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REVIEW

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Role of biofilms in neurosurgical device-related infections

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Abstract Bacterial biofilms have recently been shown to be important in neurosurgical device-related infections. Because the concept of biofilms is novel to most practitioners, it is important to understand that both traditional pharmaceutical therapies and host defense mechanisms that are aimed at treating or overcoming free-swimming bacteria are largely ineffective against the sessile bacteria in a biofilm. Bacterial biofilms are complex surface-attached structures that are composed of an extruded extracellular matrix in which the individual bacteria are embedded. Superimposed on this physical architecture is a complex system of intercellular signaling, termed quorum sensing. These complex organizational features endow biofilms with numerous microenvironments and a concomitant number of distinct bacterial phenotypes. Each of the bacterial phenotypes within the biofilm displays a unique gene expression pattern tied to nutrient availability and waste transport. Such diversity provides the biofilm as a whole with an enormous survival advantage when compared to the individual component bacterial cells. Thus, it is appropriate to view the biofilm as a multicellular organism, akin to metazoan eukaryotic life. Bacterial biofilms are much hardier than free floating or planktonic bacteria and are primarily responsible for device-related infections. Now that basic research has demonstrated that the vast majority of bacteria exist in biofilms, the paradigm of biofilm-associated chronic infections is spreading to the clinical world. Understanding how these biofilm infections affect patients with neurosurgical devices is a prerequisite to developing strategies for their treatment and prevention.

Keywords Biofilms · Central nervous system infections · Neurosurgery · Medical devices

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Introduction

The practice of neurosurgery has seen an explosion in the number of devices employed to treat patients. The potential benefits of neurosurgical devices must be weighed against the ever-present specter of device-related infections. Coping with these types of infections can be frustrating because of an ancient prokaryotic survival strategy characterized by biofilm formation. First described by Costerton et al. in 1978, biofilms represent a new paradigm for device-related infections [13, 16]. Bacterial biofilms are "self-assembling multicellular communities" [15] that behave very differently from their free floating (planktonic) counterparts. When bacteria are organized in this way, they are very resistant to standard methods of treatment apart from removing the device or tissue that is engulfed by the biofilm. The realization of the importance of biofilms in human disease in general, and in particular in neurosurgical infections, is very recent and of great importance. Although there is relatively scant literature describing the role of biofilms in neurosurgical infections, it is becoming increasing clear that biofilms play an important role in postoperative infections involving neurosurgical devices such as complex spinal instrumentation, pulse generators used during functional and epilepsy surgery, indwelling silastic catheters for the diversion of cerebral spinal fluid (CSF),

and bone flaps after delayed cranioplasty. This review describes what a biofilm is and how it forms, and then explores the implications of the biofilm phenotype in the context of neurosurgical device-related infections.

What is a biofilm?

Biofilms are organized communities of bacteria attached to surfaces, including implanted medical devices and host mucosal tissues. These bacterial populations are embedded in a slime-like matrix composed of polysaccharides, nucleic acids, and proteins known as extracellular polymeric substances (EPS). Even the most ancient lineages of bacteria preferentially exist in biofilms [37, 61]. There is evidence of biofilm formation in early fossil records over 3 billion years ago [60]. Biofilm formation is an integral characteristic of prokaryotic survival and has been observed in virtually all species of bacteria (except obligate intracellular parasites such as *Chlamydia* sp. and *Mycoplasma* sp.), including organisms associated with neurosurgical device-related infections such as *Staphylococcus epidermidis*, *S. aureus*, *Streptococcus* sp., and *Pseudomonas aeruginosa*.

The gene expression profiles of bacteria in biofilms are quite different compared with the expression profiles of the same strains when growing planktonically. Great effort has been expended over the past several years to identify novel genes that are uniquely expressed in biofilm envirovars [9, 17, 19, 27, 28, 76]. Such genes include those responsible for regulation and/or expression of surface adhesion proteins, appendages such as fimbriae, pili or flagella, and EPS in phenotypes that are distinct from their planktonic counterparts. Recent studies have also shown that there is a greatly increased rate of horizontal gene transfer among bacteria living within a biofilm [32, 79]. This reassortment of genes among biofilm bacteria is a continuous process with important contributions to evolutionary fitness and survival.

What are the five stages of biofilm development?

Recently, proteomic studies of *P. aeruginosa* biofilms have delineated a highly regulated developmental sequence that includes five stages: reversible attachment, irreversible adhesion, aggregation, growth and maturation, and detachment [65, 71]. Biofilm formation begins with attachment of bacteria to a surface [30, 31], followed by a cascade of differential gene expression resulting in the "biofilm phenotype" [71]. Biofilm microcolonies recruit other free-floating bacteria via extracellular small molecule signals that lead planktonic bacteria to find a suitable surface for attachment [20]. Biofilm formation can also be facilitated by formation of an organic conditioning layer which may include compounds released by the host inflammatory response [30]. After the initial reversible contact with a surface, bacteria then exhibit robust irreversible adhesion and extreme resistance to shear stress. Biofilms exhibit a viscoelastic response that permits stretching without dislodgement under sudden increases in shear stress. During sustained increases in shear force, the biofilm will remodel itself to tolerate even higher levels of shear stress [69]. These rheological properties of biofilms have been recently reviewed [71]. Amazingly, experiments conducted on military aircrafts have shown biofilm survival after exposure to extreme shear forces at high altitudes [16].

The third and fourth stages in the biofilm lifecycle involve, respectively, aggregation followed by growth and maturation. During these stages, bacterial biofilms can be flat or mushroom-shaped depending on the nutrient source [30, 71]. Confocal laser scanning microscopy (CLSM) has demonstrated that these colonies are complex, many of them replete with water channels resembling a primitive circulatory system [2, 12, 16, 42, 71]. Indeed, bacterial biofilm formation is similar to survival strategies employed by self-assembling eukaryotes such as cellular slime molds [30] (Fig. 1).

The fifth stage of biofilm development is detachment, or the dispersal of single bacterial cells, or aggregates of bacteria, into the surrounding environment. This process may be the result of external forces, or be caused by internal intercellular messengers [70, 68]. This "showering" of planktonic bacteria or the release of multicellular bacterial emboli leads to bacteremia and possible sepsis, depending on the host. Even if antibiotic treatment kills the circulating bacteria, the original nidus survives in the biofilm.

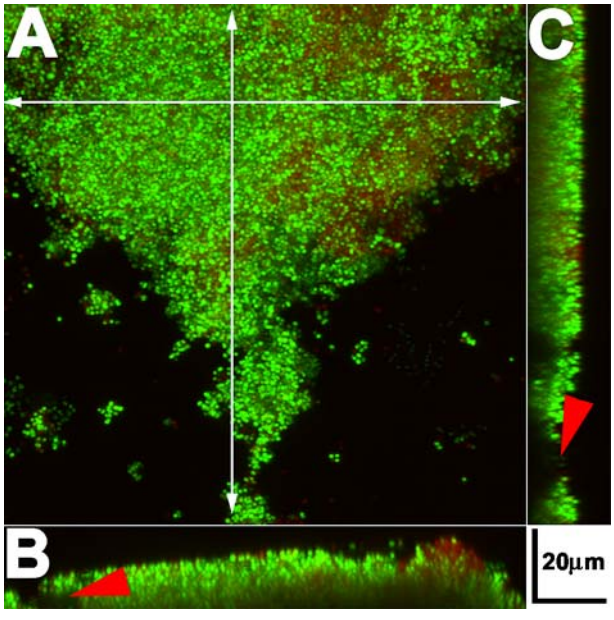

Fig. 1 Confocal laser scanning microscopic (CLSM) image of a *Staphylococcus aureus* biofilm growing on the internal surface of an in-vitro venous catheter model. **a** Plan view showing a large cell cluster containing thousands of cocci stained with the LIVE/DEAD BacLight kit (Molecular Probes). Live cells are stained green with Syto 9 dye and dead cells are stained red with propidium iodide. The biofilm is characteristically patchy with cell clusters separated by voids (*black areas*). **b**, **c** Side views through the biofilm in the XZ and YZ planes, respectively. *Red arrows* show channels penetrating the biofilm. The cross-sections were taken along transects indicated by the *white lines* in **a**. Image provided by S. Wilson, Center for Biofilm Engineering, Montana State University

What advantages do bacteria gain by being in a biofilm?

Bacteria gain tremendous advantages from biofilm formation, both ex vivo and in vivo [30]. These microbial ecosystems provide protection from environmental shifts in moisture, temperature, pH, and exposure to ultraviolet light. The close proximity of bacteria in biofilms facilitates the development of cell-to-cell interactions. Aggregation in the EPS matrix makes an entity too large to be phagocytized by the host's immune system cells. In addition, biofilm bacteria are highly resistant to both host humeral defenses and standard concentrations of antimicrobial agents [4, 34, 38, 53, 82]. This is especially relevant in the central nervous system, where the blood–brain barrier limits antibiotic penetration. It was previously assumed that bacteria were more recalcitrant to antibiotics strictly because of limited diffusion or penetration into the EPS matrix; however, it is now clear that many antibiotics can readily penetrate into biofilms [78]. Two alternative mechanisms proposed to explain biofilm resistance are: (1) a decreased metabolic activity secondary to nutrient availability [3, 7, 66, 78] and (2) the presence of subpopulations of antibiotic-resistant phenotypes or "persisters"[66, 72].

Some of the characteristics of biofilms that confer resistance to antibiotics also make them difficult to culture and enumerate in vitro. Without a treatment aimed at disrupting the biofilm EPS matrix, culturing a biofilm aggregate containing thousands of cells would yield one colony rather then one colony per bacterium, thus greatly underestimating the true number of organisms actually present [14].

Types of biofilms

Biofilm formation depends on the nature of the substratum and the surrounding environmental conditions. Although biofilms were originally thought to form only on inert surfaces, recently one of us (G.D.E.) proposed that biofilms can also form on mucosal surfaces, producing chronic infections without any foreign body present. These biofilms have been termed "mucosal biofilms," [22], and recent studies have established that this is a common phenomenon [11, 18, 51]. These biofilms exhibit markedly different gene expression patterns than their counterparts on inert surfaces, and have integrated host proteins and cells into their EPS [30].

Why have historical studies focused on planktonic bacteria?

Much of the thinking pertaining to the study of bacteria as the source of infectious disease stems from principles developed by Robert Koch in the late nineteenth century. His paradigm of isolation and pure culture was highly instructive for acute bacterial infections; however, the canonization of his teachings has focused study on planktonic bacteria to the exclusion of other bacterial phenotypes. Unfortunately this focus on bacteria growing in suspension in laboratory cultures has little to do with in vivo microbial environments. Moreover, planktonic bacteria are much easier to study than biofilm bacteria, and only recently have advances in CLSM and molecular genetics allowed for the explicit identification and characterization of these sessile, often slowly metabolizing biofilm bacteria. These technologies permit us to ask and answer questions that were previously techically unfeasible, and as a result have formed the core of the data sets that led to the development of a more sophisticated concept of bacterial infection than was possible in Koch's time.

Biofilms in human disease

Biofilm-based infections have been associated with native and prosthetic valve endocarditis [12, 35], vascular catheters [56], breast implants [77], urinary catheters [23, 52], total joint replacements, and otolaryngologic infections [57, 58] to name a few; and are often present when standard bacterial culture and plating results are negative. The biofilm, although potentially harmful to the host, is often not as pathogenic as the host's own inflammatory response to the biofilm. A classic example of this is the tissue damage in cystic fibrosis that results when frustrated neutrophils continuously fire oxidative bursts at biofilms that they cannot eradicate. Planktonic bacteria shed from the biofilm, however, can cause acute systemic illness [26, 45]. Biofilms have been increasingly recognized as playing an important role in chronic human infections. The characterization of biofilms on numerous medical devices and mucosa have fueled new molecular- and material-based strategies to combat chronic and device-related infections.

Biofilms in diseases of neurosurgical interest

The biofilm paradigm is changing our understanding of chronic and device-related infections in an era of unprecedented utilization of devices in complex spinal instrumentation, functional and epileptic surgery, and CSF diversion. Chronic infections after delayed cranioplasty are also becoming more common in light of the increasing popularity of decompressive hemicraniectomy procedures for stroke and traumatic brain injury [25, 67]. A prerequisite for the rational development of strategies to combat biofilm infections is an understanding of the metabolic processes that are unique to bacterial biofilm physiology.

Spinal instrumentation infections as biofilm diseases

Major advances in surgical instrumentation for the treatment of such pathologies as fracture, neoplasm, and degeneration of the vertebral column [55, 73, 80] have resulted in the pervasive use of hardware by neurosurgeons. However, the use of these devices is not without cost, as they are clearly associated with an increased risk of

postoperative infections. Estimates of the rate of infection range from 2.1 to 8.5% in several retrospectives reviews [1, 24, 41, 44, 48]. Implant infections result in prolonged hospital stays with an average duration of 16.6 days [43], and antibiotic therapy costs which can reach $350,000. Given that these patients often require revisional surgery and additional rehabilitation therapy after discharge, the total economic impact of these infections is even higher.

The vast majority of spinal instrumentation infections are caused by *Staphylococcus aureus* and *S. epidermidis*. However, some infections are polymicrobial in nature and others do not have an identifiable organism. The source of post-implant infections depends on the timing of the infection with respect to the placement of the implant. Early infections (during the first few weeks after surgery) most likely result from an inoculation during surgery, whereas failures that occur years following implantation are probably the result of seeding from systemic infections. Since eradication of the infection always requires re-operation and often removal of the hardware [33, 62], the most successful treatment strategies are likely to be those that prevent biofilm formation.

Biofilms on pulse generators

Biofilms have been demonstrated on cardiac pacemaker leads and pulse generators [39, 47]. Such technology is finding its way into neurosurgical procedures in the form of devices aimed at stimulating structures in the motor cortex, deep brain, dorsal column, and vagal nerve. Umerura et al. reported a 3.7% incidence of deep brain stimulator infections, requiring removal of the pulse generators in all cases and the entire system in 75% of cases [75]. Similar rates of infection for dorsal column stimulators were reported at 3.4% in a recent meta-analysis of 2972 cases [10]. In rare instances, these device-related infections can lead to serious sequelae such as paralysis or life-threatening sepsis [50, 74]. However, any biofilm infection can be locally deleterious to the patient, and all are very resistant to antibiotic treatment. Moreover, the interior of leads that run from the stimulator to the pulse generator are inaccessible to host defense mechanisms and antibiotics. With expanding indications for neurostimulators ranging from depression to obesity on the horizon [59, 63], device-related infections will continue to frustrate neurosurgeons and patients.

Biofilms in CSF shunts

Of the nearly 18,000 ventriculoperitoneal (VP) shunts placed annually, approximately 25% must undergo revision due to biofilm growth [6, 49]. Several studies have shown direct evidence of biofilm formation on VP shunts [21, 40, 64, 81], and in reality probably all cerebral spinal fluid (CSF) shunts support biofilms. Each year approximately 122,000 ventriculostomy catheters are placed for a wide variety of indications, ranging from acute hydrocephalus caused by hemorrhage or neoplasm to ICP monitoring and management in the setting of neurotrauma. A potentially life-threatening consequence of this procedure is ventriculitis resulting from microbial infection of these devices. Infections related to ventriculostomy catheter insertion have been reported to vary between 0 and 22%, but a common average is about 10% [46]. Strategies to prevent bacterial colonization of catheters have included impregnation of the catheter material with antibiotics, altering the chemical composition of the polymer, and changing the physical surface properties. Unfortunately, all of these approaches have met with limited success in reducing biofilm formation [5, 8, 40]. Future treatments should focus on preventing the formation of biofilms initially, modulating the biofilm bacteria or the EPS, and/or inducing the bacteria to

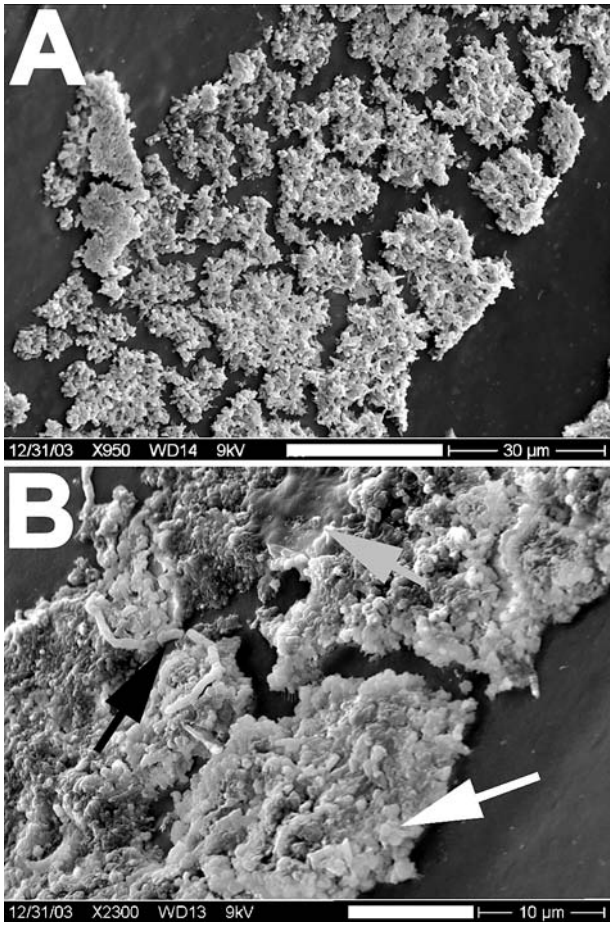

Fig. 2 Scanning electron microscopic (SEM) images of biofilms growing on the inner lumen of an infected ventriculoperitoneal shunt. **a** Lower power image showing a layer of rod shaped bacteria. The cracks are an artifact caused by dehydration of the specimen during fixation. Scale bar=30 µm. **b** Higher power image showing a biofilm formed of bacterial rods (*black arrow* indicates chain of rods) and possible cocci (indicated by *white arrow*). These distinct morphologies suggest that the infection was polymicrobial in nature, and are consistent with culture results in which both *Corynebacterium* sp. (Gram positive filamentous rods) and *Staphylococcus epidermidis* (Gram positive cocci) were isolated. The *grey arrow* indicates possible extracellular polymeric slime matrix (EPS) which is a hallmark feature of biofilms. Scale bar=10 µm

transform from the biofilm phenotype to the much more treatable planktonic form.

Biofilms in bone flap infections

Bacterial biofilm formation is fundamental to the pathogenesis of osteomyelitis. Direct scanning electron microscopy (SEM) of material obtained after surgical removal of osteomyelitic bone has revealed that the infecting bacteria grew in a pervasive biofilm that obscured the bone surfaces [29]. These adherent biofilms resist antibiotic penetration and provide protection from antibodies and other host clearance mechanisms (Fig. 2).

A major complication of delayed autologous bone flap cranioplasty is infection [36, 54]. All of the infected cryopreserved bone grafts studied had negative bacterial cultures prior to implantation [36]. However, when viewed in light of the biofilm paradigm, it is possible these implants were simply contaminated with culture-resistant biofilms. Conventional plating and culture techniques seem outdated as our knowledge of biofilms increases and an urgent need exists to adopt state-of-the-art imaging technologies and molecular diagnostics.

Conclusion

An unprecedented number of biological discoveries and engineering advances have resulted in greatly increased utilization rates of medical devices in the setting of neurologic diseases. These advances are accompanied by higher rates of postoperative infections, which are undoubtedly associated with the formation and persistence of bacterial biofilms that act as complex differentiated multicellular organisms akin to simple eukaryotic metazoans.

The bacterial biofilm paradigm encompasses four cardinal concepts: (1) bacteria prefer to exist in an organized community enshrouded in a slimy EPS matrix; (2) biofilms periodically release either emboli containing clumps of bacteria embedded within a matrix that can then metastasize, or planktonic bacteria that can produce acute systemic disease; (3) biofilm bacteria are highly resistant to antibiotics that are bactericidal against planktonic bacteria; and (4) culturing of biofilm bacteria either results in massive underestimates or is completely unsuccessful, leading to a false diagnosis of sterility. The development of the biofilm paradigm of chronic bacterial infections represents new hope for the development of novel therapies aimed at biofilm-specific metabolic processes to reduce the incidence and morbidity associated with device-related infections.

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