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.

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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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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^2), 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 extrapolation, 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