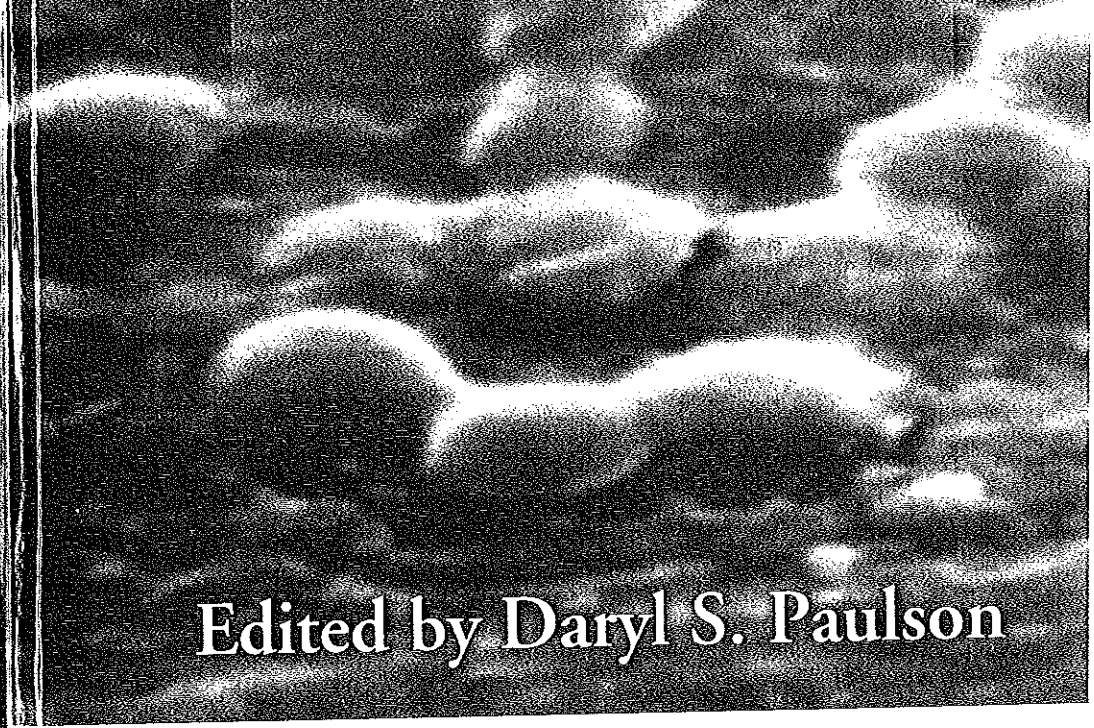
Applied Biomedical Microbiology

A Biofilms Approach



Edited by Daryl S. Paulson

CRC Press
Taylor & Francis Group

Contents

The Editor vii
Contributors ix

Chapter 1 **Biofilms before biofilms** 1
W. Curtis White

Chapter 2 **Efficacy of preoperative antimicrobial skin preparation solutions on biofilm bacteria** 9
Daryl S. Paulson

Chapter 3 **Oral biofilms and transferable antibiotic resistance** 23
Adam P. Roberts

Chapter 4 **Inhibition of foundation colonization of biofilm by surface modification with organofunctional silanes** 45
Robert A. Monticello and W. Curtis White

Chapter 5 **Biofilms and device implants** 59
Elinor deLancey Pulcini and Garth James

Chapter 6 **A simulated-use evaluation of a strategy for preventing biofilm formation in dental unit waterlines** 81
James W. McDowell, Daryl S. Paulson, and John A. Mitchell

Chapter 7 **Biofilms in hospital water distribution systems** 93
Judy H. Angelbeck, Kirsten M. Thompson, and Scott L. Burnett

Chapter 8 **Disinfection and its influence on the ecology of biofilms in drinking water distribution systems** 109
Fernando M. G. Matias, V. Susan Springthorpe, Syed A. Sattar, Marsha Pryor, and Graham Gagnon

Chapter 9 **Understanding the importance of biofilm growth in hot tubs** 133
Darla M. Goeres

Index 149

chapter five

Biofilms and device implants

Elinor deLancey Pulcini and Garth James

Contents

Introduction 59
The statistics on device-related infections 61
Bacterial growth characteristics in a biofilm 62
Detachment 63
How to analyze for the presence of biofilms on a surface 64
Surfaces 65
Cohesion 66
Bacterial strains 66
In vitro models to study biofilm formation 67
Problems with detection of biofilm on implanted devices 68
Other potential tools for detecting biofilm infections 69
Resistance to antimicrobials 70
Evasion of host defenses 71
Device-associated biofilms 71
Central venous catheters 71
Urinary catheters 72
Cerebrospinal fluid shunts 73
Biofilms and diagnostic instruments 73
Transrectal probes 73
Sublingual probes 74
Endoscopes 74
The bottom line 74
References 75

Introduction

Microorganisms attach to surfaces and form biofilms (Figure 5.1). From an historical perspective, biofilms first became an accepted entity as well as an accepted problem in environmental settings, particularly in industry. The Center for Biofilm Engineering at Montana State University–Bozeman in 1990, funded through the National Science Foundation's

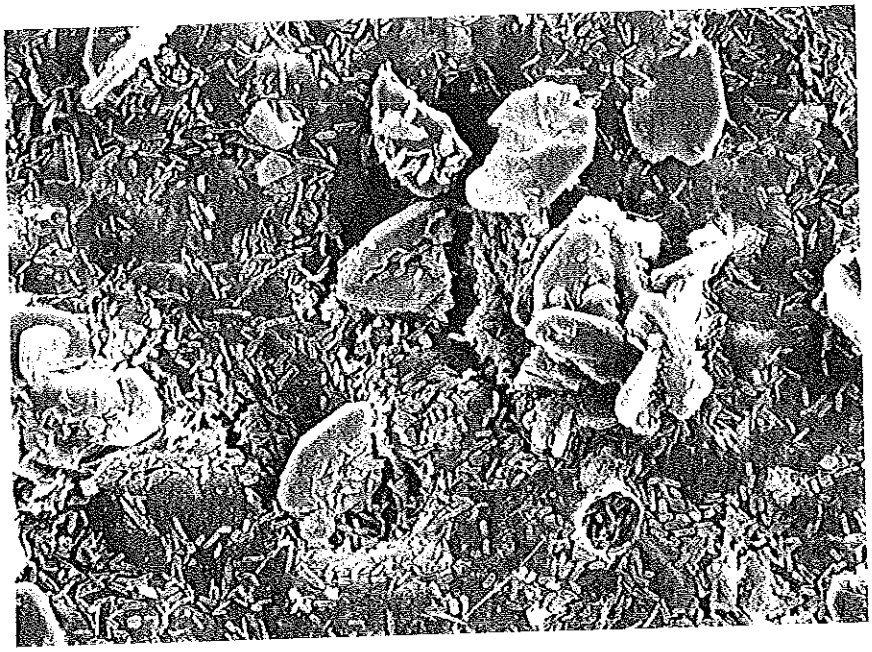


Figure 5.1 Scanning electron micrograph (SEM) of *E. coil* growing in a flow through urinary catheter model system. Image shows clusters of rod-shaped bacterial cells. Larger objects are crystals deposited on the tubing surface.

Table 5.1 Summary of Scirus Search for Articles Containing the Search Term *Biofilm* Compared with the Search Term *Medical AND Biofilm*

Years	*Biofilm*	*Medical AND biofilm*	Percent of *medical AND biofilm* articles compared to *biofilm*
1980–1985	119	8	7%
1985–1990	411	51	14%
1990–1995	1,086	176	19%
1995–2000	3,754	581	18%
2000–2005	8,376	1,579	23%
2005–2006	4,354	1,027	31%

Engineering Research Center Program, was established primarily to study environmental biofilms. The fact that biofilms were growing and clogging water pipes and cooling towers was easily accepted because the effects of the biofilms were readily apparent in the reduction and eventual loss of flow through a system.

The existence of biofilms in the medical setting has taken somewhat longer for acceptance. Using the Scirus database (http://www.scirus.com/srsapp/) to compare the search term *biofilm* with the search term *medical*

AND biofilm (abstracts and articles in any format) shows that while articles using the word *biofilm* increased dramatically from 1980 to 2006, articles for the use of the term *medical AND biofilm* has lagged (Table 5.1). During the 5-year periods from 1990 to 1995 and from 1995 to 2000, approximately 18 to 19% of the biofilm articles published contained the terms *medical* and *biofilm*. The articles containing the terms *medical* and *biofilm* published from 2000 to 2005 rose slightly to 23%. What is most exciting, however, is that the percentage of articles with the terms *medical* and *biofilm* published in 2005–2006 has increased to 31% of all biofilm articles published for that 1 year. While this is not a formal statistical analysis of publications, it does show a trend toward an increasing awareness and acceptance of the presence of medical biofilms. This increased rate of medical biofilm articles indicates two phenomena. First is an increased acceptance of the role of biofilms on medical devices and in chronic infections by the medical community. Second is the actual increase in rate of biofilm-associated device infections due to the increased use of the devices.

With advances in medicine comes a corresponding increase in the number of medical devices that are placed in patients. These can be s -duration devices, such as endoscopes and arthroscopes or urinary catheters placed for surgery; long-term devices, such as central venous catheters placed for chemotherapy or total parenteral nutrition; or permanent devices, such as artificial hips.

The baby boomer generation, which has already impacted school size and job availability, is now starting to impact the medical establishment with problems associated with aging. As this population ages, it is experiencing an increase in immune suppression rates, which is a normal effect of increasing chronological age. Couple that with increased needs for medical devices (short term to long term) and the environment is perfect for an increase in biofilm-related infections.

In this chapter, we will focus on *bacterial* biofilms on medical devices. This is not to imply that fungal biofilms or fungi in mixed-species biofilms are not medically relevant, but only that we have not studied fungal biofilm formation as extensively as bacterial biofilms.

The statistics on device-related infections

The Centers for Disease Control and Prevention estimated for 2002 that hospital-acquired infection accounts for 2 million infections, 90,000 deaths, and $4.5 billion in excess health care costs annually [1–3]. In a study undertaken at Cook County Hospital, Illinois, published in 2003, the estimated average cost of health care for patients with a suspected hospital-acquired infection increased by $6,767. For those patients with a confirmed hospital-acquired infection, the cost creased to $15,275 [4].

The reporting of hospital-acquired infection rates may not always delineate the types of infections. However, hospitals are starting to change their reporting policies. If the rates for the entire hospital are assessed rather than just the ICU, there are an estimated 250,000 cases annually of bloodstream infections due to infected central venous catheters, with costs estimated in the millions to billions of dollars for treatment [5]. Pennsylvania hospitals reported that of the total 11,668 hospital-acquired infections in 2004, 6,139 were urinary tract infections, 1,317 were surgical site infections, and 945 were multiple infections [6].

Certainly, not all these infections are biofilm infections on devices. However, such device infections may take longer to develop than an average hospital stay. One estimate is that there are 1.32 million prosthetic devices that become infected in the United States each year [7]. For example, of the more than 3 million permanent pacemakers and approximate 180,000 implanted cardiac defibrillators worldwide, it is estimated that the infection rates on these devices range from 0.13% to almost 20% [8].

Bacterial growth characteristics in a biofilm

Bacterial attachment to a surface is a complex, multistep process. Often, bacterial cells will establish a transient attachment to a surface prior to becoming irreversibly attached. As cells attach to the surface, they begin to divide and form microcolonies, which will eventually lead to the development of a mature biofilm [9].

Surface-associated changes are rapid. Proteomic analysis of *Pseudomonas aeruginosa* revealed that protein expression can change in as little as 10 min after initial attachment to a surface. In the span of 3 h post-attachment, the expression of approximately twenty proteins is changed [10]. Most of the proteins fall into the category of DNA regulation and carbon metabolism, which indicates the cell is preparing itself for the lifestyle change from planktonic to sessile.

Biofilm formation is a complex process in which there appears to be no single biofilm gene or operon. A number of studies have searched for the holy grail of biofilm formation, the biofilm phenotype, the existence of which would have great value as a diagnostic tool. Thus far, the results of proteomic and genomic analyses of biofilm vary according to species and biofilm formation conditions [11,12].

These studies illustrate the inherent adaptability of microorganisms. A study of sixty-seven clinical isolates of *Staphylococcus epidermidis* recovered from medical devices showed a range of genetic expression with respect to biofilm formation capability. Some of the clinical isolates capable of biofilm formation possessed the genes previously implicated in biofilm formation in this species, the *ica*ADBC operon. However, other isolates, also capable of biofilm formation, lacked the operon, and some of

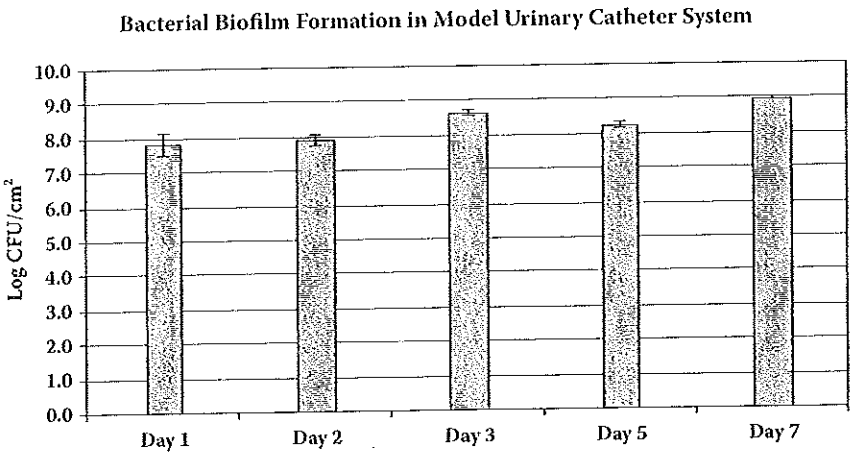


Figure 5.2 Growth of attached *E. coli* in a flow through urinary catheter model system. Prior to sampling, the catheter sections were rinsed to remove planktonic and loosely attached cells. Biofilms were disrupted by vortex, sonication vortex, and the cell suspension was placed for viable cell counts.

the isolates shown to produce low quantities of polysaccharide intercellular adhesion (PIA) contained the operon [13].

Under laboratory conditions and depending on the bacterial species, incubation conditions, and medium used, bacteria can attach to a surface and form a biofilm with viable cell counts of 10^4 to 10^8 cfu/cm^2 in 24 h. Over time, biofilms achieve a quasi-steady population state in which growth and attachment rates are equal to the rate of detachment. Growth curves of *E. coli* grown in artificial urine over a 7-day period show that the biofilms achieved a steady population of 10^8 cfu/cm^2 (Figure 5.2) [14]. With no change in parameters such a flow rates and nutrient and oxygen availability, the biofilm can likely maintain this population indefinitely. Changes in the environment can result in detachment or sloughing of a substantial portion of the biofilm, or can result in the dispersal of single cells or shedding from the matrix surface.

Detachment

Detachment of a portion of the biofilm can have serious ramifications for the transfer of infection throughout the body. The detached biofilm cell clusters retain the characteristics of a biofilm, including antimicrobial resistance and cohesiveness, because the bacterial cells are still encased in a matrix material. Using time-lapse microscopy of an *in vitro* catheter model of *S. aureus* biofilms, the extent of antibiotic resistance was directly proportional to the size of the detached biofilm particle or embolus [15].

In addition, these cell clusters are, in a sense, primed for reattachment at some other site. In some cases, the detached clumps of biofilm have been shown to roll along a surface that appears to be an important factor for dissemination, especially in nonmotile bacterial species [16].

How to analyze for the presence of biofilms on a surface

The presence of bacteria may not necessarily indicate the presence of a biofilm. So, it is crucial that a combination of analytical techniques be utilized in order to verify the presence of a bacterial biofilm. Visual analysis of a surface can reveal the presence of a bacterial biofilm (i.e., surface-associated cells covered with a matrix material), but it is difficult to quantify the number of cells. In the Medical Biofilms Laboratory at the Center for Biofilm Engineering, Montana State University, we find that use of traditional microbiological quantification techniques combined with visual analysis of the surface is the best means to both quantify the cells in a biofilm and show that a biofilm is indeed present on the surface.

For viable cell counts (plate counts) to be a reasonable estimation of the number of cells present on the surface, the biofilm must be removed and then disrupted without killing the cells in the process. Methods for removal include vortexing, sonication, and scraping, or combinations of these methods. (*Note:* Extreme care must be taken to avoid the production of aerosols when vortexing and sonicating, especially when processing clinical samples.) Cells subsequently suspended in sterile solution are then serially diluted and plated onto an appropriate medium and incubated. Final counts of the cells are expressed as cell forming units per square centimeter (cfu/cm^2) of surface area sampled. Controls can be used to estimate biofilm removal and disaggregation efficiency.

Visualization of the biofilm is done by either epifluorescent microscopy, confocal scanning laser microscopy (CSLM), or scanning electron microscopy (SEM). All three visualization methods have advantages and disadvantages. Both microscopy and CSLM combined with stains or antibody-specific probes can effectively visualize cells within a biofilm. Physiologic stains designed to indicate cell viability can indicate effects of treatments (especially when used in conjunction with viable cell counts). Fluorescent *in situ* hybridization (FISH) probes, which bind specific DNA sequences, can be used to fluorescently label cells of specific species of bacteria within a biofilm. Epifluorescent microscopy is best used on thinner biofilms; however, if the biofilm is patchy, it can become problematic to search for and find a sparse biofilm on a surface. CSLM is best used on thicker biofilms in order to utilize the sectioning capability of this microscope. Another advantage to CSLM imaging is the amenability to the use of computer image processing tools. CSLM can take a series of images

through the layers of the biofilm at predetermined thicknesses. This series can be recombined into a three-dimensional image, which can give important information on biofilm thickness and heterogeneity, as well as cell position within the biofilm matrix. With SEM, it is possible to visualize small cells and patches of biofilm on the surface of a device that may not be easily detected with microscopy or CSLM. In addition, SEM can visualize thin biofilms on a surface, whereas CSLM relies more on the presence of thicker biofilms in order to be an effective tool. SEM resolution capabilities allow for the visualization of single cells on a surface. A disadvantage to SEM lies in the processing, which involves vacuum desiccation and sputter coating of the surface with silver or gold. This flattens the biofilm, and thus reduces the information available regarding the spatial heterogeneity of the biofilm, and may introduce artifacts. SEM also only allows only for visualization of the outer surface of the biofilm. Variable-pressure SEM enables the visualization of samples under low-vacuum conditions as well as elemental analysis of the surface. This has proven to be a useful analytical tool, for example, in the examination of the crystals on a urinary catheter. Environmental SEM reduces the amount of processing required, thus a' 'ing for imaging of fully hydrated samples at high resolution.

Surfaces

As yet, there is no known surface to which bacteria cannot attach. Biofilm literature regarding surfaces examined includes a variety of materials and polymers, including metals (stainless steel, iron, copper), polymers (silicone, polystyrene, polycarbonate), glass (glass wool, slides), cement, paint on paintings, and minerals (goethite, hydroxy apatite) [17–25]. Some bacterial species do appear to preferentially attach to surfaces; this can vary by bacterial species tested and the type of surface tested. For example, initial adhesion and biofilm formation on various surfaces by *S. epidermidis* was found to vary by strain with no consistent phenotype [26].

Bacterial detachment or strength of attachment may vary according to surface roughness. *P. aeruginosa*, for example, will attach to either glass slides or hydroxy apatite (HA)–coated slides, growing biofilms that are similar in both cases in terms of length of time to form, thickness, and cfu/cm^2. However, biofilms formed on glass slides are easily removed, whereas those formed on HA slides will still have bacteria present on the surface even after scraping of the slide [27]. Rough surfaces have been shown to adsorb more proteins (i.e., conditioning layer) as well as more bacteria [28]. While surface characteristics such as hydrophobicity may play a role in initial adhesion of bacteria to that surface, these surface characteristics may be rather quickly ameliorated by chemical conditioning layers formed on the surface by proteins or host immune cells in the human body. Once the conditioning layer is established, much of

the surface characteristics have been substantially altered, thus changing the surface environment "seen" by the bacterial cells. A survey of nine clinical strains of *S. epidermidis* showed no relationship between the cell surface hydrophobicity and the ability of the cell to preferentially bind to hydrophobic or hydrophilic surfaces [26]. Surface characteristics appear to determine the chemical composition of the conditioning layer. For example, hydrophobic surfaces adsorb more albumin [29]. More agglutinins and proline-rich proteins attached to rough surfaces than to smooth surfaces in a study to assess the effects of surface characteristics on *in vitro* biofilm formation by oral bacteria [28]. These subtle differences in conditioning film composition may affect the types of bacteria that ultimately attach to that surface as well as the patterns of attachment [30].

Devices implanted in the body are coated with a layer of fibrin and fibronectin in a relatively short period of time. Urinary catheters can become coated with proteins and electrolytes to the point that the catheter surface may contain crystalline encrustations along with the biofilm [30].

Cohesion

Cohesion or "stickiness" of the biofilm matrix is a physical aspect of biofilm formation and maintenance that needs to be further studied. Exopolysaccharide (EPS) polymers produced by biofilm bacteria can vary in composition according to carbon availability, bacterial species, and environmental cues such as stress [32]. The stickier the biofilm matrix, the more firmly attached are the cells within the biofilm. The extent of cohesive characteristics within a biofilm matrix has potential implications for the ability of detached clumps of biofilm to efficiently withstand challenge by antibiotics or host immune cells, and thus be able to spread infective bacterial cells from a biofilm-impacted device to other sites in the body [33].

Bacterial strains

Care must be taken regarding the use of bacterial strains to assess biofilm formation and product efficacy in the laboratory setting. Strains that have been subcultured numerous times tend to lose virulence [34]. For medical biofilm work, it is important that stains of bacteria obtained from a clinical setting and minimally subcultured be used in order to fully characterize biofilm formation by clinically relevant bacterial species that still retain much of their virulence expression. A study of the reduction of bacterial genome size using *Salmonella typhimurium* passaged at least twenty times indicated that large-scale genome reduction can take place in a relatively short period of time. Results showed that the bacteria were discarding DNA that was no longer functional for their environment. In this serial

passage experiment, deletions in DNA ranged in size from approximately 1,200 base pairs (bp) to over 170,000 bp [35]. A study of diversity over time in *P. aeruginosa* clones grown in drip flow reactors showed phenotypic diversity in 2 to 7 days of biofilm growth. Phenotypic variation was found to increase with increased biofilm growth duration [36]. This phenomenon was observed in *Pseudomonas aeruginosa* cultured from cystic fibrosis patient lungs, which show an emergence of mucoid variants as well as small-colony variants [37].

In vitro *models to study biofilm formation*

There is no model system that is an exact replicate of the system being modeled. Despite this limitation, it is still possible to develop a model that can provide valuable data in a short period of time. The use of *in vitro* models is the crucial first step in the process of the evaluation of a product, which then progress to animal models and finally to clinical trials.

A number of different model types have been developed in order to grow reproducible biofilms. A good *in vitro* model must show minimal variation of biofilm formation criteria, such as bacterial cell counts within

in, and also from one experimental run to another. It is also important that the model be easily manipulated by the technician for treatments and analysis. The more complex the model, the more chance there is for the introduction of contaminating bacteria. It is also important that the surfaces on which the biofilm is grown be easily removed and used for microscopic analysis.

Different model types using the same bacterial strains, media, growth conditions such as temperature, and biofilm formation duration will grow different types of biofilm that respond differently to analysis and treatment.

With any model it is important to have matched controls. Despite the caveat for the need of minimal run-to-run variation, there can still be enough variation that matched controls must be utilized within each experimental run. It is best to use an untreated control, which provides information as to how well the biofilm formed in that particular run, as well as treatment controls. If, for example, the treatments being tested are dissolved in a specific carrier or solvent, then a treatment control of that carrier or solvent without any additional treatment compounds must also be included.

In vitro biofilm models based on the ninety-six-well format are able to generate data in a relatively short period. This format allows for numerous replicate samples utilizing small volumes, so these are excellent choices for the preliminary screening of compounds in which the compounds are expensive or difficult to make. A disadvantage to this model is that it is such a small-volume system that it may be affected more by variations in such parameters as temperature, oxygen, or shaking.

The basic ninety-six-well format has been used successfully [38,39]; however, a criticism of the model is the fact that the biofilm is formed on the bottom on the wells. This means the biofilm can contain both biofilm bacteria and bacterial cells that settled on the bottom of the well as a result of sedimentation.

The minimal biofilm eradication concentration (MBEC) model was designed to overcome some of these limitations [40]. It is based on the ninety-six-well system, in which the top plate contains ninety-six pegs and the bottom plate can be either a trough or a ninety-six-well plate. Advantages to this system are that biofilm tested is attached to the pegs, so it is relatively ensured that the tests are performed on attached cells. In addition, the lid can be easily placed into a fresh plate for media changes.

Disc reactors have proven to be a useful model for growing biofilms under flowing conditions. Two model types are commonly used, the rotating disc reactor (RDR) and the CDC reactor [41,42]. The RDR reactor has spaces for six removable coupons, while the CDC reactor has twenty-four coupons. These coupons can be made from a range of materials, from glass to polystyrene to metals. The biofilms formed in these systems are hardier in that they are more firmly attached to the coupon surface, as a result growing under conditions with shear. Both reactors provide replicate samples.

Flow-through systems use flow cells or tubing in which the media is pumped through the system [16]. Flow rates (fluid shear forces) can be more easily and precisely controlled in this system. The use of glass flow cells has shown to be a valuable instrument for real-time visualization of biofilms and treatment effects using the CSLM. This type of system can also be easily modified for testing of actual urinary catheters.

The drip flow reactor (DFR) is designed to model a low-shear environment and has been used for both single species and mixed species biofilm research [43]. The standard DFR model can hold four removable slides that serve as a substratum for biofilm attachment. We have observed that biofilms grown in a DFR tend to be thicker than those grown in other biofilm model systems. Presumably, bacteria within these biofilms have greater variations in physiologic and metabolic states. Some biofilms grown in this reactor have shown to be very resistant to treatments such as 10% bleach, with which there was only a 2 log reduction in bacterial counts after a 10 min treatment [44]. We have used both glass and hydroxy apatite (HA)–coated glass slides and have found that biofilms adhere better to the HA-coated glass surface. A variety of other materials can also be used as a substratum.

Problems with detection of biofilm on implanted devices

Bacteria attached to a medical device within the human body may escape detection by traditional clinical methodologies used to determine

infection. Biofilm infections tend to remain subacute and chronic [45,46]. While the biofilm itself may contain very high cell numbers (10^4 to 10^8 cfu/cm^2), there may be no indication in the patient of such high population of bacteria because the bacteria are embedded in a matrix and because few bacteria are sloughing or shedding. In some cases, the patient may present with a low-grade infection. Patients suffering from catheter-related bloodstream infections often present with clinical symptoms of sepsis but with no apparent source of infection [47].

Blood culture is the standard method for the detection of infection (sepsis). Unfortunately, this method does not always detect an infection on an implanted medical device. Bacteria may be sloughed or shed from an established biofilm into the bloodstream, usually in much lower numbers than the total number of bacteria attached to the device surface. Those bacteria shed from the biofilm as single cells may be killed by host cells or antibiotics. In other words, the presence of a biofilm-infected implanted medical device can be easily overlooked since accepted clinical methods may not detect the presence of an infection. The patient may experience recurrent low-grade infections that may be temporarily eased by the administration of antibiotics. Or the patient may appear to be healthy for quite some time until there is an event that compromises the patient's immune system. If any compromise or suppression of the patient's immune system occurs, then the dissemination of the infection from the nidus of the biofilm-infected device may prove to be rapid and fatal.

Other potential tools for detecting biofilm infections

Environmental microbiology has led the way in the development of innovative tools to look for microorganisms that may be difficult to culture in the lab [48]. The use of molecular tools, including polymerase chain reaction (PCR) to amplify DNA from complex samples, denaturing gradient gel electrophoresis (DGGE) to assess patterns of primer-selected DNA, and BLAST analysis of DNA sequences, has shown a complexity in the microbial world that up until the molecular age was unknown. Researchers have applied these molecular techniques to the clinical setting in the successful search of causative agents for diseases for which no causative bacterial agent could be detected [49]. While not a biofilm infection, the discovery of the causative agent for Whipple's disease is a case in point. It was presumed that Whipple's disease was caused by a bacterial agent due to ancillary data such as patient response to antibiotic treatment. The use of molecular techniques showed that Whipple's was caused by a previously uncharacterized pathogen. Thus, the use of molecular techniques has provided valuable information regarding the microbial diversity of disease.

This illustrates that not all clinically relevant microorganisms are culturable despite the long history of Koch's postulates to successfully pinpoint causative agents of disease. Biofilms formed in the human body may be single species or a multispecies community. These biofilm communities are complex and usually go through a process of ecological succession, with certain species of microorganisms acting as the pioneer species, establishing the biofilm and later species inhabiting it. The complexity of dental plaque is an excellent example of how complex these biofilms can be, with an estimated 500+ species inhabiting the human oral cavity [50].

Microorganisms subjected to antibiotics may not always be killed, but instead may be injured. These bacteria may not be detectable under normal culture conditions but may be capable of resuscitation with time, and thus become a source of reinfection [51]. Even bacteria not exposed to antibiotics may be difficult to detect using culture methods. In a metabolic state, termed viable nonculturable (VNC), bacteria are alive but do not grow in culture. A study of urine specimens obtained from healthy mice showed the presence of bacteria in urine considered to be sterile [52]. The VNC state has been studied in bacteria in water and food-related environments, but little research has been done in clinically relevant settings. A number of studies of *Vibrio cholerae* have detected the presence of VNC strains in water [53] and have examined the presence of pathogens such as *Campylobacter* in chicken [54]. *Salmonella typhimurium* cells in sewage effluent were shown to enter a VNC state and retain their virulence after treatment with peracetic acid [55]. An important point concerning the VNC state of bacterial cells is that the effects of treatment may be overestimated because these cells are only capable of growth under certain conditions. Since bacteria in biofilms exist in different physiologic states, it is possible that VNC cells exist in clinically relevant biofilms.

Resistance to antimicrobials

It is well established that biofilms are resistant to antimicrobials [56]. It is important to note, however, that in most cases, the antimicrobial resistance seen in biofilms is not due to a genetic change. If the biofilm is disrupted, the bacterial cells are rendered susceptible to the antimicrobial agent. Depending on the species present and the antimicrobial used, this resistance may be due to different mechanisms. The matrix itself may provide a physical barrier to prevent the penetration of the antimicrobial through the biofilm. In other cases, however, it has been shown that certain antimicrobials can penetrate biofilm quickly and efficiently, but there is minimal effect of the antimicrobial on the cells within the biofilm. Bacterial cells within an established biofilm exist in different metabolic states. Cells at

the surface of the biofilm experience an environment higher in nutrients and oxygen than cells contained deep within the matrix, where nutrients and oxygen may be severely limited. This leads to variations in metabolic and physiologic activities of the same species of bacteria within the biofilm, thereby affecting those mechanisms that certain antimicrobials may utilize for bactericidal effect. Bacteria not in an active metabolic and growth state, for example, are not susceptible to certain antibiotics, such as beta-lactams [57].

Evasion of host defenses

In addition to intrinsic resistance to antibiotics, biofilms have also been shown to be resistant to host defense mechanisms. A study characterizing the interaction of purified human neutrophils with *Pseudomonas aeruginosa* PAO1 biofilms showed that the neutrophils in contact with the biofilm became phagocytically engorged, partially degranulated, immobilized, and rounded, and showed only a slight increase in the soluble concentration of hydrogen peroxide [58].

Strains of *Serratia marcescens*, a common colonizer of contact lenses, have been shown to not induce a respiratory burst from polymorphonuclear leukocytes (PMNs) and to resist phagocytosis regardless of opsonization. This phenomenon was shown to increase when bacteria were grown as a biofilm on a contact lens [59].

It has been hypothesized that resistance to antimicrobial peptides may be due to interaction with biofilm and capsule exopolymers [60]. *Staphylococcus epidermidis* produces poly-*N*-acetylglucosamine (PIA) as a component of its exopolysaccharide during biofilm formation. PIA has been shown to play a crucial role in *S. epidermidis* resistance to neutrophil phagocytosis and human antibacterial peptides [61].

Device-associated biofilms

Any medical device can become colonized with bacteria. As the colonization process proceeds, biofilms can form in a relatively short period of time. Once established, biofilms are difficult to eradicate. The following discussion highlights some selected device-related biofilm infections.

Central venous catheters

There are approximately 5 million central venous catheters (CVCs) implanted yearly in the United States. Of those, it is estimated that 12 to 25% become infected, and 3 to 8% of patients with infections die [5]. Unfortunately, these numbers may well represent a gross underestimation of the problem. Reported numbers are usually only based on ICU

infections and may not include patients using CVCs on an outpatient basis or in a home health care setting, such as for chemotherapy or for total parenteral nutrition. In addition, there are different types of vascular catheters (peripheral venous, central venous, arterial), and then the nomenclature becomes further complicated by duration of placement, insertion site (subclavian, femoral, etc.), the insertion pathway (tunneled, nontunneled), physical length, and other characteristics, such as the number of lumens or the presence of a cuff. Thus, the definition of the catheter may impact the report of infection numbers [5].

Catheter-related bloodstream infections (CRBSIs) are defined as the isolation of the same microorganisms from cultures of the distal catheter segment and patient blood cultures [47]. CRBSI is considered to be a major complication for patients with central venous catheters [62]. Rates for CRBSI are up to 13% of CVC and may be dependent on the catheter placement. Studies indicate that femoral catheters have a higher risk of infection in adults than other placement sites [63].

The sources of infection in a CVC are from the skin at the site of insertion, contamination of the device, spread of bacteria from another infection site through the blood (hematogenous), or infusion of contaminated fluid [5]. In addition, patients who test positive as nasal carriers for *S. aureus* have been shown to have a greater risk for CRBSI than patients who are noncarriers [5,64].

Urinary catheters

More than 30 million urinary catheters are placed yearly in the United States, with approximately 10 to 30% of those becoming infected, making them one of the most common causes of nosocomial infections. Fortunately, unlike CVCs, less than 5% of infections from urinary catheters result in death [65]. Visible biofilm containing countable bacterial numbers (10^4 cfu/cm^2) were detected in a patient catheter at 19 h postinsertion [66]. Despite the fact that the bladder is considered to be a sterile environment, the introduction of a urinary catheter can rapidly change this balance. Even if the perineum is swabbed before the catheter is inserted, it is only disinfected and not sterilized. As the catheter is inserted, it can drag bacteria from the outside skin surface to the bladder. The presence of the catheter also can trigger an inflammatory response and allows for pooling of urine in the bladder or catheter [65].

A number of strategies have been examined for the prevention of infection and biofilm formation on the catheter surface. Different types of materials, such a siliconized surfaces, and impregnating the surfaces with antimicrobial agents have been shown to be somewhat effective for patients undergoing short-term catheterization [65]. However, for patients with chronic indwelling catheters, such as the elderly or persons with

spinal cord injuries, these strategies are not as successful. In most cases, these strategies merely delay the onset of biofilm formation.

Cerebrospinal fluid shunts

Cerebrospinal fluid (CSF) shunts are commonly used in the treatment of hydrocephalus to alleviate fluid pressure. There are approximately 70,000 hospital admissions for hydrocephalus, with the number of CSF shunt placements reaching tens of thousands annually [67,68]. Infection rates associated with CSF shunts are estimated to range from 3 to 30% [67]. An estimated 40% of the CSF shunts placed in pediatric patients fail within a year of implantation [68].

Sources of infection in CSF shunts may be related to the cause of the hydrocephalus, such as meningitis. However, in some cases, the placement of the shunt tip in the peritoneum has been implicated, especially in patients with appendicitis [67]. Thus, prevention of infection in CSF shunts is also complicated by the reason for the placement.

Scanning electron micrographs (SEMs) of CSF shunts removed from patients exhibiting symptoms of chronic infection indicate biofilm formation on the inner and outer surfaces of the shunts. In some cases, the presence of both cocci and bacilli indicates that the biofilms may be multispecies in nature [69].

Biofilms and diagnostic instruments

Cluster infections discovered in patients who are undergoing the same routine diagnostic exam at the same hospital or clinic are usually the first indicators of the presence of a biofilm in diagnostic instruments. The bacteria associated with these infections are resistant to routine disinfection procedures and are often associated with processing fluids, thus implicating biofilms. A variety of tests are usually performed to determine the infection source. These tests include routine swabbing and culture methods to find the source of bacterial contamination. In addition, molecular analyses are often performed to verify the strain of infecting bacterial species is the same in the affected patients as well as on the instruments in question.

Transrectal probes

Transrectal ultrasound-guided prostate biopsies are performed in men to diagnose prostate cancer. The procedure involves the use of an ultrasound probe to visualize the prostate, and then a needle biopsy is performed. In one case of cluster infections, the narrow lumen of the needle guide after it was removed from orthophthaldehyde (OPA) disinfectant was found to

be culture positive for *P. aeruginosa*. The procedure to detect *P. aeruginosa* in the needle guide required scraping of the guide with a sterile needle, thus implicating the presence of a bacterial biofilm resistant to disinfection [70].

Sublingual probes

Sublingual probes are used to monitor tissue carbon dioxide levels in hospital patients. A cluster infection of *Burkholderia cepacia* was traced to the buffered saline solution in which the probes are packaged. Further testing of the saline revealed not only *B. cepacia*, but other gram-negative rods, thus implicating the processing water at the manufacturer [71].

Endoscopes

Endoscopic procedures are minimally invasive diagnostic techniques that include a range of procedures, such as colonoscopies, laproscopies, and rhinoscopies. Cluster infections as a result of endoscopic procedures have been reported and have led to septicemia and cholangitis or infection of the bile ducts. Bacterial species detected include *P. aeruginosa*, *Mycobacterium tuberculosis*, *M. intracellulare*, and *M. avium-intracellulare* [72,73].

The manual cleaning of endoscopes is laborious due to the design of the endoscope, which contains small lumens, relatively long tubes, and angles. In addition, standard disinfection methods recommend the use of glutaraldehyde, and thereby can present a chemical risk hazard to health care personnel in charge of manually cleaning the scopes. Often, then, endoscopes are routinely cleaned and reprocessed in automated machines specially designed for that purpose. These machines use a detergent solution rinse, a disinfection step that may include glutaraldehyde, phenol, or some combination of disinfectants, and a final rinse, which is often performed with tap water. Biofilms have been found in these reprocessing machines in the detergent-holding tanks, hoses, and air vents [72,73]. Recently, biofilm-targeted approaches have been shown to provide better disinfection and cleaning than standard protocols [74].

The bottom line

A common theme for the prevention of biofilm formation on medical devices includes prevention of device contamination. There must be rigorous attention paid to aseptic technique in surgery, clinics, and home health care situations. Hand washing is the most common procedure described in the prevention of cross-contamination [2]. For infected devices, the best

option is often to remove the device as soon as possible. The use of tap water, which is not sterile, for the rinsing or processing of medical devices should be strongly discouraged.

At this point in time, there is no magic bullet that prevents the formation of biofilms on surfaces. Once bacteria become established as a biofilm on a medical device, the prognosis becomes somewhat grim, especially in the case of immune-compromised or -suppressed patients. For the present, the best protocol is the prevention of contamination of the device in order to prevent those contaminating bacteria from attaching to a surface and developing into a biofilm.

References

1. Graves, N., Economics and preventing hospital-acquired infection, *Emerg Infect Dis*, 10, 561, 2004.
2. McCaughey, B., Unnecessary deaths: *The human and financial costs of hospital infections*, Committee to Reduce Infection Deaths, www.hospitalinfection.org, 2005.
3. Wenzel, R.P., Edmond, M.B., The impact of hospital-acquired bloodstream infections, *Emerg Infect Dis*, 7, 174, 2001.
4. Roberts, R.R., Scott, R.D., 2nd, Cordell, R., Solomon, S.L., Steele, L., Kampe, L.M., Trick, W.E., Weinstein, R.A., The use of economic modeling to determine the hospital costs associated with nosocomial infections, *Clin Infect Dis*, 36, 1424, 2003.
5. O'Grady, N.P., Alexander, M., Dellinger, E.P., Gerberding, J.L., Heard, S.O., Maki, D.G., Masur, H., McCormick, R.D., Mermel, L.A., Pearson, M.L., Raad, I.I., Randolph, A., Weinstein, R.A., Guidelines for the prevention of intravascular catheter-related infections, *MMWR Recomm Rep*, 51, 1, 2002.
6. Guadagnino, C., Pennsylvania's hospital-acquired infection battle, *Physician's Digest*, www.physiciansnews.com/cover/206.html, 2006.
7. Braxton, E.E., Jr., Ehrlich, G.D., Hall-Stoodley, L., Stoodley, P., Veeh, R., Fux, C., Hu, F.Z., Quigley, M., Post, J.C., Role of biofilms in neurosurgical device-related infections, *Neurosurg Rev*, 28, 249, 2005.
8. Uslan, D.Z., Sohail, M.R., Friedman, P.A., Hayes, D.L., Wilson, W.R., Steckelberg, J.M., Baddour, L.M., Frequency of permanent pacemaker or implantable cardioverter-defibrillator infection in patients with gram-negative bacteremia, *Clin Infect Dis*, 43, 731, 2006.
9. Caiazza, N.C., O'Toole, G.A., SadB is required for the transition from reversible to irreversible attachment during biofilm formation by *Pseudomonas aeruginosa* PA14, *J Bacteriol*, 186, 4476, 2004.
10. de Lancey-Pulcini, E., Effects of initial adhesion events on the physiology of *Pseudomonas aeruginosa*, PhD dissertation, Montana State University, 2001.
11. Ghigo, J.M., Are there biofilm-specific physiological pathways beyond a reasonable doubt? *Res Microbiol*, 154, 1, 2003.
12. O'Toole, G., Kaplan, H.B., Kolter, R., Biofilm formation as microbial development, *Annu Rev Microbiol*, 54, 49, 2000.

13. Petrelli, D., Zampaloni, C., D'Ercole, S., Prenna, M., Ballarini, P., Ripa, S., Vitali, L.A., Analysis of different genetic traits and their association with biofilm formation in *Staphylococcus epidermidis* isolates from central venous catheter infections, *Eur J Clin Microbiol Infect Dis*, 25, 773, 2006.
14. de Lancey-Pulcini, E., James, G., unpublished data, 2006.
15. Fux, C.A., Wilson, S., Stoodley, P., Detachment characteristics and oxacillin resistance of *Staphyloccocus aureus* biofilm emboli in an in vitro catheter infection model, *J Bacteriol*, 186, 4486, 2004.
16. Rupp, C.J., Fux, C.A., Stoodley, P., Viscoelasticity of *Staphylococcus aureus* biofilms in response to fluid shear allows resistance to detachment and facilitates rolling migration, *Appl Environ Microbiol*, 71, 2175, 2005.
17. Costerton, J.W., Montanaro, L., Arciola, C.R., Biofilm in implant infections: Its production and regulation, *Int J Artif Organs*, 28, 1062, 2005.
18. Hall-Stoodley, L., Brun, O.S., Polshyna, G., Barker, L.P., *Mycobacterium marinum* biofilm formation reveals cording morphology, *FEMS Microbiol Lett*, 257, 43, 2006.
19. Heyrman, J., Verbeeren, J., Schumann, P., Swings, J., De Vos, P., Six novel *Arthrobacter* species isolated from deteriorated mural paintings, *Int J Syst Evol Microbiol*, 55, 1457, 2005.
20. Oosthuizen, M.C., Steyn, B., Theron, J., Cosette, P., Lindsay, D., Von Holy, A., Brozel, V.S., Proteomic analysis reveals differential protein expression by *Bacillus cereus* during biofilm formation, *Appl Environ Microbiol*, 68, 2770, 2002.
21. Sanchez-Moral, S., Luque, L., Cuezva, S., Soler, V., Benavente, D., Laiz, L., Gonzalez, J.M., Saiz-Jimenez, C., Deterioration of building materials in Roman catacombs: The influence of visitors, *Sci Total Environ*, 349, 260, 2005.
22. Steinberg, D., Rozen, R., Bromshteym, M., Zaks, B., Gedalia, I., Bachrach, G., Regulation of fructosyltransferase activity by carbohydrates, in solution and immobilized on hydroxyapatite surfaces, *Carbohydr Res*, 337, 701, 2002.
23. Teughels, W., Van Assche, N., Sliepen, I., Quirynen, M., Effect of material characteristics and/or surface topography on biofilm development, *Clin Oral Implants Res*, 17, 68, 2006.
24. Verran, J., Whitehead, K., Factors affecting microbial adhesion to stainless steel and other materials used in medical devices, *Int J Artif Organs*, 28, 1138, 2005.
25. Wingender, J., Flemming, H.C., Contamination potential of drinking water distribution network biofilms, *Water Sci Technol*, 49, 277, 2004.
26. Cerca, N., Pier, G.B., Vilanova, M., Oliveira, R., Azeredo, J., Influence of batch or fed-batch growth on *Staphylococcus epidermidis* biofilm formation, *Lett Appl Microbiol*, 39, 420, 2004.
27. de Lancey-Pulcini, E., James, G., unpublished data, 2006.
28. Carlen, A., Rudiger, S.G., Loggner, I., Olsson, J., Bacteria-binding plasma proteins in pellicles formed on hydroxyapatite in vitro and on teeth in vivo, *Oral Microbiol Immunol*, 18, 203, 2003.
29. Uyen, H.M., Schakenraad, J.M., Sjollema, J., Noordmans, J., Jongebloed, W.L., Stokroos, I., Busscher, H.J., Amount and surface structure of albumin adsorbed to solid substrata with different wettabilities in a parallel plate flow cell, *J Biomed Mater Res*, 24, 1599, 1990.

30. Bruinsma, G.M., van der Mei, H.C., Busscher, H.J., Bacterial adhesion to surface hydrophilic and hydrophobic contact lenses, *Biomaterials*, 22, 3217, 2001.
31. Stickler, D.J., Jones, S.M., Adusei, G.O., Waters, M.G., A sensor to detect the early stages in the development of crystalline *Proteus mirabilis* biofilm on indwelling bladder catheters, *J Clin Microbiol*, 44, 1540, 2006.
32. Sutherland, I., Biofilm exopolysaccharides: A strong and sticky framework, *Microbiology*, 147, 3, 2001.
33. Klapper, I., Dockery, J., Role of cohesion in the material description of biofilms, *Phys Rev E Stat Nonlin Soft Matter Phys*, 74, 031902, 2006.
34. Somerville, G.A., Beres, S.B., Fitzgerald, J.R., DeLeo, F.R., Cole, R.L., Hoff, J.S., Musser, J.M., In vitro serial passage of *Staphylococcus aureus*: Changes in physiology, virulence factor production, and agr nucleotide sequence, *J Bacteriol*, 184, 1430, 2002.
35. Nilsson, A.I., Kugelberg, E., Berg, O.G., Andersson, D.I., Experimental adaptation of *Salmonella typhimurium* to mice, *Genetics*, 168, 1119, 2004.
36. Boles, B.R., Thoendel, M., Singh, P.K., Self-generated diversity produces "insurance effects" in biofilm communities, *Proc Natl Acad Sci USA*, 101, 16630, 2004.
37. Haussler, S., Biofilm formation by the small colony variant phenotype of *Pseudomonas aeruginosa*, *Environ Microbiol*, 6, 546, 2004.
38. Pitts, B., Hamilton, M.A., Zelver, N., Stewart, P.S., A microtiter-plate screening method for biofilm disinfection and removal, *J Microbiol Methods*, 54, 269, 2003.
39. O'Toole, G.A., and Kolter, R., Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: A genetic analysis. *Mol Microbiol*, 28, 449, 1998.
40. Harrison, J.J., Turner, R.J., Ceri, H., High-throughput metal susceptibility testing of microbial biofilms, *BMC Microbiol*, 5, 53, 2005.
41. Goeres, D.M., Loetterle, L.R., Hamilton, M.A., Murga, R., Kirby, D.W., Donlan, R.M., Statistical assessment of a laboratory method for growing biofilms, *Microbiology*, 151, 757, 2005.
42. Zelver, N., Hamilton, M., Goeres, D., Heersink, J., Development of a standardized antibiofilm test, *Methods Enzymol*, 337, 363, 2001.
43. Adams, H., Winston, M.T., Heersink, J., Buckingham-Meyer, K.A., Costerton, J.W., Stoodley, P., Development of a laboratory model to assess the removal of biofilm from interproximal spaces by powered tooth brushing, *Am J Dent*, 15, 12B, 2002.
44. de Lancey-Pulcini, E., James, G., unpublished data, 2006.
45. Thomas, C., Cadwallader, H.L., Riley, T.V., Surgical-site infections after orthopaedic surgery: Statewide surveillance using linked administrative databases, *J Hosp Infect*, 57, 25, 2004.
46. Ehrlich, G.D., Stoodley, P., Kathju, S., Zhao, Y., McLeod, B.R., Balaban, N., Hu, F.Z., Sotereanos, N.G., Costerton, J.W., Stewart, P.S., Post, J.C., Lin, Q., Engineering approaches for the detection and control of orthopaedic biofilm infections, *Clin Orthop Relat Res*, 437, 59, 2005.
47. Gastmeier, P., Geffers, C., Prevention of catheter-related bloodstream infections: Analysis of studies published between 2002 and 2005, *J Hosp Infect*, 64, 326, 2006.

48. Amman, R.I., Ludwig, W., Schleifer, K.H., Phylogenetic identification and in situ detection of individual microbial cells without cultivation, *Microbiol Rev*, 59, 143, 1995.
49. Maiwald, M., Relman, D., Whipple's disease and *Tropheryma whippelii*: Secrets slowly revealed, *Clin Infect Dis*, 32, 457, 2001.
50. Kolenbrander, P.E., Oral microbial communities: Biofilms, interactions, and genetic systems, *Annu Rev Microbiol*, 54, 413, 2000.
51. Arrese, J.E., Goffin, V., Avila-Camacho, M., Greimers, R., Pierard, G.E., A pilot study on bacterial viability in acne. Assessment using dual flow cytometry on microbials present in follicular casts and comedones, *Int J Dermatol*, 37, 461, 1998.
52. Anderson, M., Bollinger, D., Hagler, A., Hartwell, H., Rivers, B., Ward, K., Steck, T.R., Viable but nonculturable bacteria are present in mouse and human urine specimens, *J Clin Microbiol*, 42, 753, 2004.
53. Binsztein, N., Costagliola, M.C., Pichel, M., Jurquiza, V., Ramirez, F.C., Akselman, R., Vacchino, M., Huq, A., Colwell, R., Viable but nonculturable *Vibrio cholerae* O1 in the aquatic environment of Argentina, *Appl Environ Microbiol*, 70, 7481, 2004.
54. Wolffs, P., Norling, B., Hoorfar, J., Griffiths, M., Radstrom, P., Quantification of *Campylobacter* spp. in chicken rinse samples by using flotation prior to real-time PCR, *Appl Environ Microbiol*, 71, 5759, 2005.
55. Jolivet-Gougeon, A., Sauvager, F., Bonnaure-Mallet, M., Colwell, R.R., Cormier, M., Virulence of viable but nonculturable *S. Typhimurium* LT2 after peracetic acid treatment, *Int J Food Microbiol*, 112, 147, 2006.
56. Stewart, P.S., Mechanisms of antibiotic resistance in bacterial biofilms, *Int J Med Microbiol*, 292, 107, 2002.
57. Anderl, J.N., Zahller, J., Roe, F., Stewart, P.S., Role of nutrient limitation and stationary-phase existence in *Klebsiella pneumoniae* biofilm resistance to ampicillin and ciprofloxacin, *Antimicrob Agents Chemother*, 47, 1251, 2003.
58. Jesaitis, A.J., Franklin, M.J., Berglund, D., Sasaki, M., Lord, C.I., Bleazard, J.B., Duffy, J.E., Beyenal, H., Lewandowski, Z., Compromised host defense on *Pseudomonas aeruginosa* biofilms: Characterization of neutrophil and biofilm interactions, *J Immunol*, 4329, 2002.
59. Hume, E.B., Stapleton, F., Willcox, M.D., Evasion of cellular ocular defenses by contact lens isolates of *Serratia marcescens*, *Eye Contact Lens*, 29, 108, 2003.
60. Otto, M., Bacterial evasion of antimicrobial peptides by biofilm formation, *Curr Top Microbiol Immunol*, 306, 251, 2006.
61. Vuong, C., Kocianova, S., Voyich, J.M., Yao, Y., Fischer, E.R., DeLeo, F.R., Otto, M., A crucial role for exopolysaccharide modification in bacterial biofilm formation, immune evasion, and virulence, *J Biol Chem*, 280, 12064, 2005.
62. Bacuzzi, A., Cecchin, A., Del Bosco, A., Cantone, G., Cuffari, S., Recommendations and reports about central venous catheter-related infection, *Surg Infect*, 7, S65, 2006.
63. Lorente, L., Santacreu, R., Martin, M.M., Jimenez, A., Mora, M.L., Arterial catheter-related infection of 2,949 catheters, *Crit Care*, 10, R83, 2006.
64. von Eiff, C., Becker, K., Machka, K., Stammer, H., Peters, G., Nasal carriage as a source of *Staphylococcus aureus* bacteremia, *N Engl J Med*, 344, 11, 2001.
65. Trautner, B.W., Darouiche, R.O., Role of biofilm in catheter-associated urinary tract infection, *Am J Infect Control*, 32, 177, 2004.

66. de Lancey-Pulcini, E., James, G., unpublished data, 2006.
67. Fux, C.A., Quigley, M., Worel, A.M., Post, C., Zimmerli, S., Ehrlich, G., Veeh, R.H., Biofilm-related infections of cerebrospinal fluid shunts, *Clin Microbiol Infect*, 12, 331, 2006.
68. Kulkarni, A.V., Drake, J.M., Lamberti-Pasculli, M., Cerebrospinal fluid shunt infection: A prospective study of risk factors, *J Neurosurg*, 94, 195, 2001.
69. Drake, J.M., et al., Randomized trial of cerebrospinal fluid shunt valve design in pediatric hydrocephalus, *Neurosurgery*, 43, 294, 1998.
70. CDC, *Pseudomonas aeruginosa* infections associated with transrectal ultrasound-guided prostate biopsias—Georgia, 2005, *MMWR*, 55, 776, 2006.
71. CDC, Nosocomial *Burkholderia cepacia* infections associated with exposure to sublingual probes—Texas, 2004, *MMWR* 53, 796, 2004.
72. CDC, Bronchoscopy-related infections and pseudoinfections—New York, 1996 and 1998, *MMWR*, 48, 557, 1999.
73. CDC, Nosocomial infection and pseudoinfection from contaminated endoscopes and bronchoscopes—Wisconsin and Missouri, *MMWR*, 40, 675, 1991.
74. Marion, K., Freney, J., James, G., Bergeron, E., Renaud, F.N., Costerton, J.W., Using an efficient biofilm detaching agent: An essential step for the improvement of endoscope reprocessing protocols, *J Hosp Infect*, 64, 136, 2006.