo and furuncles have been identified as ideal surfaces for biofilm formation by contaminating and colonizing bacteria. Acute and chronic otitis media, chronic tonsillitis, osteomyelitis, bone fragments or sequestra, and exposed bone are susceptible tissues for biofilm formation. Biofilms are found on heart valves, dental enamel, intestinal mucosa, between toes and in armpits, and are associated with rheumatoid arthritis and genitourinary disease.

**Medical devices.** Because metal plates or screws, artificial joints, indwelling catheters, internal fixation devices, sutures, and other internal medical devices may be wet or moist surfaces, they are subject to biofilm formation. Biofilms also form on bone...
TABLE 1
GLOSSARY OF TERMS

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Aerobic bacteria</td>
<td>have an oxygen-based metabolism and grow only in the presence of air or free oxygen</td>
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<tr>
<td>Anaerobic bacteria</td>
<td>grow without air or free oxygen. Bacteria are sensitive to oxygen and unable to grow in aerobic conditions</td>
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<tr>
<td>Antimicrobial</td>
<td>an agent that is destructive to or prevents the development of micro-organisms</td>
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<tr>
<td>Bactericidal</td>
<td>destructive to or destroying micro-organisms</td>
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<td>Bacteremia</td>
<td>bacteria in the blood</td>
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<td>Biocide</td>
<td>a chemical substance capable of killing different forms of living organisms. Sometimes used interchangeably with antimicrobial</td>
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<td>Conditioning film</td>
<td>trace organics molecules adsorbed to a surface that neutralize excessive surface charge and free energy that may prevent a bacterial cell from approaching near enough to initiate attachment</td>
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<td>Conventional culturing</td>
<td>refers to both aerobic and/or anaerobic cultures from swabs or tissue samples with gram staining in an approved clinical laboratory</td>
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<td>Glycocalyx (biofilm)</td>
<td>a loose, mesh-like framework of a self-produced glue-like slime matrix (sticky polymers) of primarily an exopolysaccharide and some lipids, proteins, and nucleic acids excreted by bacteria to hold the biofilm together, to facilitate attachment to surfaces, and to trap nutrients. This coating protects bacteria within from biocides, the host immune system, and other toxic substances</td>
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<tr>
<td>Intracellular</td>
<td>inside a cell</td>
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<tr>
<td>Micro-organism</td>
<td>a minute living organism too small to be visible to the naked eye</td>
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<td>Nidus of infection</td>
<td>a central point or place, nest, or focus where an infection originates</td>
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<td>Planktonic</td>
<td>free-floating micro-organisms whose movements are controlled by tissue fluids or water (opposite of sessile)</td>
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<td>Polymicrobial</td>
<td>two or more micro-organisms</td>
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<td>Quorum sensing</td>
<td>population density-dependent gene expression. Based on cell-to-cell signaling micro-organisms secrete and sense signaling molecules that accumulate in the growth medium as cells multiply to a high population density. When these micro-organisms reach a critical cell population density, they use these signaling molecules for diverse genetic cellular processes including biofilm formation, virulence, and non-growth periods of quiescence. High population or critical cell population density-dependent processes known as quorum sensing regulate these signaling molecules</td>
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<tr>
<td>Sessile</td>
<td>attached to solid substrate and not able to easily move about (opposite of planktonic)</td>
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<tr>
<td>Signaling molecule</td>
<td>in order for bacteria to communicate among each other, they release a chemical molecule (a signaling molecule) that passes to a receptor site on another bacterial cell and triggers a specific response by that cell. Micro-organisms use these signaling molecules for diverse genetic cellular processes, including biofilm formation, virulence, and non-growth periods of quiescence</td>
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Gene expression. Gene expression plays an important role in biofilm production, pathogenicity, and virulence. High population or critical cell population density-dependent processes known as quorum sensing provide bacteria the ability to communicate, coordinate, and respond to gene expression via signaling molecules once a threshold population density has been reached. Bacteria produce and detect signaling molecules (autoinducers) and use them to communicate cell-to-cell and coordinate gene expression (behavior). When bacteria multiply to a high population density (a quorum) and produce enough autoinducer (reach and sense a threshold level), the group responds by coordinating its gene expression with a population-wide gene expression, coordinating the behavior of the entire community of bacteria. For example, bacteria may colonize a wound, but they do not become a pathogenic-causing disease until they reach a certain population density such as 10^5 bacteria per gram of tissue. Micro-organisms use quorum sensing for diverse genetic cellular processes, including biofilm formation.

Survival. Micro-organisms within the biofilm may be in a low metabolic state but remain fit and able to survive under stress on a minimal amount of nutrients. An anerobic layer of bacteria may develop beneath the aerobic layer where oxygen levels are low enough to sustain their survival.
Infection. Bacteria in biofilms are commonly responsible for recurring infections after repeated trials of antibiotics. The micro-organisms may be slow to produce clinical symptoms and may remain dormant for weeks or years before causing local or systemic signs and symptoms of infection. Because the biofilm is a dynamic environment, the integrity of part or all of the biofilm may fail at any time. The micro-organisms no longer within the biofilm may quickly multiply and disperse, causing rapid increases in bacterial counts and random showers of bacteria.

Detachment or separation and dispersal of bacteria from the biofilm, such as after an injury, can act as a nidus of infection. The micro-organisms that are now planktonic, or free-floating, may cause a bacteremia or a chronic recurring infection such as osteomyelitis, cellulitis, sinusitis, or urinary tract infections. Biofilm colonies are often polymicrobial — the same bacteria may not always cause the recurrent infection.

Biofilm Resistance

Biofilms are complex and depending on a variety of factors, including bacterial strain and dosage of the antibiotic, may decrease or may have little or no impact on the effectiveness of antimicrobials. Appropriate antibiotics act on the bacteria outside the biofilm while the same species of bacteria inside the biofilm are effectively protected from most antimicrobials and the host's defense mechanisms.

MBC concentration. Bacteria inside the biofilm have a much higher minimum bactericidal concentration (MBC) than the same strain of bacteria outside the biofilm. Bacteria within a biofilm may require levels of antibiotics up to 5,000 times the MBC to kill all of their biofilm-protected bacteria compared to MBC levels that kill free-floating cells of the same strain. In a microbiological survey of automated water systems, Dreeszen reported bacteria in biofilms 3,000 times more resistant to free chlorine than the planktonic bacteria. This may overstate the clinical problem since most
human infections are treated successfully with antimicrobials. However, achievable safe therapeutic levels of most antibiotics have been shown to be ineffective in killing most biofilm-protected bacteria.8,81,87

The mechanism of resistance is complex and remains unclear but is known to be multifactorial and varies with the species and the strain of the microorganism. Resistance appears to depend on multicellular synergistic behavior,88 the physiological characteristics of the biofilm itself, the age of the biofilm, altered physiology of cells within the biofilm, and phenotypic changes in the cells within the biofilm.63,89–91

Synergistic behavior. Rather than conforming to individual, single-species bacterial behavior, microorganisms in a biofilm, whether or not mixed species, communicate between and among the same and different species by cell-to-cell signaling, interacting with each other and their environment, sharing resources, and exhibiting multicellular synergistic behavior with common goals similar to a community.54–56 One of the goals of these communities is to lessen the effects of biocides and antimicrobials. This behavior cannot be achieved by individual single-species microorganisms.32,90–94,97–101

Charge and age. Other physiological characteristics of the biofilm contribute to resistance. The biofilm has a negative charge and may restrict diffusion of positive-charged antibiotics such as aminoglycosides. In general, older biofilms are more developed, thicker, and more viscous.58,102–106 The biofilm may act as a mechanical barrier, resisting penetration or slowing the rate of diffusion of the biocide or antimicrobial; hence, diminishing its effect.107 Only those bacteria found in the outer layer of the biofilm may be exposed to the antimicrobials and antigens. In addition, antibiotic penetration is agent- and organism-specific.

Altered physiology. The biofilm may dilute, bind, or entrap all or part of the antimicrobial, preventing therapeutic levels of antibiotics, antibodies, or phagocytes from reaching the bacteria within the biofilm,102,106–113 limiting therapy effectiveness. Slow diffusion through the biofilm allows more time for the bacteria within the biofilm to provide a community-wide defense response to weaken the effect of the incoming antimicrobials.114

When the antimicrobials are able to penetrate the biofilm and kill the bacteria within, a few resistant strains may survive. These surviving bacteria (persisters) may be intracellular and are resistant to further antibiotic treatment.115,116 When antibiotic therapy is discontinued, the persisters reform the biofilm.

Growth rate. Bacteria in biofilms are associated with slow growth rates,117 especially bacteria in the deeper layers of the biofilm where nutrients may have difficulty penetrating the biofilm and waste product excretion may be slow.117,118–120 Many antibiotics (eg, cephalosporins) are less effective against slowly multiplying bacteria.

Colony size. Biofilm colonies are generally too large for phagocytes to engulf them. The biofilm may resist penetration by the phagocytes, reducing their effectiveness. Chemotactic activities of phagocytes may be inhibited by the biofilm.34,113,121,122

Gene expression. Gene expression plays an important role in pathogenicity and virulence of microorganisms in the biofilm.123 The phenotypic changes that occur in the microorganisms within the biofilm make them resistant to antimicrobial treatment and to the host’s immune response.89,124,125 Micro-organisms in biofilms often will express more virulent phenotypes than the same planktonic strain.125,126,127 The bacterial cell has a small number of target sites for antibiotics. It is theorized that some cells in the biofilm, using different genes than the planktonic bacteria, phenotypically alter these target sites to protect themselves.128–131 Quorum-sensing and sigma factor systems that signal bacteria to change their biochemistry regulate many of these gene expressions. Biocides have a much larger number of target sites, making it difficult for micro-organisms to develop resistance to such compounds; however, bacteria in the biofilm can and do resist biocides.

Biofilm Cultures

Micro-organisms grow almost everywhere but fewer than 10% can be grown outside their environment in the laboratory.132–137 Accurate identification and determination of micro-organism antibiotic sensitivities are important for the selection of appropriate therapy. However, micro-organisms within biofilm resist conventional culturing methods.8,73,138,139

The same strain of micro-organism outside the biofilm and micro-organisms released when the
Biofilm is disrupted lose their protection and may be cultured using conventional culturing methods. Biofilm colonies are dynamic and frequently changing. A biofilm colony containing many bacteria may detach intact, sometimes separated by an injury, and grow as a single colony on or in culture media. Because these colonies may be slow growing, the cultures may be discarded before the organisms have been identified. When biofilm colonies are cultured, contaminating or colonizing bacteria may dominate the culture and the pathogenic organism, if grown at all, may never be identified.

The micro-organisms that separate from the biofilm and are identified by conventional culturing methods may not be representative of the types, numbers, or pathogenicity of bacteria within the biofilm. Instead of identifying the pathogenic bacteria in the biofilm causing the infection, conventional cultures may identify the non-pathogenic planktonic bacteria; therefore, failure to disrupt the biofilm may result in a falsely sterile culture or the recovery of the non-pathogenic planktonic bacteria colonizing or contaminating the tissues. Sensitivity reports for these bacteria may lead to a false clinical interpretation. Consequently, long-established conventional methods of collecting micro-organisms from bone, blood, joint effusion, swabs, or soft tissue samples in sufficient numbers to be identified in cultures may be thwarted by the properties of the biofilm.

Ultrasonic oscillation (sonication) of biofilm culture specimens using low-energy, high frequency sound waves has been shown to disrupt the biofilm. Once the biofilm is disrupted or the micro-organisms leave the biofilm, the micro-organisms revert back to their original phenotype and can be identified using conventional culture techniques. Another method to identify bacteria is a culture-based independent approach to detect and identify micro-organisms in a biofilm-protected environment. These non-conventional methods are not readily available to the clinician.

**Treatment**

The treatment of biofilm-related infections is complex and beyond the scope of this paper. In addition, definitive treatment for biofilm infections for the most part remains in the sphere of research studies that have yet to reach clinical practice. Much of the data come from animal or laboratory models or industrial use and vary widely in design. Because research studies are performed under ideal conditions that are much different from the conditions encountered in the human body, they are not necessarily clinically useful. Furthermore, the biofilm and the bacteria in the biofilm are dynamic and constantly changing. Because of the complexity of this issue, the treatment of biofilm infections remains poorly understood and under investigation.

**Discussion**

Infection is rare considering that on a daily basis the human body coexists in a symbiotic relationship with 10^{14} micro-organisms from a countless number of vectors. The development of an infectious disease and the virulence of the micro-organisms involves a multitude of interrelated micro-organism and host factors that vary among species. Clinicians who understand infectious disease development recognize the importance of biofilms in this complexity.

An infectious disease does not occur every time a pathogen colonizes the body. Before causing disease, the bacteria must be able to adhere to and colonize the host’s tissues and reproduce successfully while remaining fit and overcoming the host’s defenses. A minimum number of bacteria must be present to express a coordinated sequence of genetic events resulting in infectious disease. DNA comprises instructions that dictate how bacterial pathogens evade antimicrobials and the immune system, change their virulence, and cause infection — the formation of a biofilm is one method micro-organisms use to subvert antimicrobials and the host’s immune system and enable survival in the human body.

Biofilms attach to wet or moist surfaces — a successful strategy micro-organisms have developed for their survival. Biofilm disease is difficult to eradicate, is a source of many recalcitrant infections, and resembles a multicellular organism or a community structure with many common goals for survival. In addition to those addressed in healthcare, bacterial cells in food, water, and industrial and environmental ecosystems are predominantly organized in specialized...
Biofilms that have significantly different phenotypic properties from free-floating bacteria of the same species. The microorganisms in the biofilm may remain dormant for years and may periodically shed bacteria; this phenomenon and an injury that dislodges the bacteria may release enough microorganisms to cause an infection.

Treatment of an infectious disease has typically depended on the microbiology laboratory to describe planktonic, freely suspended, rapidly growing microorganisms based on their growth characteristics in culture media and their sensitivities to antibiotics. However, the laboratory environment is not representative of how microorganisms appear and respond in their natural environment in a host; hence, conventional cultures often do not reveal all the organisms present. In addition, hundreds of bacteria in a biofilm may grow as a single colony.

Biofilms comprise slow-growing, difficult- or impossible-to-culture microorganisms. Easily grown, rapidly multiplying, contaminating, and colonizing microorganisms may overshadow biofilm microorganisms, making them difficult to recognize or easily overlooked. Thus, the quantity or variety of organisms present may be underestimated. Sonication of the biofilm will disrupt the integrity of the biofilm and release the microorganisms, providing an opportunity for culture and treatment by conventional methods.

Treatment of biofilm microorganisms can be difficult and frustrating. Biofilm infections tend to persist on medical devices, dead bone, and necrotic tissues despite antibiotics, antiseptics, and the host’s immune response. If no biofilm is present, these sites may act as sites of adhesion, the early stage of biofilm development. Biofilm resistance to antimicrobials or the failure to kill or suppress microorganisms protected in the biofilm may be an underlying dynamic in chronic osteomyelitis, cellulitis in the calf, and other recurring infections become active infections; why wounds should be healing but are not; why infected bone and wounds respond well to debridement; and why skin ulcers, wounds, burns, and other necrotic tissues respond well to frequent maintenance debridement.

Biofilms, persisters, and other unculturable (and therefore, unrecognized) pathogens underscore the inadequacy of sampling and culturing methods presently in use. Experiences with biofilms and persisters suggest the need for a culture-independent approach for pathogen identification. Current culture methods to demonstrate presence of infectious disease and antibiotic treatment based on sensitivities from these cultures may soon become obsolete. Along with other unrecognized pathogens, biofilms provide an opportunity to reconsider commonly held beliefs and assumptions regarding infection and offer new possibilities for diagnosis and treatment. The ramifications of biofilms can be widespread. To this end, clinicians should not only maintain a healthy skepticism regarding seemingly unexplainable phenomena, but also consider all possibilities, no matter how unorthodox.

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