

# BIOFILMS AND DEVICE-RELATED INFECTIONS

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With the benefit of hindsight, it is possible to detect a very gradual but profound shift in the nature of the diseases that affect patients in the developed world. Many acute diseases caused by specialized pathogens with specific pathogenic mechanisms, such as typhoid and diphtheria, have been largely eradicated by the use of effective vaccines and modern antibiotics. Their places among the “Horsemen of the Apocalypse” have been taken by a different type of infection, caused by organisms that were previously thought to be saprophytic or environmental, whose sole pathogenic mechanism is often the ability to persist in spite of host defenses and antibiotic chemotherapy. These low-grade infections often develop very slowly, with only a few symptoms, and they usually affect individuals who are compromised by some physiological defect (e.g., cystic fibrosis or diabetes) or by the implantation of a foreign body, such as a medical device. Direct observations of infected tissues from compromised individuals, and of the surfaces of medical devices that have become foci of chronic infections, have shown that the causative organisms actually grow in biofilms in which they

are embedded in copious amounts of exopolysaccharide matrix material. The study of bacterial biofilms is more advanced in the engineering field than in the medical field, but the simple realization that biofilms are involved in chronic infections opens the way for a massive transfer of valuable information from the engineering realm to the medical realm and for its application to the treatment of infectious diseases.

### INSIGHTS FROM BIOFILM MICROBIOLOGY

Serendipitously, the organism that predominates in virtually all cold-water systems (*Pseudomonas aeruginosa*) is also responsible for many device-related and other chronic infections, and biofilm microbiologists who study environmental and industrial systems are very familiar with biofilms formed by this species. Even the most anthropocentric among us cannot attribute mental processes to prokaryotic cells, so we must assume that they follow the same growth and survival strategies in the human body that have made them so very successful in the environment and in industrial systems. *P. aeruginosa* first came to the attention of biofilm microbiologists because it predominates in cold alpine streams (8) and grows predominantly (99.99%) in biofilms in this natural

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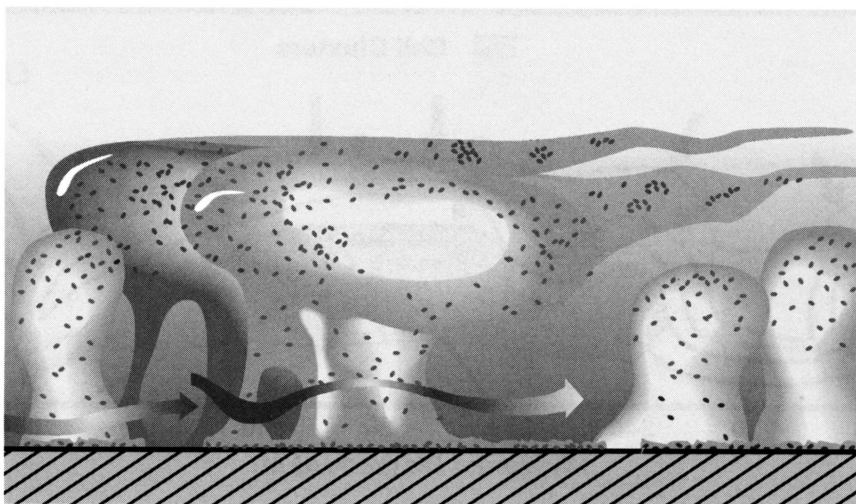
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habitat. We know that bacteria have inhabited freshwater streams for much longer than eukaryotic organisms have existed on earth, so the pronounced tendency of this ubiquitous bacterium to grow in biofilms in its real habitat sends us a clear message of importance to medical microbiology. In the original observations, two parallel teams of microbial ecologists found the rocks along the streams coated with thick biofilms ( $>10^8$  cells/cm<sup>2</sup>), while the bulk water phase of the alpine stream ecosystem contained only 8 to 10 cells/ml. This predominance of the biofilm mode of growth has been confirmed in literally hundreds of stream environments, in industrial water systems, and in hospital water and air conditioning systems (7). Basic observations of the predominance of biofilms in natural habitats have now been extended to almost all bacterial species, including gram-positive bacteria, and the only exceptions seem to be among organisms that live in mucus layers (e.g., *Campylobacter*) and among intercellular pathogens. In environmental and industrial microbiology, as in medical microbiology, bacteria that are removed from their natural ecosystems and grown in monospecies cultures in liquid media quickly adapt to this very artificial system and adopt the planktonic mode of growth almost exclusively. Two reasons for this adaptation to growth in pure culture appear to be the higher growth rate of planktonic cells, in the absence of antagonists, and the fact that biofilm cells are left behind on the walls of test tubes when liquid cultures are propagated by the traditional methods of subculture. Liquid monospecies cultures are certainly necessary for studies of the genetics or the physiology of individual species, but it is very sobering to realize that this almost universal culture method induces a mode of growth that differs profoundly from that adopted by almost all organisms in nature and in most modern infections.

Engineers in the Center for Biofilm Engineering (CBE) have defined bacterial biofilms in terms of their structures, their remarkable physiological heterogeneity, and their phenomenal resistance to antibacterial agents. Engineers favor direct observation over extrapola-

tion, and the main weapon in their arsenal for the structural examination of biofilms is the confocal scanning laser microscope (CSLM). The CSLM allows us to visualize biofilms on opaque surfaces, without fixation or dehydration, so that we can obtain clear images of living biofilms in real time. CSLM observations of living biofilms, including some formed by one to three species *in vitro* and some formed in natural ecosystems, showed unequivocally that biofilms are composed of discrete microcolonies interspersed between open water channels that communicate with the bulk fluid (Fig. 1). Some of these microcolonies are shaped like mushrooms, and some assume different shapes described as "stacks" or "towers," but all contain sessile bacterial cells embedded in a hydrated exopolysaccharide matrix whose viscoelastic properties become evident under high-shear conditions (31). The CBE has established the fact that most biofilms assume this microcolony and water channel structure, including all biofilms formed by the few gram-positive species examined to date, and the most significant consequence of this new observation is that we must now explain how these elaborate structures are established and maintained. Kolter and colleagues have shown (22) that planktonic cells of *P. aeruginosa* maneuver on a surface, following initial association, and form aggregates that develop into microcolonies when matrix formation is "switched on" (12). It is clear that these initial stages of biofilm formation are controlled by signals, analogous to the hormones and pheromones that control morphogenesis and behavior in higher organisms. The subsequent structural developments that lead to the microcolony and water channel structures of mature biofilms are even more complex, and we must invoke an even more complex set of signals to control this morphogenesis and to explain the persistence of open water channels when random growth would rapidly occlude them. Mature biofilms obviously constitute primitive multicellular organisms (7), and their signaling systems may constitute a new target for manipulation as we



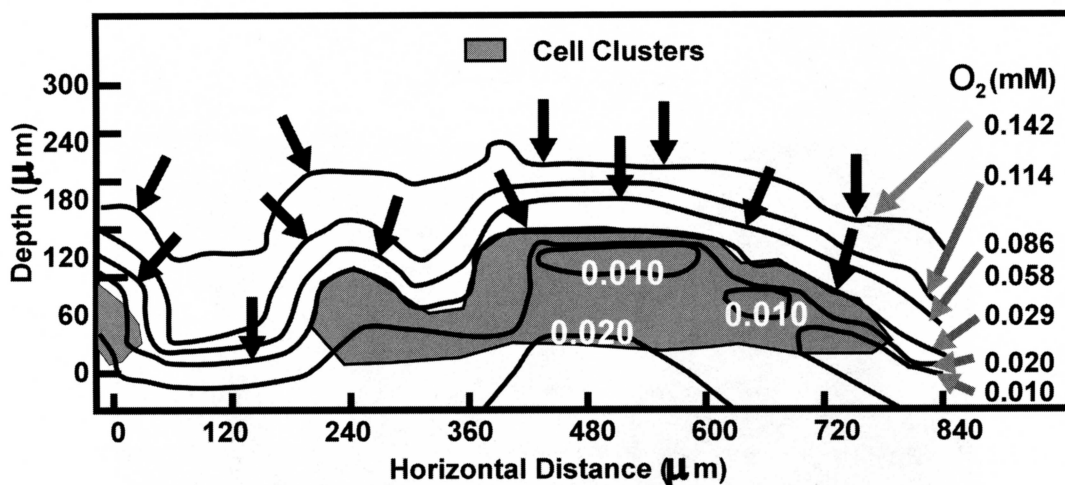
**FIGURE 1** Diagrammatic representation of the cellular structure of a microbial biofilm showing the directly observed shapes of matrix-enclosed microcolonies and intervening water channels, in which convective flow occurs.

think about methods to control their formation in infections.

The tendency of engineers to make direct observations, and their skill with miniature instruments, has shown that the complex structures of bacterial biofilms produce equally complex physiological patterns within these sessile populations. Lewandowski and his team of engineers and microbiologists have used very fine ( $<10\text{-}\mu\text{m}$ ) microelectrodes to map characteristics such as dissolved oxygen and pH within biofilms (25), and the data are at once fascinating and disturbing. A map of dissolved oxygen concentrations in a biofilm produced by cells of *P. aeruginosa* (Fig. 2) shows that some of the sessile cells that compose this sessile community grow aerobically while some experience a completely anaerobic environment. Direct observations of the rates of metabolic activity of sessile cells at various locations within biofilms, using chemical probes that measure reducing power, show an equally heterogeneous pattern (38–40). The consequence of this remarkable physiological heterogeneity within biofilms is that there are sessile cells in any mature biofilm that are growing in a huge variety of physiological states and at an equally

wide variety of rates. This physiological heterogeneity is a powerful survival mechanism for sessile communities, because any antibacterial agent must kill all of the cells growing in all of the different physiological states or the cells that survive in any given microcolony will simply propagate and reestablish the biofilm in a matter of hours.

Engineers are accustomed to thinking in terms of mass transfer when they consider the penetration of any molecule through a matrix. These concepts, and the methods that are used to support them, have been very useful in the examination of the penetration of antibacterial agents through the matrices of biofilms with the objective of killing sessile organisms. Stewart and his team of engineers and microbiologists have examined the penetration of both biocides and antibiotics through biofilms (4, 13; P. S. Stewart, F. Roe, J. Rayner, J. G. Elkins, Z. Lewandowski, U. A. Ochsner, and D. J. Hassett, submitted for publication; J. A. Anderl, P. S. Stewart, and M. J. Franklin, submitted for publication); they have concluded that the biofilm matrix presents only a minor barrier to penetration except in cases in which the agent reacts chemically with the matrix mate-

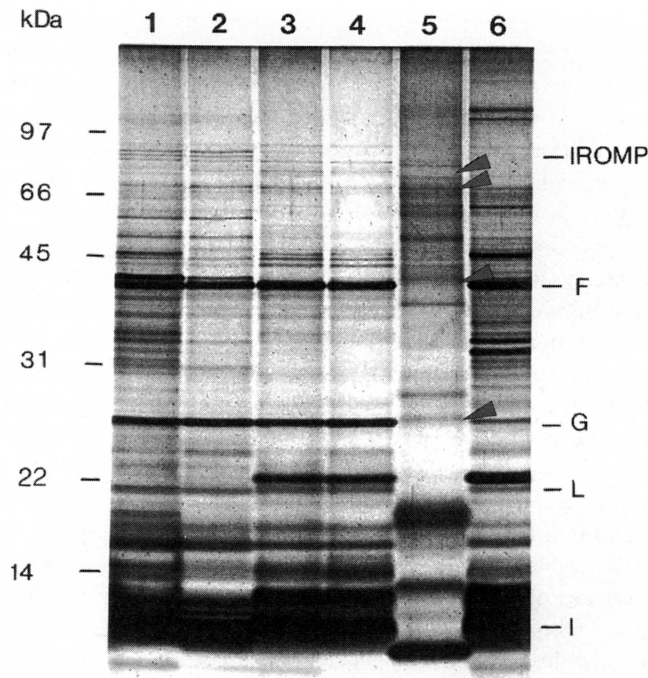


**FIGURE 2** Isobar map of dissolved oxygen concentration as measured directly in a living biofilm by the use of a microelectrode, showing that the centers of microcolonies can be essentially anoxic, even when the biofilm is growing in ambient air.

rial. The penetration of an antibacterial agent can be monitored by using the exquisitely sensitive noninvasive technique of attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) (35, 38) to determine when the molecule in question reaches the colonized substratum. The CBE team can also monitor penetration by mapping the positions of the surviving sessile cells (19). The biofilm matrix may act as a barrier to the penetration of antibacterial agents if these molecules react with or bind to the matrix material (which is usually an anionic exopolysaccharide), but this barrier can be overcome by simply increasing the concentration of the agent until it exceeds that lost to reaction or binding (30). Limitations in mass transfer are sufficient to explain some low levels of resistance of biofilms to antibiotics, but they are not sufficient to account for the 1,000- to 1,500-fold increases in resistance seen when cells of most species form these sessile populations (28). Most antibiotics affect individual bacterial cells differently, depending on the bacterial growth rate, and many authors (1, 2) have suggested that the low growth rates seen in some sessile cells may make them inherently less susceptible to the

antibiotics that penetrate the matrix of the biofilm. However, the growth rate is only one of many physiological parameters that vary significantly in the different microcolonies that constitute a biofilm, and such parameters as local oxygen tension, pH, and local expression of *rpoS* (16) may affect susceptibility to antibacterial agents equally profoundly. To grasp the clinical consequences of this physiological heterogeneity of biofilms, it is essential to remember that any sessile cell that survives the onslaught of an antibiotic finds itself embedded in a matrix containing the remnants of all of its dead neighbors, and the biofilm reforms very quickly when therapy is finished.

A recent discovery that the cells of *P. aeruginosa* assume a radically different phenotype when they form biofilms may provide a more complete explanation of the phenomenal resistance of sessile populations to antibiotics. We expected that the synthesis of alginate (the matrix material of *Pseudomonas* biofilms) would be triggered by the adhesion of these cells to a surface; we have shown that one of these genes (*algC*) is upregulated within minutes of adhesion (12), and we assume that the whole alginate cassette is expressed in this initial phase of



**FIGURE 3** Polyacrylamide gel electrophoresis gel showing the pattern of production of OMPs by cells of *P. aeruginosa* in the biofilm mode of growth (lane 5) versus production by cells in the planktonic mode of growth (lanes 1 to 4 and 6). The differences in OMP production between these cells indicate that the biofilm phenotype differs profoundly from the planktonic phenotype (H. Yu and J. W. Costerton, unpublished data).

biofilm formation. However, we were recently surprised to note that the outer membrane proteins (OMPs) of *P. aeruginosa* differ very radically (Fig. 3) between planktonic cells and biofilm cells, indicating the existence of different planktonic and biofilm phenotypes in the organism. The biofilm phenotype appears to include the expression of the *rpoS* gene (K. D. Xu, unpublished data), which is normally expressed only in the stationary growth phase by planktonic cells, and it is very significant that this gene is expressed in bacterial cells recovered directly from the infected lungs of patients with cystic fibrosis (16). If bacterial cells of other species also express a completely different set of genes when they are growing in biofilms, we must reexamine much of the work in medical microbiology that has been based on studies of planktonic cells growing in monospecies cultures. Antibiotics have been screened for their ability to kill planktonic cells, and vaccines have been produced that include the surface antigens expressed by planktonic

cells, but some pathogens actually growing in the body may express a profoundly different phenotype with different permeabilities and different surface proteins. Perhaps, then, it is not surprising that these antibiotics and vaccines have been effective against acute bacterial diseases in which the pathogens grow in the planktonic phenotype but much less effective in the control of biofilm diseases (6).

If we try to imagine the bacterial survival strategies that would have been effective in the earliest stages of the development of life on this planet, growth in stationary biofilms that were protected from unfavorable conditions would prevent bacteria from being swept into acid or boiling downstream pools and from surges of threatening water from upstream sources. Biofilms predominate in modern hot spring areas for exactly these reasons. Later in the development of life, sessile bacteria would be protected from the depredations of bacteriophage viruses and primitive amoebae when they were in the biofilm mode of growth. When we study natu-

ral mixed-species biofilms taken directly from rivers and streams, we can watch amoebae cruise over the surfaces of biofilms and even penetrate the water channels of these sessile populations, but these phagocytic eukaryotes capture and ingest only the few planktonic cells that cannot enjoy the protection afforded by the biofilm. We can, therefore, speculate that bacteria adopted the essentially defensive biofilm phenotype long before multicellular animals evolved and that they reverted to this strategy when, as pathogens in the human body, they were faced with the armamentarium of antibodies and antibiotics thrown at them by modern medicine. The large-scale use of medical devices is a relatively new development in medicine, and this provision of readily colonizable surfaces may have selected for the invasion of the body by a new class of pathogens whose main pathogenic mechanism is their ability to produce well-defended biofilms. This might explain the recent success in the hospital environment of species (e.g., *P. aeruginosa* and *Legionella pneumophila*) that were already notably successful biofilm formers in lakes and rivers or notably successful but innocuous colonizers of the human skin (*Staphylococcus epidermidis*). Medical microbiology has been very successful in the control of acute planktonic pathogens, but now it must employ some of the techniques of microbial ecology in order to deal with the new biofilm pathogens that have "crept out of the swamp" to attack those who are compromised by physiological defects or by the implantation of medical devices.

#### **BIOFILM INFECTIONS OF MEDICAL DEVICES**

Our clinical colleagues, notably Marrie and Gristina, have reported that bacterial infections associated with medical devices are generally resistant to host defense mechanisms and refractory to antibiotic chemotherapy (18). Because environmental and industrial biofilms are also resistant to phagocytic cells (amoebae) and refractory to treatment with biocides, we examined the surfaces of devices and associated tissues from these infections to determine

whether the causative organisms grow in biofilms. Even though the morphological methods available at that time (scanning and transmission electron microscopy) depended on radical dehydration of the specimens, it was immediately obvious that the bacterial cells involved in these infections grew in biofilms identical to those seen in our previous studies. The bacterial cells on the surfaces of either urinary catheters (28) or pacemaker leads that had been the foci of device-related infections were clearly seen to be embedded in fibrous material even though this matrix was severely condensed by dehydration (Fig. 4). The pivotal role of biofilms in device-related and other chronic bacterial infections was proposed (8), and subsequent examinations of literally hundreds of implanted medical devices have reinforced this observation (17, 21). Virtually all transcutaneous medical devices acquire microbial biofilms that travel into accessible internal tissues in a matter of weeks, and devices that are simply apposed to tissues (like urinary catheters and intrauterine devices [IUDs]) often acquire biofilms because they carry bacteria from colonized tissues into normally sterile organs. Implanted medical devices may acquire biofilms as a result of bacterial contamination during surgery or subsequent hematogenous spread. In the absence of a medical device, biofilms may form on tissue surfaces because of a physiological compromise (cystic fibrosis) or because of tissue damage, such as the formation of sequesterae of dead bone in the initial stages of osteomyelitis (24). In rarer cases, bacterial biofilms may form on the surfaces of healthy tissues (e.g., prostate or endocardium) as a result of a temporary failure of usually effective host defense mechanisms. A partial list of biofilm diseases that affect patients in developed countries is presented in Table 1, and it has been estimated that as many as 60% of the bacterial diseases treated in this decade are actually biofilm infections.

#### **CHARACTERISTICS OF BIOFILM DISEASES**

While a biofilm infection can give rise to an acute planktonic infection at any time, the bio-

