BIOFILMS AND DEVICE-RELATED INFECTIONS

J. William Costerton and Philip S. Stewart

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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.

J. William Costerton and Philip S. Stewart, Center for Biofilm Engineering, Montana State University, Bozeman, MT 59717.

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

Persistent Bacterial Infections, Edited by J. P. Nataro, M. J. Blaser, and S. Cunningham-Rundles, © 2000 ASM Press, Washington, D.C.

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

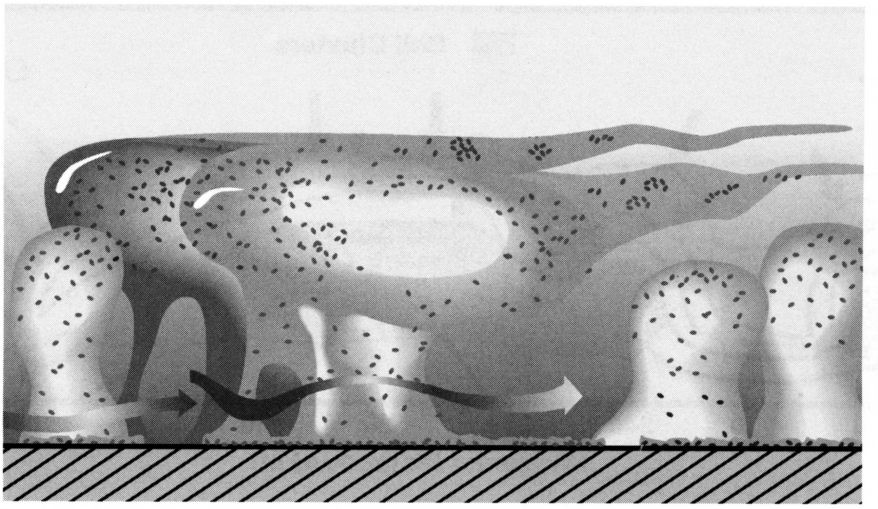


FIGURE 1 Diagrammatic representation of the cellular structure of a microbial biofilm showing the directly observed shapes of matrix-enclosed microcolonies and intervening water channels, in which convective flow occurs.

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-μm) 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-

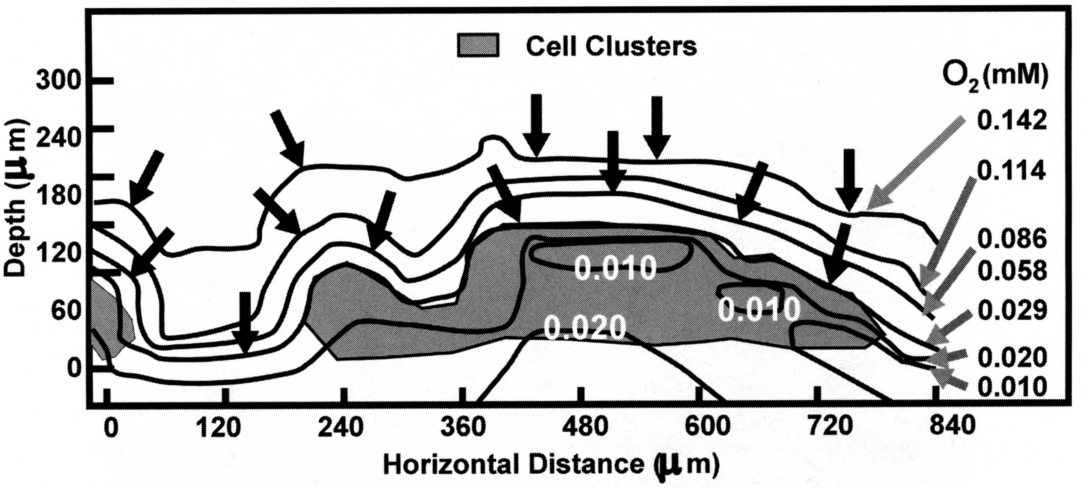


FIGURE 2 Isobar map of dissolved oxygen concentration as measured directly in a living biofilm by the use of a microelectrode, showing that the centers of microcolonies can be essentially anoxic, even when the biofilm is growing in ambient air.

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

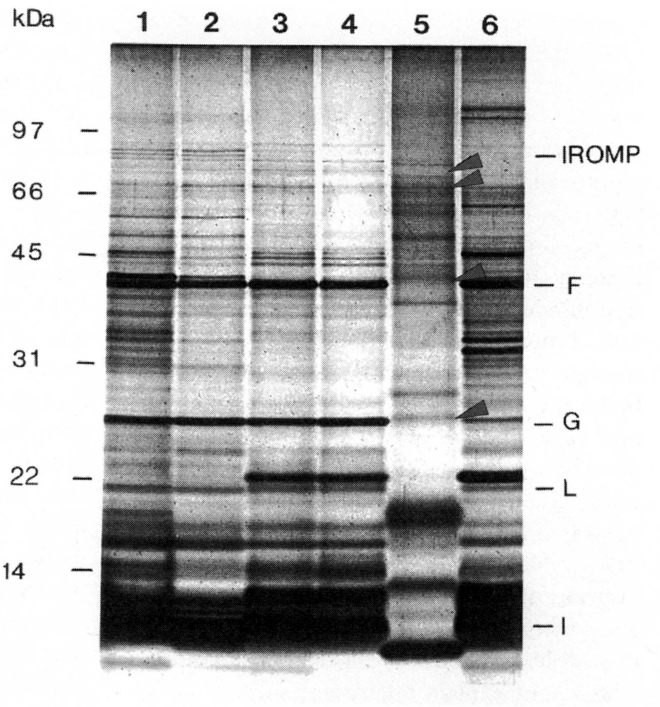


FIGURE 3 Polyacrylamide gel electrophoresis gel showing the pattern of production of OMPs by cells of *P. aeruginosa* in the biofilm mode of growth (lane 5) versus production by cells in the planktonic mode of growth (lanes 1 to 4 and 6). The differences in OMP production between these cells indicate 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 sequestrae 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-

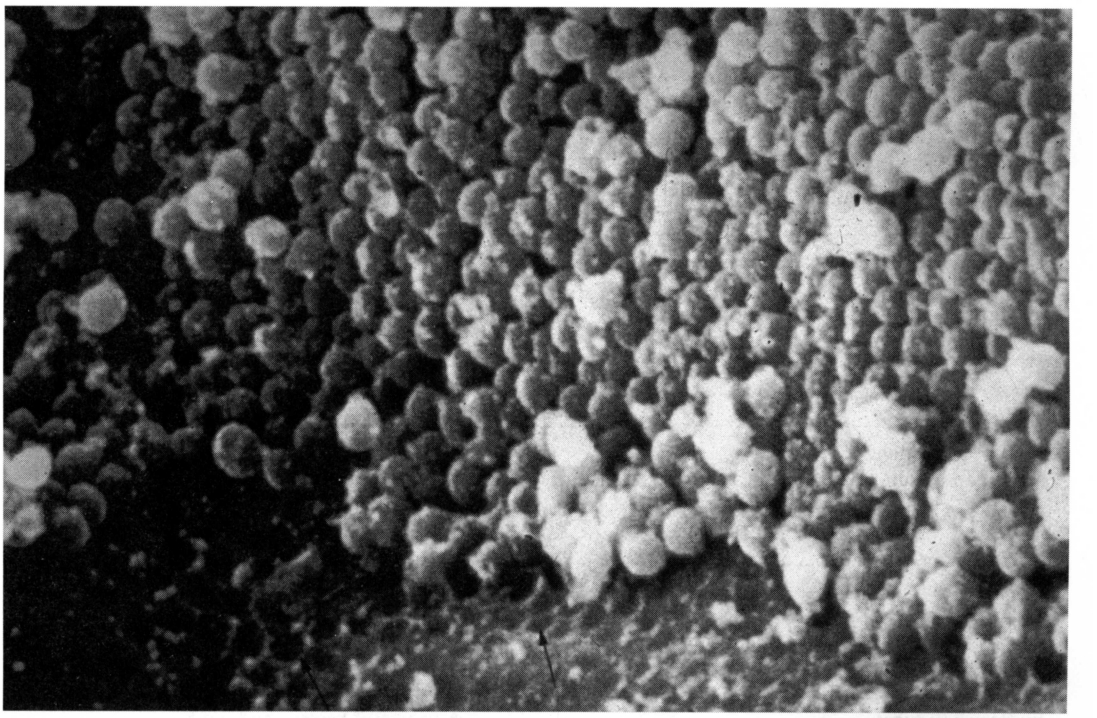


FIGURE 4 Scanning electron micrograph of an *S. aureus* biofilm on an endocardial pacemaker lead, showing spherical bacterial cells embedded in dehydration-condensed matrix material. These biofilm cells were resistant to a 6-week course of very high-dose antibiotic therapy.

film infection itself is not notably aggressive. We have found that all Tenckhoff catheters used in peritoneal dialysis (9) and all of the Hickman catheters used in cancer chemotherapy (33) are completely colonized with microbial biofilms on both their external and lumenal surfaces within 3 weeks of installation. Even though these relatively intrusive devices are completely colonized by bacterial biofilms, most patients are asymptomatic, and acute infections emanating from these sessile bacterial populations occur relatively rarely and appear to depend more on the host defenses than on the presence of the biofilms (10). Some of the most abundant biofilms that we have seen on medical devices occur on the surfaces of the copper wires that form parts of some IUDs (Fig. 5), but endometrial infections associated with these devices are very rare. The amount of surface area colonized by bacterial biofilms appears to have some bearing on the ability of these smoldering infections to trigger an acute infection, with dissemination of planktonic cells. For example, small pacemaker leads appear to be well tolerated, while the total artificial heart is not (17). The role of host defenses in controlling biofilm infections is discussed below.

The characteristic that distinguishes chronic device-related infections most clearly from acute bacterial infections is the response to antibiotic chemotherapy. While many acute infections can be eradicated by a single brief course of systemic antibiotic therapy, biofilm infections often respond incompletely and then recur. The first detailed examination of a device-related bacterial infection involved a patient who presented with a *Staphylococcus aureus* bacteremia secondary to an olecranon bursitis (26). The patient had been fitted with an endo-

TABLE 1 Partial list of human infections involving biofilms[a]

Infection or disease	Common biofilm bacterial species
Dental caries	Acidogenic gram-positive cocci (e.g., *Streptococcus*)
Periodontitis	Gram-negative anaerobic oral bacteria
Otitis media	Nontypeable strains of *Haemophilus influenzae*
Musculoskeletal infections	Gram-positive cocci (e.g., staphylococci)
Necrotizing fasciitis	Group A streptococci
Biliary tract infection	Enteric bacteria (e.g., *E. coli*)
Osteomyelitis	Various bacterial and fungal species, often mixed
Bacterial prostatitis	*E. coli* and other gram-negative bacteria
Native valve endocarditis	Viridans group streptococci
Cystic fibrosis pneumonia	*P. aeruginosa* and *Burkholderia cepacia*
Meloidosis	*Pseudomonas pseudomallei*
Nosocomial infections	
Intensive-care unit pneumonia	Gram-negative rods
Sutures	*S. epidermidis* and *S. aureus*
Exit sites	*S. epidermidis* and *S. aureus*
Arteriovenous shunts	*S. epidermidis* and *S. aureus*
Schleral buckles	Gram-positive cocci
Contact lens	*P. aeruginosa* and gram-positive cocci
Urinary catheter cystitis	*E. coli* and other gram-negative rods
Peritoneal dialysis (CAPD)[b] peritonitis	A variety of bacteria and fungi
IUDs	*Actinomyces israelli* and many others
Endotracheal tubes	A variety of bacteria and fungi
Hickman catheters	*S. epidermidis* and *Candida albicans*
Central venous catheters	*S. epidermidis* and others
Mechanical heart valves	*S. aureus* and *S. epidermidis*
Vascular grafts	Gram-positive cocci
Biliary stent blockage	A variety of enteric bacteria and fungi
Orthopedic devices	*S. aureus* and *S. epidermidis*
Penile prostheses	*S. aureus* and *S. epidermidis*

[a] Taken from reference 6 with permission.
[b] CAPD, chronic ambulatory peritoneal dialysis.

cardial pacemaker many years previously, and Marrie speculated that the planktonic bacteria that had caused the bacteremia could have colonized the plastic and metal surfaces of the pacemaker lead. Following 6 weeks of therapy with cloxacillin and rifampin, the pacemaker lead was removed and was found to be colonized by a biofilm composed of spherical cells (Fig. 4), and plating of macroscopic biofilm material scraped from these surfaces yielded $>10^8$ cells of *S. aureus*. From this case study it was clear that sessile cells of this gram-positive pathogen could survive prolonged exposure in vivo to relatively high levels of antibiotics that would readily have killed planktonic cells of the same species.

The exact extent of the inherent resistance of sessile bacteria to antibiotics required in vitro experimentation. *P. aeruginosa* biofilms that had been formed on urinary catheter material in flowing urine were treated with a variety of different antibiotics (28). These experiments, and many subsequent experiments by independent investigators, have now established that sessile bacterial cells are up to 1,000 times as resistant to antibiotics as their planktonic counterparts (7). It is clear that biofilm formation is a common survival strategy that serves prokaryotic organisms well in the human body, just as it serves them very well in environmental and industrial systems. Here, the experience of biofilm microbiologists who work in indus-

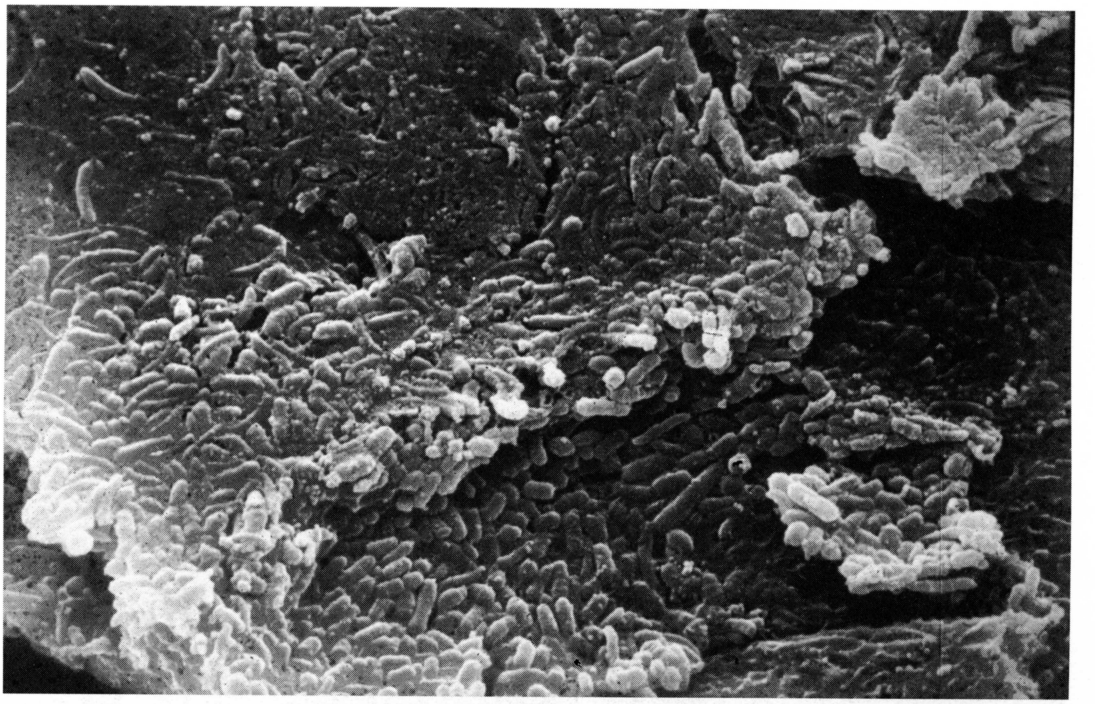


FIGURE 5 Scanning electron micrograph of a mixed-species bacterial biofilm on the copper component of a Copper 7 IUD worn by an asymptomatic patient.

trial systems can be useful in medical microbiology, because these people, who live with biofilm problems every day, have usually resorted to mechanical removal of sessile populations followed by high-dose biocide treatment. Practical clinical wisdom dictates that colonized devices must often be removed before the infections associated with them can be resolved by antibiotic therapy (21, 27).

Just as the inherent antibiotic resistance of biofilm infections correlates well with similar resistance to industrial biocides, the characteristic chronicity of these infections (6) correlates well with observations of biofilms in other ecosystems. Phagocytic white blood cells are as ineffective as wild amoebae in ingesting sessile bacteria (20), and nascent biofilms composed of only a few microcolonies of four to six cells enclosed in matrix material resist phagocytic clearance, even in fiercely defended sites like the peritoneum (36). The humoral immune system is stimulated by the presence of sessile bacteria growing in biofilms and by the frustrated response of phagocytic cells, and biofilms growing in tissues (like *P. aeruginosa* biofilms in the lung in cystic fibrosis) are seen to be surrounded by masses of immune complex (Fig. 6). Lam et al. (23) have shown that antibodies against *P. aeruginosa* are ineffective in clearing the pathogens in cystic fibrosis, and Cochrane et al. (5) have shown that the formation of immune complexes stimulates damaging cellular responses in which lysosomal enzymes are released. Generally, then, the body's host responses to biofilms are at best ineffective and at worst severely damaging to adjacent tissues. Some clinicians have resorted to immune suppression in order to dampen the body's immune reaction to the presence of sessile bacteria, which may persist for decades in such chronic diseases as cystic fibrosis and prostatitis, and thus to minimize tissue damage by frustrated phagocytosis.

The least understood characteristic of bio-

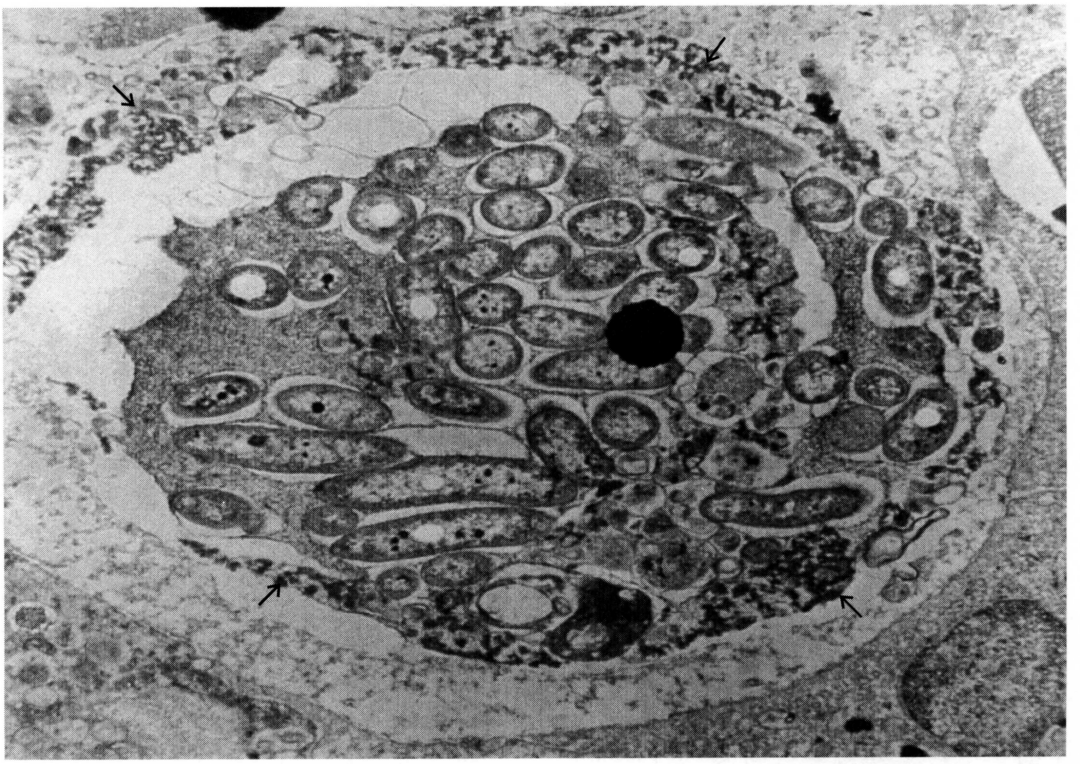


FIGURE 6 Transmission electron micrograph of a matrix-enclosed microcolony of cells of *P. aeruginosa* in the lung of a rat with a model infection designed to mimic cystic fibrosis in human patients. Note the dehydration-related shrinkage of the matrix material and the dark "crust" of immune complex material surrounding the microcolony.

film infections, including those associated with medical devices, is their cryptic, or indolent, nature. Again, this characteristic is explained by recent discoveries in biofilm microbiology. Biofilms are composed of bacterial cells (± 15% by volume) embedded in a voluminous exopolysaccharide matrix (± 85% by volume) in discrete microcolonies. Polysaccharides are generally poorly immunogenic and are not notably inflammatory, so the cellular and humoral immune systems of the body "see" only the lipopolysaccharide and protein molecules that protrude from the immunologically sequestered biofilm. For this reason, large areas of the surfaces of medical devices may be colonized without the patient suffering any detectable symptoms (10). On the other hand, biofilms are programmed to regularly release planktonic cells from the earliest stages of their formation on a surface, and the total number of planktonic cells that challenge the body's defenses depends on the size of the colonized area that is shedding these highly inflammatory cells. In our studies of transcutaneous medical devices (Tenckhoff and Hickman catheters), we have found that many patients are entirely asymptomatic, even when the total surface areas of their catheters are colonized by thick biofilms, and only a minority of these patients experience bacteremic infection.

Generally, chronic biofilm infections remain very localized, and even patients with cystic fibrosis with massive colonization of the lungs rarely experience infections in other organs. Small medical devices that are completely colonized by sessile bacteria (e.g., stitches and

staples) rarely elicit inflammation, and rarely give rise to disseminated infection. All transcutaneous devices are colonized eventually, especially on their outer surfaces, and inflammation may occur at the exit site and less often in the subcutaneous tract. These intrusive devices constitute a "bacterial highway" into internal tissues, but most are well tolerated, and the body's defenses can handle the small number of planktonic cells released from the surfaces of small devices, provided that these defenses are not radically compromised. The release of biofilm fragments occasioned by the replacement of vascular catheters over a "J wire" may damage downstream organs (e.g., lungs and brain), and the placement of a colonized Swann-Ganz catheter across an operating heart valve is profoundly unwise. Large transcutaneous devices with huge surface areas, like the Jarvik heart, cannot be tolerated when they become colonized by biofilms, but smaller devices are much less dangerous from a microbiological point of view. Devices that are inserted into organ systems, such as urinary catheters and IUDs, bypass the natural antibacterial barriers of these systems (27) and transfer external organisms into normally sterile areas. The consequences of this colonization of deep organs, and of the devices that are inserted into them, may be trivial if the organisms are well tolerated or devastating if they are not. Devices that are implanted, surgically and aseptically, may become colonized by bacterial biofilms as a result of contamination or of hematogenous spread of organisms from other sites. The microbiological risks posed by these colonizable surfaces will diminish as the surgery used in their implantation is improved and refined and as hematogenous sources (dental procedures) are controlled. Hip replacement seems to be leading the way with very low infection rates, and other very common procedures show gradually decreasing rates as surgical and management procedures are improved.

The key to improved prevention and control of biofilm infections, including those associated with medical devices, lies in the replacement of an old concept with a new one. While medical microbiologists have realized that plastic and metal devices increase the risk of infection, they have heretofore visualized the pathogens as planktonic cells, swimming or floating in tissue fluids near the device, that are essentially identical with the planktonic cells that they grow in liquid cultures. The new concept is simply that of the general biofilm, which recognizes that bacteria can grow in either of two basic phenotypes—planktonic or biofilm. Sessile cells differ profoundly from their planktonic counterparts, and these differences confer a high level of resistance to both natural host defenses and to antibiotics. Biofilm cells are partly hidden in their matrices, so they are less inflammatory than planktonic cells, and they may grow undetected for long periods of time. However, sessile populations constantly release planktonic cells that may produce symptoms or may even initiate acute infections. The basic strategy of bacteria in the production of two different phenotypes is to colonize new ecosystems with vulnerable planktonic "scouts" but to retain much of the genomic reserve of the species in a defensive sessile community, which can survive the vagaries and challenges of ecosystems in which it is already established. If we understand this microbial strategy we can craft counterstrategies that are likely to be more successful than our current reliance on vaccines and antibiotics that are chosen chiefly for their efficacy in killing planktonic cells.

CLINICAL STRATEGIES FOR THE CONTROL OF BIOFILM INFECTIONS

There is a growing conviction (6, 22) that antibiotics are losing their ability to control bacterial infections because the bacteria have mobilized all of their survival strategies in the face of this frontal attack. Strategies that were effective when these bacteria evolved near antibiotic-producing fungi in the soil are being utilized to thwart modern drugs, as are biofilm strategies that were effective in gaining predominance in primitive aquatic environments. It may be salutary to examine this question in areas in which biofilms are more completely

understood and in which biofilm microbiologists have been more successful in solving specific problems.

Biofilm Avoidance

Sessile bacteria, including matrix-enclosed fragments of biofilms, are resistant to host defenses and antibiotics surprisingly early in their development. Three- to 4-cell microcolonies on a plastic surface can withstand vigorous opsonin-induced phagocytosis (36), and 8- to 10-cell biofilm fragments can survive the pulmonary host defenses (23), even when they are deposited directly into the lower left lobe of the lung of a healthy rat or mouse. For this reason, it is imperative that medical devices be sterile when they are implanted and that they be free from surface deposits of organic material that can accelerate adhesion and biofilm formation by any planktonic cells that may be present in the operative field. The practice of soaking medical devices in a solution of an antibiotic immediately prior to their implantation may be useful in preventing subsequent biofilm formation, but we must remember that any biofilms of a resistant organism that might form during the soaking process would automatically cause an infection if the sessile population was mature enough to resist host defenses when it was installed. Medical devices therefore must be both scrupulously clean and absolutely sterile before they are implanted. Pulmonary exposure to biofilm fragments, from industrial sources or from dental water systems, must be avoided, and persistent pulmonary diseases due to these volatilized microcolonies are often presaged by the seroconversion of whole populations to specific pathogens in these aerosols.

Antibacterial Materials

Industry has spent billions of dollars on the development of plastics that inherently resist colonization by planktonic cells of adapted laboratory strains of bacteria, but these materials have done poorly in clinical trials. Wild-type bacteria use literally dozens of adhesion mechanisms, many of which are lost by adapted cells grown in culture, and no material has yet been found that inherently resists bacterial colonization and biofilm formation. Metals, such as silver and copper, are very heavily colonized by bacteria in natural and industrial systems: the thickest biofilm that we have ever found on a medical device was seen on the copper wires of an IUD (26). Modern antibacterial materials are more likely to rely on the release of toxic molecules or ions from the surface of the material, so that "incoming" planktonic bacterial cells will be killed before they have the opportunity to form a resistant biofilm. These "doped" materials often have excellent antibacterial properties, because the molecules and ions released from their surfaces are effective and because they reach the bacteria before they can adopt the resistant biofilm phenotype. Of course, these putative antibacterial materials depend on the release of specific molecules or ions from their surfaces, and this controlled release can maintain only a local bactericidal concentration of the agent for sufficient time to be clinically effective. A fast-releasing agent will necessarily give protection for a shorter time, especially if it is being simultaneously removed by either diffusion or fluid flow. Hundreds of putative antibacterial materials are being offered to the medical-device industry, and all are offered with strong data concerning release rates and efficacy. What is urgently needed to discriminate among this plethora of "antibacterial" materials is a test system that uses fresh clinical isolates, mimics the flow characteristics of the system for which the device is designed, and uses direct observations to determine the efficacy of bacterial killing. Microbiological scraping and plating techniques for the recovery of sessile bacteria from biofilms on solid surfaces are notoriously unreliable (28), and materials have been judged to be antibacterial simply because the sessile cells in biofilms on their surfaces were difficult to remove (G. Cooke, J. W. Costerton, and R. O. Darouiche, submitted for publication). The most accurate method for the assessment of antibacterial efficacy is to expose the material to cells of a wild strain of the putative pathogen, in a realistic flow regimen, and to monitor the formation of biofilms

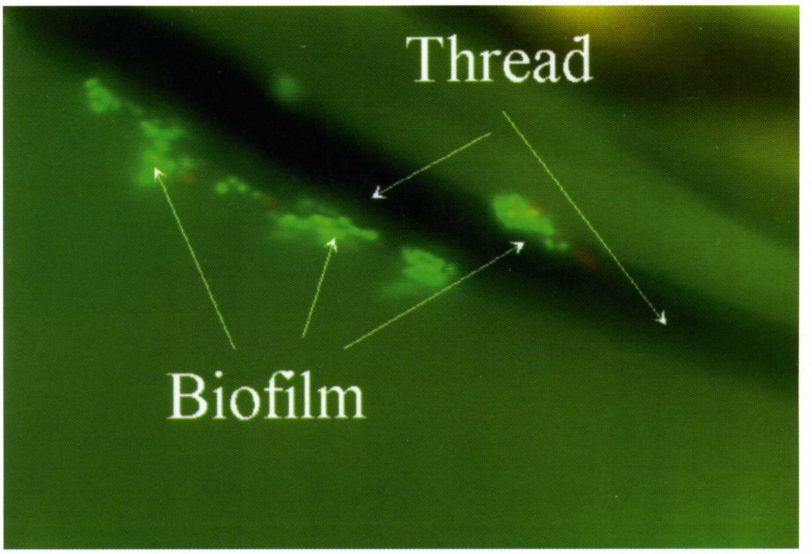


FIGURE 7 Confocal scanning laser micrograph of a silver-coated sewing cuff fabric designed for a mechanical heart valve. This thread had been exposed to cells of *S. epidermidis*, which had colonized its surface to produce matrix-enclosed microcolonies containing living (green) and a few dead (orange and red) cells in a developing biofilm.

by direct microscopy using methods that determine cell viability (34). Figure 7 shows the colonization of threads of a putative antibacterial material that have been exposed to cells of a wild strain of *S. epidermidis*, with the resultant formation of a biofilm within which the bacteria are alive, as indicated by their green appearance with the BacLite viability stain (Cook et al., submitted). Scrape-and-plate testing had yielded favorable data, but direct observation of the luxurious colonization of this material by the predominant pathogen throws the efficacy of this putative antibacterial material into some doubt.

Mechanical Removal and Adequate Antibiotic Dosage

Most clinical experts (17, 21, 27) counsel the removal of medical devices that have become obvious foci of bacterial infection. Like the industrial biofilm microbiologists who also rely on the mechanical removal of fouled surfaces from their systems, they usually combine this removal with high doses of antibacterial agents in order to kill the planktonic cells and the sessile bacteria that are inevitably released in these procedures. Similarly, high-dose antibiotic therapy is often used to protect the microbiologically vulnerable plastic and metal surfaces of recently installed medical devices from colonization by planktonic cells and biofilm fragments released by dental procedures and other disruptive manipulations. Epithelial surfaces are much more resistant to the formation of bacterial biofilms than are the plastic and metal surfaces of medical devices, so that device sites often heal well in the absence of the device and it can sometimes be reinstalled successfully when the infection has entirely subsided. Gristina called this competition between the host epithelium and the bacterial biofilm the "race for the surface" (17). It will be a fitting memorial to this investigator if we hold this image in our minds as we visualize the epithelialization of a recently implanted device and judge the time when the patient's epithelium may have won this race.

For many decades, biofilms in industrial systems thrived and caused immeasurable damage because small doses of biocides that were

known to kill planktonic cells were used continuously in the systems at risk. Billions of dollars were lost before industrial biofilm microbiologists learned that these small continuous antibiotic doses had no effect because they did not kill appreciable numbers of protected sessile organisms. In contrast, high "shock" doses of antibiotics produced complete killing. Because of the serious risks of toxicity, shock treatment is not normally an option, but clinicians have learned that prolonged systemic application of the highest tolerable doses of antibiotics has a salutary effect on biofilm diseases such as endocarditis and cystic fibrosis. Higher doses of antibiotics that have some chance of killing sessile bacteria have been used in topical applications (e.g., vancomycin for exit site infections) and in local irrigation of infected medical devices, but we must remember that biofilms are typically resistant to concentrations 1,000 times those that kill planktonic cells. The minimum biofilm elimination concentration test system developed at the University of Calgary (3) indicates to the clinician the dose of an antibiotic that will kill all of the sessile cells in a biofilm. It is sobering to reflect that a biofilm under attack by an antibiotic contains a broad spectrum of sessile cells in a wide variety of physiological states that see an equally broad spectrum of antibiotic concentrations, and it is difficult to imagine a situation more well suited to the development of classic antibiotic-resistant strains. Virtually all modern antibiotics were selected because they were useful in killing planktonic bacteria in preliminary screens, and pharmaceutical firms have only begun to search for agents that affect sessile populations preferentially. As we begin to match the agents and the selection procedures more exactly to the mode of growth and the bacterial phenotype that we see directly in modern infections, we may have more effective antibiotics to use in the fight to control device-related and other chronic bacterial infections.

Physical Manipulation of Biofilms In Situ

If a biofilm is thought of as a well-defended adherent population in which the sessile cells resist antibacterial agents by virtue of their altered phenotype and by virtue of their position in a protective matrix, the mind of the engineer turns automatically to methods to disrupt this defensive enclave. Pitt and his engineering colleagues at Brigham Young University (29) have pioneered the use of ultrasound to increase the susceptibility of sessile cells to conventional antibiotics, and they report excellent efficacy, especially with the use of pulsed untrasound. Another physical manipulation that reduces the inherent resistance of sessile bacteria to antibiotics is the application of a DC electric field across the biofilm (37). Engineers at the CBE have shown that the resistance of sessile bacteria can be reduced to approximately that of planktonic cells by the application of a DC field across the biofilm, even at field strengths as low as 2 mA/cm^2. The mechanism of this "bioelectric" enhancement of the susceptibility of sessile cells to killing by antibiotics is not fully understood, but the available evidence points to enhanced penetration of the biofilm matrix, membrane disturbance by cation depletion, and the local generation of oxidizing ions, such as peroxide. It would seem to be entirely logical to use physical manipulations to enhance the efficacy of antibiotic therapy in any device-related infection in which the device is accessible, especially when it is metallic and sufficiently conductive to act as an electrode for the establishment of a DC electric field.

Behavioral Manipulation of Biofilms by Signaling Molecules

We now realize that both the formation of biofilms by adherent cells (11) and the detachment of planktonic cells from developed biofilms are controlled by acyl homoserine lactones in a large number (perhaps a majority) of gram-negative species. Preliminary data suggest that peptide signals control these same behaviors in gram-positive species (15), and even more preliminary data suggest that some signals control the behavior of many species in a given ecosystem while other signals control the behavior of a single species. Kolter and Losick (22) have predicted that behavior modification by signal-

ing molecules and their analogs will supplement and/or replace the use of conventional antibiotics in the near future. Now that commercial entities connected with the CBE have produced acyl homoserine lactone signal analogs that prevent biofilm formation and promote the detachment of planktonic cells from mature biofilms, the way forward towards their use in the treatment of biofilm infections seems clear. We predict that signals and signal analogs that either block or reinforce signal-controlled behaviors will be used to interdict specific mechanisms by which bacterial pathogens affect their hosts. This interdiction may be as simple as adding molecular blockers of the signals that control toxin production so that a pathogen can occupy its ecological niche, much as *Clostridium difficile* occupies its niche in the large intestine, but cannot produce the toxin that is central to its attack on tissues. Another signal that affected the ability of the pathogen to integrate itself into the ecology of the tissue could then be activated, and the organism would cease to occupy its ecological niche. The strategy behind this approach is to shut off pathogenesis without putting the pathogen under a toxic frontal attack that stimulates its survival mechanisms and that produces resistant strains.

Biofilm diseases would constitute the ideal targets for the manipulation of bacterial behavior, because they depend on the sessile mode of growth as their central pathogenic mechanism. If cells of a single species (e.g., *S. epidermidis*) formed a biofilm on a medical device (e.g., the sewing cuff of a mechanical heart valve), the simple stimulation of their detachment from this colonized surface would lead to their deaths, if the patient's circulating level of antibiotics was suitably elevated. Alternatively, signal analogs that block biofilm formation or promote detachment even as bacteria start to adhere could be incorporated into the biomaterials used to produce these medical devices, and they would completely escape microbial colonization. Even in non-device-related chronic bacterial infections, such as prostatitis (27), the detachment of planktonic cells from biofilms formed by cells of *Escherichia coli* would remove the infecting sessile population and allow the clearance of these recalcitrant infections by high-dose antibiotic chemotherapy. Biofilm diseases offer the burgeoning companies that propose to use signals to manipulate bacterial behavior a target that is even more attractive than the lucrative targets offered by industrial systems. While the bacterial populations that cause fouling and corrosion in industrial water systems are composed of many species, and some universal signal analogs are already in use (14), biofilm infections often involve only one species, and the selection of suitable signals may be facilitated. There can be little doubt that Kolter and Losick (22) are correct in their predictions, and infectious-disease practitioners will soon have therapeutics at their disposal whose activities are predicated on natural signaling mechanisms.

ACKNOWLEDGMENT

This work was supported by cooperative agreement ECD-8907039 with the National Science Foundation.

REFERENCES

1. **Brown, M. R. W., D. G. Allison, and P. Gilbert.** 1988. Resistance of bacterial biofilms to antibiotics: a growth-rate related effect. *J. Antimicrob. Chemother.* **22:**777–783.
2. **Brown, M. R. W., and P. Williams.** 1985. The influence of environment on envelope properties affecting survival of bacteria in infections. *Annu. Rev. Microbiol.* **39:**527–556.
3. **Ceri, H., M. E. Olson, C. Stremick, R. R. Read, D. Morck, and A. Buret.** 1999. The Calgary biofilm device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J. Clin. Microbiol.* **37:**1771–1776.
4. **Chen, X., and P. S. Stewart.** 1996. Chlorine penetration into artificial biofilm is limited by a reaction-diffusion interaction. *Environ. Sci. Technol.* **30:**2078–2083.
5. **Cochrane, D. M. G., M. R. W. Brown, H. Anwar, P. H. Weller, K. Lam, and J. W. Costerton.** 1988. Antibody response to *Pseudomonas aeruginosa* surface protein antigens in a rat model of chronic lung infection. *J. Med. Microbiol.* **27:**255–261.
6. **Costerton, J. W., P. S. Stewart, and E. P. Greenberg.** 1999. Bacterial biofilms: a common cause of persistent infections. *Science* **284:** 1318–1322.

7. **Costerton, J. W., Z. Lewandowski, D. E. Caldwell, D. R. Korber, and H. M. Lappin-Scott.** 1995. Microbial biofilms. *Annu. Rev. Microbiol.* **49:**711–745.
8. **Costerton, J. W., G. G. Geesey, and G. K. Cheng.** 1978. How bacteria stick. *Sci. Am.* **238:** 86–95.
9. **Dasgupta, M. K., K. B. Bettcher, R. A. Ulan, V. Burns, K. Lam, J. B. Dossetor, and J. W. Costerton.** 1987. Relationship of adherent bacterial biofilms to peritonitis in chronic ambulatory peritoneal dialysis. *Peritoneal Dialysis Bull.* **7:** 168–173.
10. **Dasgupta, M. K., and J. W. Costerton.** 1989. Significance of biofilm-adherent bacterial microcolonies on Tenckhoff catheters in CAPD patients. *Blood Purif.* **7:**144–155.
11. **Davies, D. G., M. R. Parsek, J. P. Pearson, B. H. Iglewski, J. W. Costerton, and E. P. Greenberg.** 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* **280:**295–298.
12. **Davies, D. G., and G. G. Geesey.** 1995. Regulation of the alginate biosynthesis gene *algC* in *Pseudomonas aeruginosa* during biofilm development in continuous culture. *Appl. Environ. Microbiol.* **61:**860–867.
13. **de Beer, D., R. Srinivasan, and P. S. Stewart.** 1994. Direct measurement of chlorine penetration into biofilms during disinfection. *Appl. Environ. Microbiol.* **60:**4339–4344.
14. **De Nys, R., P. D. Steinberg, P. Willemsen, S. A. Dworjanyn, C. L. Gabelish, and R. J. King.** 1995. Broad spectrum effects of secondary metabolites from the red alga *Delisea pulchra* in antifouling assays. *Biofouling* **8:**259–271.
15. **Dunny, G. M., and B. A. Leonard.** 1997. Cell-cell communication in Gram-positive bacteria. *Annu. Rev. Microbiol.* **51:**527–564.
16. **Foley, I., P. Marsh, E. M. H. Wellington, A. W. Smith, and M. R. W. Brown.** 1999. General stress response master regulator *rpoS* is expressed in human infection: a possible role in chronicity. *J. Antimicrob. Chemother.* **43:**164–165.
17. **Cristina, A. G., J. J. Dobbins, B. Giamara, J. C. Lewis, and W. C. DeVries.** 1988. Biomaterial-centered sepsis and the total artificial heart: microbial adhesion versus tissue integration. *J. Am. Med. Assoc.* **259:**870–877.
18. **Gristina, A. G., and J. W. Costerton.** 1984. Bacteria-laden biofilms: a hazard to orthopedic prostheses. *Infect. Surg.* **3:**655–662.
19. **Huang, C.-T., F. Yu, G. A. McFeters, and P. S. Stewart.** 1995. Nonuniform spatial patterns of respiratory activity within biofilms during disinfection. *Appl. Environ. Microbiol.* **61:**2252–2256.
20. **Jensen, E. T., A. Kharazmi, K. Lam, J. W. Costerton, and N. Hoiby.** 1990. Human polymorphonuclear leukocyte response to *Pseudomonas aeruginosa* biofilms. *Infect. Immun.* **58:** 2383–2385.
21. **Khoury, A. E., K. Lam, B. Ellis, and J. W. Costerton.** 1992. Prevention and control of bacterial infections associated with medical devices. *ASAIO J.* **38:**M174–M178.
22. **Kolter, R., and R. Losick.** 1998. All for one and one for all. *Science* **280:**226–227.
23. **Lam, J., R. Chan, K. Lam, and J. W. Costerton.** 1980. Production of mucoid microcolonies by *Pseudomonas aeruginosa* within infected lungs in cystic fibrosis. *Infect. Immun.* **28:**546–556.
24. **Lambe, D. W., Jr., K. P. Ferguson, K. J. Mayberry-Carson, B. Tober-Meyer, and J. W. Costerton.** 1991. Foreign-body-associated experimental osteomyelitis induced with *Bacteroides fragilis* and *Staphylococcus epidermidis* in rabbits. *Clin. Orthop.* **266:**285–294.
25. **Lewandowski, Z., W. Lee, W. G. Characklis, and B. Little.** 1989. Dissolved oxygen and pH microelectrode measurements at water immersed metal surfaces. *Corrosion* **45:**92–98.
26. **Marrie, T. J., and J. W. Costerton.** 1984. Scanning and transmission electron microscopy of in situ bacterial colonization of intravenous and intraarterial catheters. *J. Clin. Microbiol.* **19:** 687–693.
27. **Nickel, J. C., J. W. Costerton, R. J. C. McLean, and M. Olson.** 1994. Bacterial biofilms: influence on the pathogenesis, diagnosis and treatment of urinary-tract infections. *J. Antimicrob. Chemother.* **33:**31–41.
28. **Nickel, J. C., I. Ruseska, J. B. Wright, and J. W. Costerton.** 1985. Tobramycin resistance of *Pseudomonas aeruginosa* cells growing as a biofilm on urinary catheter material. *Antimicrob. Agents Chemother.* **27:**619–624.
29. **Pitt, W. G., M. O. McBride, J. K. Lunceford, R. J. Roper, and R. D. Sagers.** 1994. Ultrasonic enhancement of antibiotic action on gram-negative bacteria. *Antimicrob. Agents Chemother.* **38:**2577–2582.
30. **Stewart, P. S.** 1996. Theoretical aspects of antibiotic diffusion into microbial biofilms. *Antimicrob. Agents Chemother* **40:**2517–2522.
31. **Stoodley, P., I. Dodds, Z. Lewandowski, A. B. Cunningham, J. D. Boyle, and H. M. Lappin-Scott.** 1999. Influence of hydrodynamics and nutrients on biofilm structure. *J. Appl. Microbiol.* **85:**19S–28S.
32. **Suci, P., M. W. Mittelman, F. P. Yu, and G. G. Geesey.** 1994. Investigation of ciprofloxacin penetration into *Pseudomonas aeruginosa* biofilms. *Antimicrob. Agents Chemother.* **38:**2125–2133.
33. **Tenney, J. H., M. R. Moody, K. A. Newman,**

S. C. Schimpff, J. C. Wade, J. W. Costerton, and W. P. Reed. 1986. Adherent microorganisms on lumenal surfaces of long-term intravenous catheters: importance of *Staphylococcus epidermidis* in patients with cancer. *Arch. Intern. Med.* **146:**1949–1954.

34. **Terzieva, S., J. Donnelly, V. Ulevicius, S. A. Grinshpun, K. Willeke, G. N. Selma, and K. P. Brenner.** 1996. Comparison of methods for detection and enumeration of airborne microorganisms collected by liquid impingement. *Appl. Environ. Microbiol.* **62:**2264–2272.
35. **Vrany, J. D., P. S. Stewart, and P. A. Suci.** 1997. Comparison of recalcitrance to ciprofloxacin and levofloxacin exhibited by *Pseudomonas aeruginosa* biofilms displaying rapid-transport characteristics. *Antimicrob. Agents Chemother.* **41:** 1352–1358.
36. **Ward, K. H., M. E. Olson, K. Lam, and J. W. Costerton.** 1992. Mechanism of persistent infection associated with peritoneal implants. *J. Med. Microbiol.* **36:**406–413.
37. **Wellman, N., S. M. Fortun, and B. R. McLeod.** 1996. Bacterial biofilms and the bioelectric effect. *Antimicrob. Agents Chemother.* **40:** 2012–2014.
38. **Wentland, E., P. S. Stewart, C.-T. Huang, and G. A. McFeters.** 1996. Spatial variations in growth rate within *Klebsiella pneumoniae* colonies and biofilm. *Biotechnol. Prog.* **12:**316–321.
39. **Xu, K. D., P. S. Stewart, F. Xia, C.-T. Huang, and G. A. McFeters.** 1998. Spatial physiological heterogeneity in *Pseudomonas aeruginosa* biofilm is determined by oxygen availability. *Appl. Environ. Microbiol.* **64:**4035–4039.
40. **Yu, F. P., and G. A. McFeters.** 1994. Rapid *in situ* assessment of physiological activities in biofilms using fluorescent probes. *J. Microbiol. Methods* **20:**1–10.