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BIOMATERIALS SCIENCE

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4.8 BIOFILMS, BIOMATERIALS, AND DEVICE-RELATED INFECTIONS

Bill Costerton, Guy Cook, Mark Shirtliff,
Paul Stoodley, and Mark Pasmore

INTRODUCTION

Tens of millions of medical devices are used each year and, in spite of many advances in biomaterials, a significant proportion of each type of device becomes colonized by bacteria and becomes the focus of a device-related infection. Topical devices (e.g., contact lenses) are colonized as soon as they are placed on tissue surfaces, transcutaneous devices (e.g., vascular catheters) are progressively colonized by skin organisms, and even surgically implanted devices regularly become foci of infection. Implanted devices may be colonized by bacteria at the time of surgery, or they may be colonized by organisms that gain access to their surfaces by a hematogenous route, from a distant source. The most significant factor in the development of device-related infections appears to be the skill of the surgical team, with prosthetic hips being infected in less than 0.2% of cases in large specialized clinics, and as many as 4% in less proficient facilities. Generally, large and complex medical devices that require long and complicated surgery for their placement are at high risk of bacterial infection, and transcutaneous devices in this category (e.g., the Jarvik heart) automatically become infected. In many areas of medicine, the risk of infection limits the use of devices that constitute the epitome of the engineer's skill and imagination and incorporate the finest and most sophisticated biomaterials available in this fast-moving field.

As medical devices came into more regular use, the surgeons who placed them used their well-developed observation skills to define the “classic” device-related infection. These infections were often very slow to develop, with overt symptoms sometimes being seen almost immediately and sometimes being seen months or even years after the device was installed. Inflammation and pus formation were often local, especially in transcutaneous devices, but a certain proportion of patients with device-related infections suddenly developed acute disseminated infections caused by the same species that had colonized the device. These acute exacerbations of device-related infections responded well to antibiotic therapy. However, this treatment almost never reversed the local symptoms, and colonized devices often gave rise to a predictable series of acute exacerbations, so that good medical management usually dictated their removal. The bacteria that caused device-related infections were common skin biota (e.g., *Staphylococcus epidermidis*) and common environmental organisms (e.g., *Pseudomonas aeruginosa*), and certain species predominated in infections of certain devices. Because the infecting bacteria, and occasional fungi (e.g., *Candida albicans*), were so ubiquitous in the modern human environment, device recipients always had good immunity against these low-level pathogens, but these antibodies failed to prevent infection. It was the “front-line” medical specialists (e.g., orthopedic surgeons) who gradually persuaded medical microbiologists

and infectious disease specialists that device-related infections differed from acute bacterial infections in several important respects.

The biofilm concept was developed and articulated (Costerton *et al.*, 1978) in environmental microbiology, and it was introduced into medical microbiology when Tom Marrie *et al.* (1982) examined the surfaces of devices that had failed because of bacterial infection. This concept states that bacteria, in all but the most nutrient-deprived ecosystems, grow preferentially in matrix-enclosed communities attached to surfaces (Costerton *et al.*, 1987). Electron microscopy proved to be useful in the examination of the surfaces of failed medical devices, because both scanning (SEM) and transmission (TEM) electron microscopy involve dozens of washing steps that remove floating or loosely adherent bacteria. Therefore any bacterial or fungal cells that remained on the surfaces of the device, after processing, were *bona fide* biofilm organisms. With medical colleagues leading the search (Khoury *et al.*, 1992; Marrie and Costerton, 1984; Nickel *et al.*, 1985), our morphological team examined hundreds of types of failed medical devices and found biofilms on all of their surfaces. Biofilms were seen on the surfaces of contact lenses that had been worn by volunteers (McLaughlin-Borlace *et al.*, 1998), and very extensive sessile communities were seen on the surfaces of lenses that had been stored overnight in storage cases (Gray *et al.*, 1995; McLaughlin-Borlace *et al.*, 1998). Some of the most extensive biofilms we ever saw on a medical device were found on the surfaces of intrauterine contraceptive devices (Marrie *et al.*, 1982), and teeth and dental devices were equally heavily colonized. It was in this area of topical medical devices that the distinction was made between colonization, which is the simple presence of microbial biofilms on a surface, and the infections that occur when this presence of a biofilm elicits a pathogenic response.

The surface of skin is colonized by a wide variety of bacteria and fungi, most of which are removed or killed by surgical preparations, but the deeper layers are also colonized by bacteria (mostly *S. epidermidis*) that escape skin sterilants. This cutaneous biota rapidly colonizes the surfaces of any transcutaneous device, and the biofilm moves along any device that is placed in a subcutaneous "tunnel," until the entire surface of the device is colonized. In this manner a microbial biofilm is introduced into the normally sterile environment of the peritoneum, by the Tenckhoff catheter (Dasgupta *et al.*, 1987), or into the normally sterile environment of the heart, by devices like the Hickman (Tenney *et al.*, 1986) and the Swan-Ganz (Mermel *et al.*, 1991) catheters. The inevitable colonization of transcutaneous devices, which is usually complete in 3–4 weeks, does not automatically lead to infection. All of the Hickman catheters in our National Cancer Institute study (Tenney *et al.*, 1986) were seen to be colonized, and one was even partially blocked by a very exuberant biofilm, but only four of the 81 patients experienced overt infection and bacteremia. Chronic ambulatory peritoneal dialysis (CAPD) patients all have well-developed biofilms on their Tenckhoff catheters, but many do not develop peritonitis if their humoral and cellular immune mechanisms can "keep up" (Dasgupta *et al.*, 1990) with the planktonic (floating) cells that are released from these sessile communities.

When implanted medical devices become colonized, the presence of these microbial biofilms always triggers pathogenic changes in the surrounding tissues, but symptoms are often slow to develop. Mechanical heart valves and vascular grafts can fail because biofilms on stitches that hold them in place cause inflammation, weaken the tissues involved, and lead to their detachment and displacement (Hyde *et al.*, 1998). Orthopedic devices may develop "aseptic loosening" in that the device is loosened by bone dissolution, but there are no signs of inflammation. The biofilms of the causative pathogens are so coherent that routine cultures of the device and the tissues are almost always negative. Biofilms elicit few symptoms, because their matrix-enclosed cells produce few toxins and stimulate only cursory immune responses and inflammation, but local symptoms will be produced when planktonic cells are released from these sessile communities.

The examination of failed medical devices frequently reveal microbial biofilms. Therefore, the unique characteristics of device-related infections can be explained in terms of the characteristics of biofilms (Costerton *et al.*, 1999). The slow development and asymptomatic nature of many device-related infections can be explained by the observation that biofilm bacteria produce few toxins and elicit little inflammatory response. Many device-related infections are negative in routine microbiological cultures because biofilms release a limited number of planktonic cells, large biofilm fragments grow up as a single colony on plates, and sessile cells do not grow well on agar surfaces. Common bacterial species predominate in device-related infections because they form biofilms very effectively in their natural environments (e.g., skin), and this biofilm mode of growth protects them from the immune responses that occur in all potential hosts. The biofilm mode of growth protects the causative agents of device-related infections from both humoral and cell-mediated immunity (see Chapter 4.3) (Leid *et al.*, 2002), so these infections occur in healthy individuals, and they are never resolved by even the most active host defense mechanisms. Exacerbations of device-related infections are caused by the release of planktonic cells, and antibiotics can kill these floating cells and reverse the symptoms of acute infection, but the infection persists because the causative biofilm is resistant to these antibacterial agents. Most, if not all, of the characteristics of device-related infections can be explained in terms of the characteristics of biofilms, so it may be useful to examine the burgeoning field of biofilm microbiology, as an early step in the search for new biomaterials that will control these infections.

BIOFILM MICROBIOLOGY

Many of the concepts and techniques that have served microbiologists well, in the virtual conquest of epidemic bacterial diseases caused by planktonic organisms, now serve us only poorly in the study of device-related and other chronic bacterial diseases. This section on biofilm microbiology will focus on the central fact that biofilm bacteria differ from their planktonic counterparts in so many ways that they are as different as spores are from vegetative bacteria, and it is imperative

that special biofilm methods be used in studies of the bacterial colonization of **biomaterials**.

Bacterial Adhesion to Surfaces

Often, the DLVO theory is applied to the study of bacterial adhesion to surfaces (van Loosdrecht *et al.*, 1990). This classic concept of colloid behavior visualizes a planktonic bacterial cell as a smooth colloid particle that interacts with the surface in a manner based on the charges on both surfaces, which overcome the basic repulsion of individual particles. Examinations of the surfaces of planktonic bacteria, using special preparations and electron microscopy, have clearly shown that these surfaces are not smooth. In addition to proteinaceous appendages (flagella and pili) that project 2–6 μm from the cell, the entire surfaces of planktonic cells of natural strains of bacteria are covered by a matrix of hydrophobic exopolysaccharide (EPS) fibers, and sometimes by a highly structured protein “coat.” The external EPS layer of planktonic cells is anchored to the polysaccharide O antigen fibers that project from the lipopolysaccharide (LPS) of the outer membrane of gram-negative cells, and to the polysaccharide teichoic acid fibers that project from the cell walls of gram-positive cells. Elegant freeze-substitution microscopy preparations have shown that the actual surface of planktonic bacterial cells that would be capable of interacting with the surface to be colonized is a 0.2 to 0.4- μm -thick forest of protein and polysaccharide fibers. The planktonic bacterial cell is not a smooth-surfaced colloid particle, and the actual interaction of these cells with surfaces is based on the bridging of bacterial fibers with fibers adsorbed to the surface being colonized. Thus, DLVO theory is of limited application in the study of bacterial adhesion.

Another conventional microbiology method, the reliance on pure cultures of bacteria isolated from the system of interest, but subcultured hundreds of times in rich media, also does not serve us well in biofilm studies relevant to medical devices. This method, which dates from Robert Koch in the 1850s, produces lab-adapted strains of bacteria that are selected in favor of planktonic growth, because the simple act of subculturing leaves adherent cells behind in the old culture and transfers only free-floating cells. These lab-adapted strains lack many surface structures that would be necessary for their survival in a hostile “wild” environment, but they are not challenged by antibacterial agents, so they survive in the test tube, but perish if they are released into natural ecosystems. When these lab-adapted strains are used in studies of bacterial adhesion to **biomaterials**, they come close to the smooth-surfaced colloidal particles visualized in the DLVO theory, and data that are misleading for the understanding of medical-device-centered infection are generated. Several companies have spent millions of dollars on novel **biomaterials** to which lab-adapted strains of bacteria would adhere to only very poorly, only to have these **biomaterials** heavily colonized by “wild” natural bacteria, and to find that they performed unsatisfactorily in clinical tests. Most microbiologists who focus on biofilms never do adhesion experiments on strains that are more than one transfer from an infected patient, if their objective is to assess the propensity of a biomaterial for colonization by a putative pathogen. Scientists

at the FDA and EPA are aware of this necessity to use “wild” bacteria in **biomaterials** testing.

When planktonic cells adhere to a surface, which they do with considerable avidity, they exhibit behaviors that have been divided into “reversible” and “irreversible” patterns (Marshall *et al.*, 1971). The most actively motile organisms (e.g., *P. aeruginosa*) may use their flagella as landing mechanisms, and then may use their type IV pili to produce a twitching motility that allows them to pile up into elaborate structures, some of which resemble the fruiting bodies of the myxobacteria. Other less mobile organisms produce “windrows” of cells (Korber *et al.*, 1995) following adhesion, while cells that have neither flagella nor pili simply stay in place if the location is favorable, and detach if it is not. Movies showing these post-adhesion behaviors of bacteria are available on the Center for Biofilm Engineering (CBE) Web site (www.erc.montana.edu). Biofilm engineers have generated surprising data (Stoodley *et al.*, 2001a, b) showing that many cells that adhere to surfaces also detach and leave the area, before they make the genetic switch to attach irreversibly and initiate the process of biofilm formation. Many people in the **biomaterials** field have speculated, intuitively, that key surface characteristics must favor (or inhibit) bacterial adhesion, and almost every possible combination of these characteristics has been tried in the search for colonization-resistant **biomaterials**. Wild bacteria adhere equally well to very hydrophobic (e.g., Teflon) and to very hydrophilic (e.g., PVC) surfaces, they colonize smooth surfaces as well as they adhere to rough surfaces (Marrie and Costerton, 1984; Settlemire *et al.*, 1986), and they colonize smooth surfaces in very high shear flow systems (Characklis, 2003). Thus, we have no perfect biomaterial surface that resists bacterial colonization by virtue of its inherent surface properties, but nonfouling surfaces show limited potential for this application (Chapter 2.13).

Biofilm Formation on Surfaces

When a bacterial cell has “made the decision” to colonize a surface it sets in motion a pattern of gene expression that profoundly alters its previous planktonic phenotype, to produce a unique biofilm phenotype that may differ by as much as 70% in the proteins expressed (Sauer and Camper, 2001). Some of the first genes that are up-regulated in adherent cells are those involved in the production of the EPS material that will form the matrix of the biofilm and will also anchor the cell irreversibly to the surface. In *P. aeruginosa* the up-regulation of *algC*, which is a part of the alginate synthesis pathway, occurs within 18 minutes of initial cell adhesion (Davies and Gessley, 1995), and we see the secretion of matrix material by these cells within 30 minutes of adhesion. The genes that are up-regulated in the biofilm phenotype of many bacterial species are being analyzed by proteomics (Miller and Diaz-Torres, 1999; Oosthuizen *et al.*, 2002; Sauer and Camper, 2001; Sauer *et al.*, 2002; Svensater *et al.*, 2001; Tremoulet *et al.*, 2002a, b) and by microarray analysis (Schemm *et al.*, 2003; Schoolnik *et al.*, 2001; Stoodley *et al.*, 2002; Wagner *et al.*, 2003; Whiteley *et al.*, 2001), and individual genes involved in this profound phenotype shift are being identified daily. Sauer and her colleagues

have reported that the phenotype of planktonic cells of both *P. aeruginosa* and *P. putida* differ from that of their biofilm counterparts more than they differ from that of planktonic cells of other species in the same genus. The inherent resistance of biofilm bacteria to antibiotics, all of which were selected on the basis of their ability to kill planktonic cells, is now largely attributed to the altered gene expression pattern of the biofilm phenotype. Scientists at Microbia Ltd (Boston) have identified one specific gene (fnt C) that is responsible for this inherent antibiotic resistance in biofilms formed by all staphylococcal species, and the deletion or blockage of this gene produces biofilms that are susceptible to conventional antibiotics.

Once attached cells have triggered the conversion to the biofilm phenotype, the multicellular community on the colonized surface begins to accrete larger numbers of cells by binary fission and by further recruitment of planktonic cells from the bulk water phase. As they increase in numbers and produce large amounts of EPS matrix material, the attached cells form microcolonies in which they constitute approximately 15% of the volume and the matrix occupies approximately 85% of the volume. The microcolonies assume tower-like and mushroom-like shapes (Fig. 1) in most natural and cultured biofilms, but many other morphologies may be dictated by species characteristics and by nutrient availability. The microcolonies may occupy the colonized surface, as discrete entities separated by open water channels (Fig. 1), or they may pile up in several layers to form thick sessile communities, but they always maintain their structural integrity and move independently under shear stress. As the biofilm matures and undergoes more phenotypic changes (Stoodley *et al.*, 2002), the processes of cell division and recruitment come into balance with programmed detachment of planktonic cells from the sessile community and sloughing. Most natural biofilms reach a mature thickness and

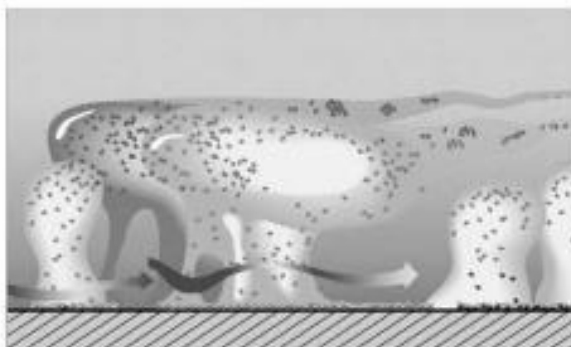


FIG. 1. Diagrammatic representation of the tower-like and mushroom-like microcolonies that are the basic structural units of the biofilms on colonized surfaces. Note that the matrix material occupies $\pm 85\%$ of the mass of these structures (while the cells comprise $\pm 15\%$), that the microcolonies can be deformed into oscillating streamers by shear forces, and that water moves through these complex communities in a convective pattern. It was the complex differentiated structure of these microcolonies, and the maintenance of the open water channels, that stimulated speculation that some form of "hormone-like" cell-cell signaling must be involved in the formation of microbial biofilms.

a stable community structure within a week or two of their initiation of colonization, and many remain relatively stable for years. Stoodley *et al.* (2002) have concluded that the biofilm phenotype would favor bacterial survival in the harsh environment of the primitive earth, and they have suggested that the planktonic phenotype may have developed considerably later, as a mechanism for dissemination.

Natural Control of Biofilm Formation on Surfaces

The complex structure of biofilm communities (Fig. 1) stimulated a lively discussion of what signals (e.g., hormones or pheromones) must be operative to allow the development of shaped microcolonies and sustained water channels. In 1998 the first demonstration that biofilm development in *P. aeruginosa* is controlled by an acyl homoserine lactone (AHL) quorum-sensing signal was published (Davies *et al.*, 1998), and it has subsequently been reported that agr, sar, and RAP (Balaban *et al.*, 1998) signals control this same process in gram-positive organisms. Additionally, it was shown that the autoinducer II signal (furanone) controls biofilm formation, and many other processes, in virtually all bacterial species (Schauder *et al.*, 2001; Xavier and Bassler, 2003). Most biofilm specialists agree that these signals are simply the tip of the iceberg, that many more signals will be discovered, and that specific blockage of many of these simple chemical signals offers a practical way to control virtually any bacterial "behavior." It has already been shown that specific signal inhibitors can block toxin production in *S. aureus*, and can even render specific bacteria essentially nonpathogenic in animal models (Balaban *et al.*, 2000).

The manipulation of biofilm formation is a very attractive target for new agents to prevent device-related infections. If bacteria that make contact with biomaterials were "locked" in the planktonic phenotype and were unable to assume the protected biofilm phenotype, they would be readily killed by host defense mechanisms and/or by antibiotic therapy. Several chemical analogs that block signal activity by interfering with the binding of the signal to its cognate receptor have been shown to be effective in inhibiting biofilm formation in specific pathogens (Balaban *et al.*, 1998). One such analog (RIP) prevents the binding of a biofilm control signal (RAP) to its receptor (TRAP). This signal blocker has been shown to prevent biofilm formation in an animal model of a device-related infection, and to allow complete eradication of the bacteria with conventional antibiotic therapy (Balaban *et al.*, 2003a, b). The researchers involved in the search for biofilm-control signal inhibitors are acutely aware of the subtle nature of the signal network in bacterial cells. It is highly unlikely that we will find a single ON/OFF switch for biofilm formation, and blockers that prevent biofilm formation may up-regulate invidious bacterial behaviors (e.g., toxin production), but we are encouraged by several observations made in natural ecosystems. Marine plants and animals control biofilm formation on their surfaces, presumably because biofilm/silt accretion would bury them and preclude photosynthesis in the plants, and at least one of the compounds that they use for this purpose is a signal inhibitor (de Nys *et al.*, 1995). In these natural systems, plant and animal surfaces are ideal locations for biofilm formation and growth,

but no bacterial mutant capable of thwarting the action of these natural biofilm control agents has emerged in millions of years of evolution.

Novel Engineering Approaches to Biofilm Control

The current therapy for device-related infections consists of trying to kill a biological entity (the bacteria) with a chemical (the antibiotic), with the only variable parameters being concentration and time of contact. Engineers have suggested that a number of physical forces could be harnessed to deliver higher concentrations of the antibiotic to the infecting organisms, or to compromise the bacteria in ways that make them more susceptible to the agents concerned. Two technologies that offer considerable promise involve the use of ultrasonic energy (Nelson *et al.*, 2002; Rediske *et al.*, 1998), and the imposition of a very weak sustained DC field (Costerton *et al.*, 1994) across the biofilm, and both have been shown to render sessile microbial populations susceptible to conventional antibiotics. Practical research is currently underway in the modification of biomaterials, and of device design, to harness the potential of these physical biofilm control technologies in our general strategies for the control of device-related infections.

BIOFILM-RESISTANT BIOMATERIALS

Biofilm-related complications have cost many lives in clinical settings. This unfortunate outcome may be reduced if the concepts and methods of modern biofilm microbiology can be inculcated into the development process for antibiofilm biomaterials. We will discuss some of the new agents that may give us effective control over the colonization of biomaterials and the incidence of device-related infections, and then we will discuss new methods for the release/delivery of these agents at the surfaces of biomaterials.

Testing for Antibacterial and Antibiofilm Properties of Biomaterials

There are serious concerns with the utility and information content of some of the methods used to assess the biofilm resistance of biomaterials. If a biomaterial gives a positive zone of inhibition test, what does it mean? It means that the biomaterial contained an antibacterial agent, which it released in the moist environment of the surface of an agar plate, and the agent killed the planktonic bacteria that had been deposited on this same surface to produce a "lawn." The major parameter operative in the test is the diffusion of the antibacterial agent through the agar, or in the fluid on the agar surface, more than the effectiveness of the agent. A very effective agent would have a very small zone, if it moves slowly through agar, and a weak agent would have an enormous zone if it diffused well through agar, or if it diffused well through water and the plate was wet. The release kinetics of the agent from the biomaterial are those of a biomaterial on a moist agar surface, which is not a common use target for biomaterials. Flask tests, in which candidate biomaterials are suspended in a medium

that is simultaneously inoculated with planktonic bacteria, are equally naive. If the biomaterials release enough of an antibacterial agent in the first few minutes of the test, all of the planktonic cells will be killed, the medium will be sterile, and there will be no organisms to colonize the biomaterial. So an antibiotic-releasing biomaterial that "dumps" all of its antibacterial agents in a few minutes will emerge from this test with flying colors! If the bacteria used in these relatively crude tests are lab-adapted by repeated subculturing, and thus defective in both antibacterial resistance and adhesion to surfaces, the biomaterials will be seen as promising. Yet both these tests are inappropriate and tend to lead to biomaterials that fail in biofilm resistance in animal and clinical trials.

The most appropriate tests are ones that mimic the conditions in the systems in which the biomaterial is targeted for use. If the biomaterial will be subjected to flow, or even to fluid exchange, the test should include these parameters. If the biomaterial will be used in a body fluid, such as blood or urine, an accurate simulation of that fluid should be used in the test, and the bacteria supplying the challenge should be adapted to the fluid. Bacteria used to challenge the putative biomaterial should be "wild" strains, recently isolated from clinical sources, and the challenge should come from fast-growing exponential-phase cells supplied by a chemostat, and not from variable cells from a "batch" culture. All of these parameters are best delivered using a flow cell, fed by a chemostat, and one of the most popular designs for such a system is given in Stoodley *et al.* (2001a). The flow cell also allows direct observation of large areas of the surface of the biomaterial, especially if the flow cell is mounted on the stage of a confocal scanning laser microscope (CSLM), and surface colonization can be monitored continuously (Cook *et al.*, 2000). Because the confocal microscope can resolve bacteria on opaque surfaces, and because this microscope allows us to examine living hydrated preparations, we can actually see the first microbial cells that adhere to biomaterial surface (Fig. 2A). If the adherent cells survive, they will initiate biofilm formation, and the adherent cells will gradually form matrix-enclosed communities (Fig. 2B) within which the cells will be separated by 3–5 μm of slime-filled space. The formation of biofilms requires that the adherent cells must be alive, so the observation of structured biofilms (Fig. 2C) on a surface that makes antibacterial and antibiofilm claims could have unfortunate clinical consequences (Cook *et al.*, 2000).

The observation of cells on the surface of a biomaterial is not necessarily negative data, especially if the cells are not very numerous and have not formed biofilms, because some antibacterial agents kill "incoming" planktonic cells and the dead cells remain on the surface (Fig. 2D). Even though we prefer biomaterials whose active agents kill "incoming" bacteria and do not retain these dead cells on the surface, agents that kill and retain bacterial cells are of some interest. For this reason, one of several available live/dead probes to ascertain the viability of adherent bacterial cells on biomaterials can be used. All of these methods give "snapshot" data, in that they necessitate the termination of colonization and the removal of biomaterial from the test system, but they yield accurate and useful data. The BacLite Live/Dead probe (Cook *et al.*, 2000) distinguishes live cells on the basis of membrane integrity, and live cells stain green while dead cells stain red (Fig. 2B). Living cells can also

question immediately before the device is installed. This tactic has backfired in many cases in which bacteria resistant to the antibiotic have started to grow in the fluids of the vessel in which the device is being soaked, have formed a biofilm on its surface, and have caused serious infections. We must be fastidious in the installation of medical devices, in that no preformed biofilms must ever be implanted, because preformed biofilms automatically give rise to biofilm infections (Ward *et al.*, 1992). It is equally important that the surfaces of biomaterials be absolutely clean, because any residue of dead biofilm or other organic accretion radically increases the colonization of that surface by planktonic bacteria and increases the chance of a biofilm infection. Also, some gram-negative bacterial cell-wall residues (endotoxin) can lead to inflammatory reactions.

The most commonly employed strategy in infection prevention is the impregnation of biomaterials with recognized antibacterial ions or molecules, with the intent of killing planktonic bacteria before they can colonize the material concerned. Whenever an ion or molecule is loaded into or onto a polymer, Fick's laws dictate that large amounts will be released in the early time frame, and that the release will taper off during the long period in which the concentration in this reservoir is being depleted (see Chapter 7.14). Many biomaterial designers have become adept at manipulating the initial concentration of the agent and the release rate, but we are always left with certain "spectra" of concentrations and polymer configurations that require choices of the "Hobson's" variety (that is, no real choice). If we put a large amount of ionic silver on a surface and release it quickly, we are flirting with silver toxicity. If we put a very stable form of silver on a surface and silver ions are released very slowly, bacteria will grow all over the silver coating (Fig. 2C) just as they colonize metallic copper (McLean *et al.*, 1993) if few copper ions are present. Westain Biomedical, Inc., has introduced an exciting new silver coating for burn bandages that uses a galvanic combination of silver and copper and releases silver and copper ions at a steady rate that control bacterial colonization for a useful period of time. The galvanic potentials set up by these side-by-side "lakes" of copper and silver may also have an inhibitory effect on bacterial adhesion, biofilm formation, and the inherent resistance of biofilm bacteria to antibacterial agents.

Because many modern antibiotics are much less toxic than metal ions, the release patterns of these agents from biomaterials pose a different problem. We can obtain high and very effective concentrations of antibiotics, in the immediate vicinity of devices, for lengths of time that have already been found to be effective in certain clinical situations. These biomaterials are useful, but we cannot control the low-level release of the agent for months or years after this effective time frame. This produces a prolonged period in which the agent is present at a sublethal concentration, near the device and sometimes in the whole body, and raises the specter of the development of acquired antibiotic resistance in many potentially dangerous bacterial species. A new development at the University of Washington Engineered Biomaterials (UWEB) Engineering Research Center has addressed this problem. Biomaterials can be coated with a molecular "skin," a self-assembled surface layer, that completely contains molecules loaded into an

underlying plastic and can be temporarily deranged (by ultrasonic energy) to yield a controlled release of the molecule in question (Kwok *et al.*, 2001). This coating has been used to deliver insulin, in controlled pulses, and the UWEB and the CBE are currently adapting this ultrasonic-sensitive coating for the controlled release antibacterial agents (and bacterial manipulation agents) from implanted biomaterials. High concentrations of these agents could be released at the surfaces of medical devices, perioperatively or at any preliminary signs of device-related infection, and no further release would occur if the coating was not stimulated ultrasonically.

Many surgeons have expressed an interest in being able to sterilize a medical device *in situ*, after it has been installed and before the operative wound is closed. This strategy is rational, because the device is accessible, and any planktonic bacteria present in the operative field will initiate colonization of the surface of the device if they are not killed or manipulated to preclude biofilm formation. Irrigation with antibiotics is presently used, biofilm-inhibiting signals and signal blockers are being developed, and this *in situ* procedure may be the perfect opportunity for the use of ultrasonic energy and/or DC electric fields to enhance the killing of bacteria in nascent biofilms. We can readily contaminate sham animal operations with bacteria and determine the efficacy of several possible procedures for *in situ* sterilization by using the live/dead probe to examine the surfaces of devices recovered at intervals after the procedure. The final proof of the efficacy of the *in situ* sterilization of medical devices would be obtained in clinical tests, in which significant reductions in device-related infections could be documented.

SUMMARY

The concept that has been distilled from decades of clinical experience with device-related infections has now been fused with the biofilm concept, which states that bacteria live predominantly in matrix-enclosed protected communities. This fusion is reassuring, and intellectually satisfying, because it asserts that bacteria employ the same strategy for survival in the human body that they use in all other ecosystems. The mental image that is invoked is one in which a biofilm forms on the surface of a biomaterial, and that it has all of the properties of the sessile communities that predominate in industrial and environmental systems. Its cells express the distinct biofilm phenotype: They are resistant to antibacterial agents and to uptake by phagocytes, most of them grow slowly and adopt many different metabolic strategies, and they detach planktonic cells and biofilm fragments in a programmed manner. The clinical consequences of this mode of bacterial growth are that antibiotics are useful in treating acute planktonic exacerbations, but that these agents cannot clear the biofilm reservoir on the biomaterial, and the device must usually be removed to resolve the infection.

As we fuse the device-related infection concepts with biofilm concepts, we can discard several older methods that have been used to assess the efficacy of putative antibacterial biomaterials. New biofilm methods allow us to visualize bacteria on opaque surfaces, to determine the viability of these organisms, and even to identify the cells by genus and species. We realize that freshly