

Biofilm Formation: A Clinically Relevant Microbiological Process

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Microorganisms universally attach to surfaces and produce extracellular polysaccharides, resulting in the formation of a biofilm. Biofilms pose a serious problem for public health because of the increased resistance of biofilm-associated organisms to antimicrobial agents and the potential for these organisms to cause infections in patients with indwelling medical devices. An appreciation of the role of biofilms in infection should enhance the clinical decision-making process.

In nature, microorganisms exist primarily by attaching to and growing upon living and inanimate surfaces. These surfaces may take many forms, including those found in soil and aquatic systems, those on the spectrum of indwelling medical devices, and those of living tissues such as tooth enamel, heart valves, or the lung, and middle ear. The common feature of this attached growth state is that the cells develop a biofilm. Biofilm formation is a process whereby microorganisms irreversibly attach to and grow on a surface and produce extracellular polymers that facilitate attachment and matrix formation, resulting in an alteration in the phenotype of the organisms with respect to growth rate and gene transcription. Figures 1 and 2 show scanning electron micrographs of biofilms.

Biofilms have great significance for public health, because biofilm-associated microorganisms exhibit dramatically decreased susceptibility to antimicrobial agents. This susceptibility may be intrinsic (as a natural outcome of growth in the biofilm) or acquired (due to transfer of extrachromosomal elements to

susceptible organisms in the biofilm). The susceptibility of biofilms to antimicrobial agents cannot be determined by means of standard microdilution testing, since these tests rely upon the response of planktonic (suspended) rather than biofilm (surface-associated) organisms. Instead, susceptibility must be determined directly against biofilm-associated organisms, preferably under conditions that simulate conditions in vivo.

Many bloodstream infections and urinary tract infections are associated with indwelling medical devices and, therefore, (in most cases) biofilm associated. The most effective strategy for treating these infections may be removal of the biofilm-contaminated device. A better understanding of the process of biofilm formation may impact clinical decision-making by affecting the way blood samples and catheter-tip samples are collected and examined or by providing a clearer picture of the limitations of conventional therapies for treating biofilm-associated infections. This article presents an overview of the process of biofilm formation and its implications for the health care practitioner.

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THE NATURE OF BIOFILMS ON MEDICAL DEVICES

Table 1 provides a partial listing of medical devices on or in which biofilms have been shown to develop. Evidence of the occurrence of biofilms on medical devices has come from studies in which the devices either were examined upon removal from the patients or were tested in animal or laboratory systems. Raad et al. [1] used the scanning electron microscope to demonstrate that central venous catheters removed from patients were universally colonized by biofilms. These and other



Figure 1. Scanning electron micrograph depicting a developed biofilm (A), the substratum (B), and an attached cell (C). (Image by Rodney Donlan and Donald Gibbon, from the American Society for Microbiology MicrobelLibrary and used with permission of the authors.)

investigators also used culture media and various processing techniques to remove, culture, and quantify the biofilm-associated organisms from medical devices [2–6]. Tunney et al. [7] examined artificial hip prostheses, using a sonication procedure to remove biofilm aggregates from the device surface. The confocal laser scanning microscope was used to visualize these biofilm aggregates and document the occurrence of biofilms on the device surface. Other imaging techniques such as echocardiography have been used to document the occurrence of biofilms (termed “vegetations”) on mechanical and prosthetic heart valves [8].

The types of organisms that develop biofilms are quite broad and include a number of known pathogenic bacteria and fungi. Microorganisms commonly isolated from indwelling medical devices are shown in table 2. These organisms may form both pure-culture and polymicrobial biofilms.

BIOFILM FORMATION

Biofilms have been studied extensively over the past 20 years, and much is known about the process of microbial attachment and initial biofilm formation. To understand attachment, the first stage in biofilm formation, it is necessary to examine closely the properties of both the substratum and the cell surface. Substrata run the gamut from very hydrophobic materials, such as Teflon (DuPont), various plastics, latex, and silicone, to highly charged hydrophilic materials, such as glass and various metals. Certain materials are quite rough or textured (e.g., water pipes, environmental surfaces) while others are much smoother (e.g., silicone or Teflon catheters). Some materials also

have antimicrobial properties that need to be considered (e.g., antibiotic-impregnated catheters or heart valve sewing rings and metal pipes constructed of copper or copper-containing alloys).

The characteristics of the substratum may have a significant effect on the rate and extent of attachment by microorganisms. In general (although there are exceptions), the rougher and more hydrophobic materials will develop biofilms more rapidly [9–12]. The situation becomes more complicated when it is considered that any substratum placed into a fluid environment (whether the open ocean, the bloodstream, or the urinary tract) acquires a conditioning film or coating comprised of primarily proteinaceous material that is present in the fluid in that environment. This conditioning film will confer chemical properties on the surface of the substratum that may completely mask the properties of the underlying substratum itself.

In addition to the characteristics of the substratum, the characteristics of the cell surface are also important. For example, the presence of flagella, pili, fimbriae, or glycocalyx may impact the rate of microbial attachment. This is because the microbial cell, once drawn to the surface, must overcome the repulsive forces common to all materials, and these appendages enable the cell to remain attached until more permanent attachment mechanisms are in place. Korber et al. [13] compared mutant and wild-type organisms and showed that the presence of flagella facilitated attachment of gram-negative bacteria to surfaces. Another study [14] demonstrated the importance of fimbriae (which are external proteinaceous structures of bacteria) for attachment. Cell surface hydrophobicity has also been shown to be very important for attachment [15].

BIOFILM GROWTH

The cells that attach irreversibly to surfaces (i.e., those not removed by gentle rinsing) will begin cell division, form mi-

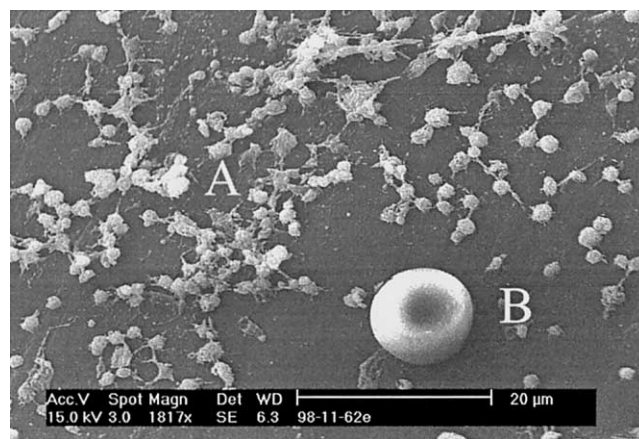


Figure 2. Staphylococcal biofilm (A) containing an RBC (B) on the surface of a needleless connector. (Scanning electron microscope micrograph by Janice Carr, Centers for Disease Control and Prevention [Atlanta]).

Table 1. Indwelling medical devices on which biofilms may develop.

Central venous catheters
Central venous catheter needleless connectors
Contact lenses
Endotracheal tubes
Intrauterine devices
Mechanical heart valves
Pacemakers
Peritoneal dialysis catheters
Prosthetic joints
Tympanostomy tubes
Urinary catheters
Voice prostheses

crocolonies, and produce the extracellular polymers that define a biofilm. These extracellular polymeric substances (EPSs) consist primarily of polysaccharides and can be detected microscopically and by chemical analysis. EPSs provide the matrix or structure for the biofilm. They are highly hydrated (98% water) and tenaciously bound to the underlying surface. The structure of the biofilm is not a mere homogeneous monolayer of slime but is heterogeneous, both in space and over time, with “water channels” that allow transport of essential nutrients and oxygen to the cells growing within the biofilm [16]. Biofilms have a propensity to act almost as filters to entrap particles of various kinds, including minerals and host components such as fibrin, RBCs, and platelets.

Biofilm-associated organisms grow more slowly than planktonic organisms [17], probably because the cells are limited by nutrient and/or oxygen depletion. Cells detach from the biofilm as a result of either cell growth and division or the removal of biofilm aggregates that contain masses of cells. It is possible for

these detached cells to cause a systemic infection, depending on a number of factors, including the response of the host immune system.

RESISTANCE TO ANTIMICROBIAL AGENTS

The biofilm mode of growth confers on the associated organisms a measurable decrease in antimicrobial susceptibility. For example, Ceri et al. [18] found that biofilm-associated *Escherichia coli* required >500 times the MIC of ampicillin to provide a 3-log reduction. Williams et al. [19] found that *Staphylococcus aureus* biofilms required >10 times the MBC of vancomycin to provide a 3-log reduction. The effect on susceptibility may be intrinsic (i.e., inherent in the biofilm mode of growth) or acquired (i.e., caused by the acquisition of resistance plasmids).

There are at least 3 reasons for the intrinsic antimicrobial resistance of biofilms. First, antimicrobial agents must diffuse through the EPS matrix to contact and inactivate the organisms within the biofilm. EPSs retard diffusion either by chemically reacting with the antimicrobial molecules or by limiting their rate of transport. Hoyle et al. [20] showed that the EPSs of *Pseudomonas aeruginosa* was capable of binding tobramycin; dispersed cells were 15 times more susceptible to this agent than were cells in intact biofilms. Second, biofilm-associated organisms have reduced growth rates, minimizing the rate that antimicrobial agents are taken into the cell and therefore affecting inactivation kinetics. DuGuid et al. [21] found that an increase in growth rate resulted in an increase in susceptibility of *Staphylococcus epidermidis* biofilms. DuGuid et al. [22] also showed that ciprofloxacin activity was influenced by the cell cycle; newly formed daughter cells were more susceptible than other populations in the biofilm. Third, the environment immediately surrounding the cells within a biofilm may provide

Table 2. Biofilm-associated microorganisms commonly isolated from selected indwelling medical devices.

Indwelling medical device	Organisms
Central venous catheter	Coagulase-negative staphylococci, <i>Staphylococcus aureus</i> , <i>Enterococcus faecalis</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , <i>Candida albicans</i>
Prosthetic heart valve	Viridans <i>Streptococcus</i> , coagulase-negative staphylococci, enterococci, <i>Staphylococcus aureus</i>
Urinary catheter	<i>Staphylococcus epidermidis</i> , <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Enterococcus faecalis</i> , <i>Proteus mirabilis</i>
Artificial hip prosthesis	Coagulase-negative staphylococci, β -hemolytic streptococci, enterococci, <i>Proteus mirabilis</i> , <i>Bacterioides</i> species, <i>Staphylococcus aureus</i> , viridans <i>Streptococcus</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i>
Artificial voice prosthesis	<i>Candida albicans</i> , <i>Streptococcus mitis</i> , <i>Streptococcus salivarius</i> , <i>Rothia dentocariosa</i> , <i>Candida tropicalis</i> , <i>Streptococcus sobrinus</i> , <i>Staphylococcus epidermidis</i> , <i>Stomatococcus mucilaginosus</i>
Intrauterine device	<i>Staphylococcus epidermidis</i> , <i>Corynebacterium</i> species, <i>Staphylococcus aureus</i> , <i>Micrococcus</i> species, <i>Lactobacillus plantarum</i> , group B streptococci, <i>Enterococcus</i> species, <i>Candida albicans</i>

conditions that further protect the organism. Tresse et al. [23] found that agar-entrapped *E. coli* demonstrated a decreased susceptibility to aminoglycoside antibiotics as a result of decreased uptake of the antibiotic by the oxygen-deprived cells.

With regard to acquired resistance, research has shown that plasmids can be exchanged in biofilms under a number of conditions. Plasmids are extrachromosomal circles of DNA that may encode resistance to a number of antimicrobial agents, including β -lactams, erythromycin, aminoglycosides, tetracycline, glycopeptides, trimethoprim, and sulfonamides [24]. A large number of bacterial species have been shown to transfer plasmids to other bacterial species [25]. Ehlers and Bouwer [26] demonstrated plasmid transfer by conjugation between different gram-negative bacteria growing in biofilms. The rates of horizontal plasmid transfer were several orders of magnitude higher in biofilms than in liquid cultures of the same organisms. Other investigators have demonstrated a similar phenomenon [27–29]. The reasons for enhanced transfer of plasmids in biofilms may include both the greater probability of contact between cells and the negligible effect of shear forces in either disrupting cell-to-cell contact or damaging the pili required for conjugation.

SUSCEPTIBILITY TESTING

Standard antimicrobial susceptibility testing, which challenges planktonic (suspended) cells with an antimicrobial agent, will not accurately predict the efficacy of an agent against biofilm-associated organisms. The National Committee for Clinical Laboratory Standards (NCCLS) does not have an approved method for evaluating the effectiveness of antimicrobial agents against biofilm-associated organisms. However, a variety of protocols for testing antimicrobial agents against biofilms have been suggested [17]. An acceptable method would be one that can predict the susceptibility of the biofilm-associated organisms in vivo, is reproducible, is inexpensive, provides relatively rapid turnaround of results, and is easy to use.

Ceri et al. [18] developed the Calgary Biofilm Device and used it to test several antimicrobial agents against biofilms of *E. coli*, *S. aureus*, and *P. aeruginosa*. The device grows biofilms on replicate “pegs” and then tests these biofilms by means of a standard 96-well plate apparatus. Results from this device showed that biofilm growth on pegs was reproducible (there was no statistically significant difference between results for different rows of pegs), and the device was relatively easy to use and inexpensive. Domingue et al. [30] used a tubular flow-through device called the Modified Robbins Device to grow biofilms of selected organisms on replicate membranes in flowing conditions, then removed the membranes with biofilms from the device and exposed them to various antimicrobial agents. By comparing results from these tests with results ob-

tained with use of the NCCLS procedures, it was possible to compare the susceptibility of biofilm and planktonic phenotypes of an organism.

However, a concern with any of these biofilm susceptibility testing protocols is how closely they can approximate an in vivo or in situ biofilm. How reliably does the method predict the efficacy of an antimicrobial agent against biofilms on a catheter or prosthetic heart valve, for example? Model systems that closely simulate an implanted medical device might allow better estimates. Murga et al. [31] developed a system for growing biofilms on central venous catheter needleless connectors that simulated the characteristics of the device when in use with respect to flow rate, temperature, composition of the medium, presence of a blood-conditioning film on the device surface, and the organisms present. Similar systems could be developed and used to evaluate the effectiveness of antimicrobial agents against biofilms on these devices. A logical next step might be to compare results obtained from the in vitro model with results from an in vivo animal model.

BIOFILMS AND CLINICAL DECISION-MAKING

Several aspects of biofilms make their formation a clinically relevant process: (1) they are resistant to antimicrobial agents; (2) they may be a persistent source of infection; (3) they may harbor pathogenic organisms, and (4) they may allow exchange of resistance plasmids. The clinician who is informed about the relevance of biofilms in infection can use this knowledge to make sound decisions that affect patients' health and safety. Several specific suggestions follow.

Determining the Biofilm Link

Collect paired blood samples. For a patient with a central venous catheter, more clinically relevant results will likely be obtained if blood for culture is drawn from a peripheral vein rather than through the catheter. If the catheter is colonized with a biofilm, cells may detach from the biofilm when blood is drawn through the catheter, thereby contaminating the sample and providing misleading results. This is also why venous sampling is recommended if a blood sample is drawn from a catheter.

Repeated negative results for samples may not imply absence of biofilms. Because antibiotic concentrations sufficient to kill or inactivate planktonic organisms are inadequate to kill biofilm-associated organisms, organisms might be absent from blood samples and still survive on the catheter within the biofilm.

The coagulase-negative staphylococci dilemma. Coagulase-negative staphylococci are probably the organisms most commonly isolated from biofilms on medical devices. When these organisms are isolated from blood samples, there is a

tendency to question the clinical significance of the finding, since they are common skin organisms that can readily contaminate samples. If consecutive culture-positive samples confirm the significance of coagulase-negative staphylococci, then the presence of biofilms as well as the possibility of contamination should be considered for patients with any indwelling medical device. The attending physician and an infectious disease specialist should always be consulted regarding difficult interpretations.

Detecting Biofilms on Medical Devices

Methods that detect and quantify biofilms on the inner (luminal) as well as outer surfaces of catheters will provide the only true picture of biofilm colonization. A widely practiced procedure to detect bacterial colonization on catheter tips is the roll plate method developed by Maki [32]. This technique is based on the premise that biofilm-associated bacteria on the outside of the catheter tip can be reproducibly recovered by rolling the tip over the surface of an agar plate. However, organisms that are not removed by contact with the agar or organisms on the inner lumen of the catheter are not detected, and any attempt to relate roll-plate data to biofilm colonization is questionable.

A much more reliable, more quantitative method is to use mechanical forces (e.g., sonication or vortexing) to remove the biofilm-associated organisms, which can then be quantified by means of plate count or fluorescent staining techniques. Examples of such methods are described in articles by Tenney et al. [33], Sherertz et al. [34], and Donlan et al. [35]. Kite et al. [36] also proposed an endoluminal brush technique for the quantification of biofilm-associated organisms on catheters. Any of these procedures should be useful for the recovery and quantification of biofilms on other medical devices, such as prosthetic joints and mechanical heart valves. However, once the catheter tip is removed, some symptoms may resolve. For all patient populations, the roll-plate method may provide clinically relevant data, or it may not, particularly if culture of a venous blood specimen is unavailable for comparison with catheter-tip culture results.

Treating Infections That Involve Biofilms

Standard NCCLS broth microdilution testing methods using pure cultures will not enable prediction of antimicrobial efficacy against biofilms. As already discussed, the concentrations of antimicrobial agents required to inactivate biofilm-associated organisms are much higher than the concentrations sufficient to inactivate systemic organisms in the standard in vitro microdilution test. In addition to the issue of concentration differences, it is possible that certain categories of antibiotics may be more effective against biofilm-associated organisms than are others. For example, Ceri et al. [18] showed that

ciprofloxacin and trobramycin were more effective as treatment against biofilms of *P. aeruginosa* than were a number of other antibiotics, such as piperacillin, imipenem, and ceftazidime. Gentamicin was more effective against *S. aureus* biofilms than were any of the other agents tested, including oxacillin and vancomycin. If antimicrobial therapy is considered a viable option against biofilm colonization, then susceptibility testing should be performed with biofilm-associated organisms. It may, in fact, be difficult or impossible to achieve inhibitory concentrations of the antimicrobial within the biofilm at the site of infection (i.e., tissue or blood).

Biofilm age may influence susceptibility. If an indwelling medical device is colonized by a biofilm, the problem will inevitably get worse, and the aging biofilm will become increasingly difficult to treat against. Old biofilms have been shown to be even less susceptible to antimicrobial agents than are younger biofilms. In addition, if organisms with acquired resistance are present in the biofilm, the probability of resistance-plasmid transfer might increase over time.

Evaluate the efficacy of "coated catheters" on the basis of laboratory and clinical studies. Several of the currently used coated catheters used in treatments with the combinations of minocycline and rifampin [37] or chlorhexidine and silver sulfadiazine [38] have been evaluated only in clinical studies. Evaluation of these coating treatments should include laboratory studies that use model system biofilms. This would allow the investigator to determine whether the coating prevents or merely impedes biofilm formation, whether the treatment effects are organism-specific, the rate of biofilm formation and cell detachment from the treated surface, and whether long-term use of coated catheters will affect the antimicrobial resistance characteristics of the biofilm-associated organisms. Ideally, a coated device should be evaluated in a laboratory model and in an animal model prior to human clinical trials.

CONCLUSIONS

The tendency of microorganisms to develop biofilms has been well documented for a number of medical devices. This process is particularly relevant for the clinician because biofilm-associated microorganisms are much more resistant to antimicrobial agents than are planktonic organisms and because colonization of a medical device with a biofilm may be associated with infection. Although the mechanisms of biofilm formation, growth, and antimicrobial resistance have been investigated by the research community, there is still a need for effective treatments against biofilm-associated organisms. A clearer understanding of the role of biofilms in infection should enhance clinical decision-making and provide the foundation for further research on novel control strategies.

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