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Orthopaedic biofilm infections

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Abstract

A recent paradigm shift in microbiology affects orthopaedic surgery and most other medical and dental disciplines because more than 65% of bacterial infections treated by clinicians in the developed world are now known to be caused by organisms growing in biofilms. These slime-enclosed communities of bacteria are inherently resistant to host defenses and to conventional antibacterial therapy, and these device-related and other chronic bacterial infections are unaffected by the vaccines and antibiotics that have virtually eliminated acute infections caused by planktonic (floating) bacteria. We examine the lessons that can be learned, within this biofilm paradigm, by the study of problems (e.g. non-culturability) shared by all biofilm infections and by the study of new therapeutic options aimed specifically at sessile bacteria in biofilms. Orthopaedic surgery has deduced some of the therapeutic strategies based on assiduous attention to patient outcomes, but much can still be learned by attention to modern research in related disciplines in medicine and dentistry. These perceptions will lead to practical improvements in the detection, management, and treatment of infections in orthopaedic surgery.

Keywords

chronic infection; biofilm; antibiotic resistance; surgical debridement; diagnosis

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THE BIOFILM HYPOTHESIS

The biofilm hypothesis, which was first promulgated in 1978,¹ states that bacteria in all nutrient sufficient ecosystems grow predominantly in matrix-enclosed surface-associated communities, within which they are protected from a wide variety of antibacterial factors. The extension of this hypothesis into medicine occurred shortly thereafter,² with the description of a biofilm formed by cells of *Staphylococcus aureus* on a pacemaker lead in a patient with a bacteremia secondary to an orthopaedic injury (Figure 1). The sessile cells within this biofilm survived 6 weeks of very intensive antibiotic therapy, and the protected microbial community served as a nidus for recurrent bacteremias each time that antibiotic therapy was discontinued.

This device-related infection was eventually resolved by the removal of the device, and the notion of biofilms as etiological agents was added to the general paradigm of chronic device-related infections that resist clearance by antibiotics and host defenses. As more and more chronic bacterial infections were shown to have a biofilm etiology,³ and as the huge morbidity and mortality of these chronic infections were documented,⁴ we recognized a new category of “biofilm infections.” The biofilm paradigm demands that we think of the bacteria that cause chronic infections as adherent communities of slime-enclosed organisms, instead of swarms of independent cells, and it suggests that the strategies we have evolved to deal with the planktonic (floating) bacteria that cause acute infections must be modified to deal with this new threat.⁵ The premise that biofilms are the etiological agents of all device-related and other chronic bacterial infections³ introduces the concept that practical clinical strategies for the detection and treatment of these recalcitrant infections can be shared amongst medical and dental disciplines. For this reason, we must recognize that observations (e.g. that biofilm diseases rarely yield positive cultures) that are made in connections with middle ear infections⁶ and bacterial prostatitis⁷ may be very useful in understanding similar problems in orthopaedic surgery.

BIOFILMS IN ORTHOPAEDIC INFECTIONS

When Gristina and Costerton⁸ applied the biofilm hypothesis to device-related orthopaedic infections as early as 1984, it became abundantly clear that the clinical “earmarks” of these chronic bacterial infections could be rationalized in terms of their biofilm etiology. Like all biofilm infections, these infections of prostheses and fixation devices could develop over months or years, with few signs of inflammation, and they usually remained localized to the immediate vicinity of the colonized prosthesis.⁹ Antibiotic therapy resolved the symptoms triggered by floating single “planktonic” cells shed from these biofilms, as we had shown in biofilm systems cultivated *in vitro*, but the stationary slime-enclosed “sessile” cells in the biofilm niduses were not affected by these concentrations of antibacterial agents, and the infections persisted.¹⁰ Even in cases in which the innate and adaptive immune capabilities of the host were intact, device-related orthopaedic infections rarely resolved spontaneously, and this characteristic of these chronic diseases could be rationalized by our *in vitro* data documenting the inherent resistance of biofilms to antibodies¹¹ and activated phagocytes.¹² We used direct microscopic methods to examine tissues from non-device-related human osteomyelitis and from animal models of this disease,¹³ and we found equally persuasive evidence that the causative bacteria grew in very extensive biofilms (Figure 2).

TREATMENT STRATEGIES FOR ORTHOPAEDIC BIOFILM INFECTIONS

Even before the general study of biofilm infections indicated that surgical removal of these slime-enclosed communities was a necessary precondition for the resolution of device-related infections, orthopaedic surgeons had deduced this fact and adopted this approach. Device removal and thorough debridement is best done before more tissues are destroyed by

spreading biofilms, and the biofilm-based management of orthopaedic infections has served as an example for the management of other device-related infections.¹⁴ A cogent example of this general principle in the management of foreign body infections occurred in a total elbow arthroplasty, in which the failure to remove even the smallest fragments of tobramycin impregnated spacer cement that harbored bacterial biofilms (Figure 3) resulted in the recurrence of symptoms and ultimately in a failed total elbow arthroplasty.

These direct observations of bacterial biofilms growing in association with orthopaedic devices, or even with small residual of these devices, can provide the intellectual basis of a rational approach to treatment. Because bacterial biofilms are inherently resistant to host defenses and to conventional antibiotic therapy, these biomaterials and their adherent biofilms must be completely removed by fastidious surgery before the infection can be resolved.

LESSONS FROM RELATED DISCIPLINES

Mandibular Osteomyelitis

In our recent examinations of osteomyelitis secondary to tooth extraction in the human mandible we have found a biofilm (Figure 4) that had resisted aggressive antibiotic therapy for more than 4 years.

This coherent biofilm was composed of cells of a single morphotype, suggestive of an actinomycete species. This sessile community occupied an area of thousands of cubic microns within the affected tissue and appeared to be under attack by the patient's phagocytes (Figure 4, arrows). When seen by scanning electron microscopy (SEM), the cells that comprised this sessile community were separated by matrix material that had condensed upon dehydration to reveal a very extensive network of fine nanowires. These nanowires have been shown in pure cultures of other species to conduct electrical energy,¹⁶ and their presence in this pathogenic biofilm raises the possibility that these sessile cells can communicate by electrical signals in addition to the chemical signals that they undoubtedly also use.¹⁷ This example from maxillofacial surgery teaches us that bacterial biofilms growing in bone can form communities with remarkable communication mechanisms and that these biofilms may resist antibiotic therapy for years if they are not removed by surgery.

Osteonecrosis Secondary to Bisphosphonates

Similar direct observations of mandibular bone affected by long-term use of bisphosphonates reveals a unique etiology of a chronic osteomyelitis, in that the mixed-species biofilms occupy large spaces in the bone (Figure 5), very likely as a result of bacterial mediated bone resorption. We speculate^{18,19} that bisphosphonates may facilitate the colonization of bone by any bacteria that are present as a result of dental manipulations and that these organisms may form biofilms that eventually occupy large areas within this compromised tissue. This osteonecrosis of the jaw is remarkably nonaggressive, and simply excision of the affected bone usually serves to resolve the infection without any spread to adjoining tissues, but the biofilms that cause the infection persist in spite of active host defenses and aggressive antibiotic therapy.

This example teaches us that patients who have used bisphosphonates over long periods of time may have a very nonaggressive invasion of the lacunae in bone, in which osteocytes have been replaced by bacterial biofilms, with consequent resorption of some bone matrix. This condition has only been described in the mandible and maxilla,^{18,19} but it may of some interest to examine this possible mechanism of bone compromise in other skeletal elements.

PROBLEMS SHARED WITH RELATED DISCIPLINES

Detection of Bacteria in Natural Ecosystems

The gold standard of the detection of bacteria by culture methods has served us well for more than 150 years, but it has recently been shown to be ineffective in the detection of biofilm bacteria. This phenomenon was first discovered in marine microbiology in which the majority of organisms were described as “viable but nonculturable,”²⁰ and only a minority of planktonic cells yielded colonies when plated on agar. Less than 1% of the bacteria seen in seawater actually produce colonies on agar, and we have discovered²¹ that clumps of the natural biofilms of *Staphylococcus aureus* that populate the human vagina fail to grow when placed on the surfaces of media on which planktonic cells always form colonies. We suggest that the failure to grow and produce colonies, when biofilm fragments are placed on the surface of agar plates, devolves from the profoundly different phenotype that sessile cells adopt very early in the course of biofilm formation.⁵ In cases in which biofilms shed planktonic cells, and the planktonic cells survive in the presence of host defenses and ambient concentrations of antibiotics, positive cultures are obtained, but the number of colonies that grow only reflect the number of planktonic cells that are present.

Detection of Bacteria in Biofilm Infections

The medical consequences of this difficulty in detecting biofilm bacteria by culture methods have been profound and especially damaging because they have been discussed separately in each of several medical disciplines. Biofilms complicate sampling, by their very nature, because any fluid sample only contains planktonic cells that have been released from these sessile communities or fragments of the biofilm that have detached because of mechanical stress. Some planktonic cells may be killed by local defenses or by residual antibiotics, and biofilm fragments may not be evenly distributed so that body fluids only yield reliable culture data in fulminating acute infections caused by swarms of planktonic bacteria. As recently as 1995, the failure of culture methods to detect bacteria in otitis media with effusion (OM-E), led to speculations concerning a viral etiology or a sterile inflammation.²² However, Post *et al.*²² of the Center for Genomic Sciences (CGS), showed the presence of bacterial DNA in the effusions and proved that bacterial pathogens could be both detected and identified at a species level by molecular methods. Objections concerning the viability of these bacteria were overcome when the CGS team detected short-lived bacterial mRNA in the effusions,²³ and the issue was fully resolved when the imaging arm of the center showed biofilms in the effusions and on the epithelia of the middle ear.^{24,25} Similar etiological questions have arisen in urology in which the bacterial role in prostatitis and in trigonitis has been challenged because of the paucity of positive cultures, and direct observations have shown the presence of biofilms in affected tissues.⁷

Detection of Bacteria in Orthopaedic Infections

This difficulty in detecting biofilm bacteria was discussed in the orthopaedic context by Trampuz *et al.*²⁶ when they were able to increase the proportion of positive cultures from overt infections of total joint prostheses by sonicating tissues and biomaterials recovered at revision. We hypothesize that some biofilm cells reverted to the planktonic phenotype during and after sonication and that these planktonic cells produced colonies on agar plates. Our own experience with patients scheduled for surgical revision of total joint prostheses is that cultures were positive in only 8 of 40 patients analyzed by routine culture techniques (unpublished data). This impression is reinforced by the fact that parallel analysis of specimens from these 40 patients, by the independent RNA-based fluorescence *in situ* hybridization (FISH) technique, showed the presence of extensive biofilms composed of clearly resolved bacterial cells in the tissues of patients whose intraoperative cultures were consistently negative.

The greater sensitivity of molecular methods^{22,23,27} for the detection of bacterial RNA and DNA and their ability to detect bacteria in the biofilm phenotype suggest that these methods will replace cultures as the gold standard in bacterial detection in cases in which an infection is suspected. Most molecular detection methods begin with the extraction and amplification of the bacterial nucleic acids, and this amplification usually uses the polymerase chain reaction (PCR) to produce many copies of the sections of the DNA (or RNA) that are defined by the use of specific primers. Multiplex PCR of panels of genes can identify the presence of known pathogens²⁸ and provide information concerning antibiotic sensitivity by detection of genes, such as the *mecA* gene that identifies methicillin resistant *S. aureus* (MRSA).²⁹ The new and more powerful technique of “deep” 16 S rRNA sequencing, using the 454 pyrosequencing system,³⁰ detects all bacterial species quantitatively, and we rely heavily on this system to detect all bacterial species whether they are known or unknown. We have suggested¹⁴ that the clinical management of orthopaedic infections can be based on the basic tenets of the biofilm hypothesis, and we now suggest that preoperative antibiotic therapy and revision strategies can be predicated on data from modern DNA and RNA-based technologies for the detection and identification of bacteria.³¹ The practical result of these advances in modern molecular techniques will be that preoperative aspirates will be analyzed for the presence of bacteria, with an accurate determination of the species present and their sensitivity to common antibiotics, and these data will be available to surgeons within 12 hours of sampling. The presence or absence of bacteria will be confirmed by the molecular analysis of intraoperative samples, and treatment strategies can then be predicated on accurate microbiological information.

BIOFILM-BASED TECHNOLOGIES FOR THE TREATMENT OF ORTHOPAEDIC INFECTIONS

Between 1990 and 2001, the National Science Foundation invested more than \$20 million in the study of microbial biofilms, with the stated objective of solving practical problems caused by these sessile populations in natural and engineered ecosystems. The Center for Biofilm Engineering (CBE) gradually developed an accurate spatial model of biofilms (Figure 6) based on direct observations of these communities, and we realized that their structural and functional complexity virtually demanded some form of chemical signaling.³²

Figure 6 represents a “snapshot” of the complex architecture of a typical biofilm, but the dynamics of the formation and maturation of these communities is best understood by examining the movies available under “image library” on the CBE website (www.erc.montana.edu).

The Use of Signals and Signal Inhibitors to Block Biofilm Formation

When we examined the quorum-sensing signal systems that control many activities of virtually all bacteria,¹⁷ we discovered that any deletions or inhibitions of these universal systems affected biofilm formation.³³ Specifically, the deletion or inhibition of the acyl homoserine lactone system in gram negative organisms,³³ or of the cyclic octapeptide system in gram positive organisms,³⁴ obviated the whole process of biofilm formation in these bacteria. The universal search for signals or signal analogues that block biofilm formation has been facilitated by the discovery of many natural compounds that prevent the formation of these sessile populations in natural ecosystems,³⁵ and several biofilm inhibitors have been submitted for approval by the FDA. Most of these biofilm inhibitors prevent biofilm formation and restrict bacteria to the planktonic mode-of-growth, in which they are susceptible to host defenses and antibiotics, so that their inclusion in coatings used on orthopaedic devices would reduce device-related infections. This dividend from biofilm microbiology will be available for use in orthopaedics, and a detachment signal that triggers

the natural detachment of cells from pre-formed biofilms also may be available in the near future.³⁶

The Use of Ultrasound and Electrical Fields to Kill Biofilm Bacteria

As the engineers in the CBE began to widen the search for means to control microbial biofilms, several turned to physical methods of killing sessile bacteria or of making them susceptible to practical concentrations of antibiotics. Rediske *et al.*³⁷ discovered that ultrasound, at specific frequencies and intensities, killed some cells and made the remainder exquisitely sensitive to antibiotics, and this technology is being explored in a medical context. At the same time, Blenkinsopp *et al.*³⁸ discovered that the sensitivity of sessile biofilm bacteria to conventional antibiotics was markedly increased if these agents were used in conjunction with a DC field³⁹ of only 2 mAmps/cm². Ehrlich *et al.*³⁰ proposed that DC electrical fields can be used to prevent and control biofilm infections associated with prostheses, and the electrically-conductive metallic nature of these devices may favor this application. Subsequently, McLeod⁴⁰ found that sessile bacteria within biofilms could be killed by practically attainable concentrations of conventional antibiotics, if these agents were used in conjunction with low-frequency varying magnetic fields. Research in these technologies has been sponsored by the manufacturers of orthopaedic prostheses, and their eventual application to infection control will depend on the sustained iterative interaction of orthopaedic clinicians and biofilm engineers.

CONCLUSIONS

A recent analysis of the morbidity and mortality associated with biofilm infections has revealed that over 12 million people are affected, and that 400,000 people die as a result of these infections in the USA each year.⁴ Orthopaedic infections are amongst the most devastating of these biofilm infections because of osteomyelitis and its limb-threatening sequelae. The number of patients affected is expected to rise with the increasing reliance on total joint replacement. In this review we have attempted to prove that orthopaedic infections are archetypical biofilm infections, and our central thesis is that the detection and management of these conditions will be materially improved by the adoption of concepts and methods that have been useful in the control of biofilms in other medical and dental systems.

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Figure 1. Scanning electron micrograph (SEM) of spherical cells of *Staphylococcus aureus* in a biofilm formed on an endocardial pacemaker in a patient with a bacteremia secondary to an elbow injury. Note the negative impressions (arrow) of these cells in the slimy matrix within which this thick biofilm was enclosed.

Figure 2. Transmission electron micrograph (TEM) of the biofilm formed in the femur of a rabbit infected with *Staphylococcus aureus*, in an animal model in which a surgical nail was inserted to compromise the bone. Note the extent to which the sessile bacteria in this extensive community are surrounded by electron dense matrix material.

Figure 3.

Biofilm clusters of live bacterial cocci (yellow), identified as *S. aureus* from reverse transcriptase PCR (RT-PCR) analysis, associated with an infected total elbow arthroplasty. The nuclei of host cells are stained red. Specimens were rinsed and observed under fully hydrated conditions demonstrating that the cell clusters were coherent and strongly adhered to the cement and surrounding tissue. (A) Biofilm clusters (representative cluster shown by arrow) associated with tobramycin-impregnated cement (blue) used as a spacer. (B) Biofilm clusters (arrow) were also associated with reactive tissue and aspirate associated with the cement spacer. Diffuse green staining between the cocci suggests the presence of extracellular DNA (eDNA) in the biofilm slime matrix. Preoperative aspirates were culture negative.¹⁵

Figure 4. Scanning electron micrograph of a single species biofilm that formed in the mandible of a patient, secondary to a tooth extraction, and persisted for longer than 4 years in spite of aggressive antibiotic therapy. Note the intrusion of human phagocytes into the biofilm (arrows), and the very extensive ramifying network of fine nanowires connecting the cells to each other.

Figure 5.

Scanning electron micrograph of mandibular bone from a patient with osteonecrosis secondary to the use of bisphosphonates. Note that very large spaces within the bone (bottom left) are filled with bacterial biofilms, whose fine structure and morphology can be clearly discerned in the main image, and that as much as 50% of the volume of the bone is occupied by these microbial communities.

Figure 6.

This illustration shows the "towers" and "mushrooms" whose complex structures and persistent water channels suggested that some form of cell-cell signaling must be involved in the development of biofilms. This perception led to the discovery of the signals that control this process and to the notion that signal analogues can be used to block biofilm formation (Reproduced with permission from Center for Biofilm Engineering).