The Role of Bacterial Biofilms and the Pathophysiology of Chronic Rhinosinusitis

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Current Allergy and Asthma Reports 2008, **8:**227–233
Current Medicine Group LLC ISSN 1529-7322
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The earliest description of a bacterial biofilm is likely centuries old. However, only in the past few decades has a wealth of knowledge developed pertaining to this bacterial form of existence. Biofilms have been implicated mainly in chronic disease states, and the current available treatment modalities for infection have demonstrated limited efficacy against bacteria in this form. There is evidence associating bacterial biofilm formation in chronic infections of the upper airway, and therefore we examine the possible role of a bacterial biofilm in chronic rhinosinusitis while drawing parallels with recent data from other bodily regions. Lastly, directions for contemporary biofilm research are reviewed and highlighted in terms of their application to chronic rhinosinusitis.

Introduction

Although the discussion and investigation of bacterial biofilms specific to diseases of the head and neck has intensified over the past several years, the actual discovery of a bacterial biofilm may be centuries old [1]. However, only recently was evidence realized for bacterial biofilm formation and control [2,3]. Since then, an intensive development of scientific knowledge has occurred regarding biofilm growth and behavior. In concordance with these developments, the medical community is beginning to recognize the role of biofilms in the setting of chronic infections [4]. Specific to otolaryngology, a great deal of knowledge has developed implicating bacterial biofilms in recurrent adenotonsillar infection, otitis media, cholesteatoma, and chronic rhinosinusitis (CRS) [5,6]. In this article, we highlight some parallels between findings in CRS and other organ systems, and we focus on our current knowledge and understanding of the role of bacterial biofilms in CRS.

What Is a Biofilm?

A biofilm consists of an organized community of bacteria adherent to a mucosal surface or foreign body, situated in an extensive extracellular polymeric substance (glycocalyx) composed primarily of polysaccharides, but also containing protein and DNA [7]. The glycocalyx is a mosaic of bacterial colonies that possesses varying phenotypes and different physiochemical properties. Not only does it protect its bacterial inhabitants, but it also serves to modulate the microenvironment of these colonies through its numerous canals via a process of interbacterial signaling called quorum sensing [7].

The concept of bacteria living in the organized communities that compose bacterial biofilms is a radical departure from the classic notion of bacterial infection and bacterial behavior. Conventional theory dictates that bacterial infections are caused by isolated bacteria (bacteria in planktonic form) that can be identified by conventional culture techniques, treated with antibiotics, or prevented with specific vaccines. This theory has formed the foundation of bacterial infectious disease practice for decades, but this approach may not be appropriate to explain the increasing role that bacterial infection or colonization is believed to play in human disease. In contrast, bacterial biofilms appear to be relevant in chronic or much more complex infections, which are common manifestations of chronic human illnesses. When in the form of a biofilm, the causative bacteria are difficult to culture and are largely resistant to current antimicrobial therapy. To date, no specific medical treatment exists to specifically target biofilms in the human host. Similarly, the identification of a biofilm has relied upon the analysis of tissue samples with electron microscopy or DNA identification with polymerase chain reaction (PCR). No simple clinical test is available for detecting the presence of biofilms.

Biofilm formation

The formation of a bacterial biofilm is theorized to occur in several concurrent steps. However, before this process can start, a supportive nutrient-rich microenvironment must be present to allow the survival and replication of the biofilm-initiating planktonic organisms. This prerequisite microenvironment is provided by essentially all bodily fluids. However, endothelial surfaces may be more resistant to biofilm initiation due to their innate antimicrobial defenses and, in some cases, by the presence of symbiotic native bacteria whose biofilm inhibits the adhesion of other planktonic organisms. When successful biofilm formation does occur, the initiating event is the attachment of planktonic bacteria to a surface due to several generally weak reversible physical forces. If these bacteria are not immediately separated from this surface, they will undergo a phenotypic change that allows for yet stronger binding, using adhesins. Once adhered, through the process of quorum sensing (cell-to-cell signaling), they facilitate the binding of other bacteria to the infected surface and/or to the initial colonists, thereby forming bacterial colonies, while undergoing phenotypic change. During this period, the protective glycocalyx also forms, enveloping the bacteria.

Cell division and recruitment ensure the continued growth of the biofilm. As this cycle continues and the biofilm grows, maturation of the biofilm occurs with the formation of an underlying architecture of microcolonies and channels. Eventually, a dynamic equilibrium is reached where planktonic or free bacteria are present at the biofilm surface; these bacteria can then be redistributed or seeded through a number of mechanisms to other areas of the host surface, where further biofilm growth can be initiated.

Mechanisms of antibiotic resistance in biofilms

The recognition of bacterial biofilms was important to explain the occurrence of postantibiotic persistence of infection with implanted medical devices. These infections would appear to be clinically resolved, yet would return shortly after cessation of antimicrobial therapy. Thus, bacterial biofilms appear able to evade host defenses while at the same time resisting current antimicrobial chemotherapeutic agents. Although traditional mechanisms of bacterial resistance to antibiotics may operate in bacterial biofilms, it is likely that nonconventional mechanisms are responsible for biofilm antibiotic resistance. Similarly, biofilm-associated antimicrobial resistance is likely multifactorial and may vary among organisms.

The simplest explanation of antibiotic resistance is that biofilms form a physical barrier to antibiotics, perhaps as a result of charge affinity between the antibiotic and polymers in the biofilm matrix [8]. However, this is likely not the predominant explanation because numerous mechanisms exist by which biofilms may limit antimicrobial efficacy [8–10]. One possible contributory mechanism of resistance is related to nutrient and oxygen status. Nutrient and oxygen depletion, or waste-product accumulation, within the biofilm may lead bacteria to enter a quiescent, low metabolic state termed the general stress response. These cells then show increased resistance to a wide variety of physical and chemical agents, and because of this reaction to extreme nutrient stress are therefore less susceptible to growth-dependent antimicrobial killing [2,4]. A further theory is the differentiation of bacterial species into a resistant phenotype to antimicrobial products because of osmotic stress and the resultant downregulation of transmembrane channels [8]. The existence of efflux pumps or the expression of biofilm-specific antimicrobial resistance genes that are not required for biofilm formation may also play a role in biofilm persistence [10]. Quorum sensing, in biofilms of both gram-negative and gram-positive bacteria, is yet a further method of protection, resistance, and biofilm proliferation. In its simplest form, quorum sensing may allow the "pooling" or sharing of genetic information within the bacterial community via intercellular signaling. Following a specific contact, peripheral cells can send a signaling molecule to other cells located deeper in the glycocalyx labyrinth, which when bound to its receptor can induce transcription of its target genes [9]. This allows the bacteria in biofilms to have access to a wealth of genetic material, such as resistance genes, without having to bear their associated metabolic cost and negative selection pressure.

Biofilms in Otolaryngology

Otitis media

Otitis media is a very common pediatric infection. As a result, myringotomy and ventilation tube insertion are frequently performed surgical procedures in North America and Europe to treat otitis media with effusion (OME) [11]. The evidence for implicating biofilms in the pathophysiology of OME is relatively new, as is that for most chronic infections involving the upper airway. The contribution of a bacterial pathogen to the occurrence of a chronic middle ear effusion had been suspected for several years [12,13]. However, initial investigations using culture techniques could only implicate bacterial involvement in the persistence of effusion in about one third of OME cases [14–16]. The advent of PCR technology has enabled further investigation and detection of bacteria as a possible cause of persistent OME.

The use of PCR-based techniques has demonstrated a significantly higher detection rate of bacterial pathogens in OME when compared with conventional culture techniques alone [17]. In many cases, DNA from pathogenic bacteria could be detected using PCR in ears that were culture negative [18]. Furthermore, to determine that DNA sequences being identified with PCR were indeed from live bacteria rather than the fossilized remains of prior infec-

tive species, one study used PCR to detect the presence of mRNA from bacterial pathogens in OME [19]. Given the extremely short half-life of mRNA, its presence was evidence that viable metabolically active bacterial organisms were likely present in OME. Additional indirect evidence for bacterial presence was attained by the identification of endotoxin, secreted by gram-negative bacteria, in middle-ear aspirates [20]. Most convincing was the demonstration in a chinchilla model that live bacteria, although nonculturable, could persist in OME for weeks, whereas DNA strands and DNA from intact but nonviable bacteria could not exist for more than a day [21]. Therefore, in the OME model the use of PCR facilitated the recognition that 1) a persistent middle-ear effusion could be related to the presence of bacteria; 2) bacteria did exist in a live form in OME; and 3) these bacterial species were largely undetectable by conventional culture techniques.

The identification of biofilms elsewhere in the body led to the realization that these same organisms could be present in OME. With evidence collected through the use of PCR, searching for the presence of bacterial biofilms in OME was a logical next step. Using the chinchilla model, investigators showed that when *Haemophilus influenzae* is introduced into the middle-ear space, viable bacterial biofilms form in as little time as 1 day. These biofilms remained throughout the observation period, and achieved a greater density with time, indicating the possible implication of biofilms in OME [22,23]. Similarly, in a primate model, ears inoculated with *Pseudomonas aeruginosa* have been shown to produce a biofilm [24]. Finally, direct demonstration of bacterial biofilm in middle-ear mucosal biopsies from a series of patients with OME, recurrent acute otitis media, and undergoing myringotomy and tube insertion showed that 92% of these specimens had bacterial biofilms, as detected by confocal scanning laser microscopy (CSLM) [25]. In contrast, the positive culture rate was only 22% in the children with middle-ear effusions in this study.

In summary, a role for bacteria in the occurrence of OME has long been suspected. The discovery of bacterial biofilms provides further evidence linking bacteria to this disease. Because the bacteria likely exist in their nonplanktonic form, this helps explain the difficulty of initial investigators in demonstrating a bacterial presence in this disease. However, the exact mechanisms by which bacteria in the form of a biofilm induce OME remain to be determined.

Adenotonsillar disease

Similar to OME, the presence of a high bacteria load has been identified in adenotonsillar hypertrophy and chronic disease [26,27]. The findings from surface and core cultures have been used to infer that the chronic disease state and/or adenotonsillar hypertrophy is related to the presence of identified bacterial pathogens. The demonstration of bacterial biofilms in these disease states further supported these insights. In chronic tonsillitis, anatomic evidence exists for the presence of both gram-positive and gram-negative bacterial biofilms [28]. Recently, bacterial biofilms have been detected in 70% of patients with chronic tonsillitis at the time of tonsillectomy [29]. Similarly, in a group of pediatric patients with CRS, adenoidectomy specimens revealed that nearly 95% of their surface was covered by mature bacterial biofilms. At the same time, specimens from control patients revealed very little coverage (< 2%) [30]. Finding these bacterial biofilms in patients with chronic adenotonsillar disease supports the notion that these chronic illnesses may be secondary to bacterial infection in a nonplanktonic form, which has been shown to be difficult to eradicate with current antimicrobial therapies.

Biofilms and CRS

Chronic rhinosinusitis is a chronic disease of the paranasal sinuses that affects a significant number of individuals in North America and the developed world. Numerous etiologies for this heterogeneous disease have been posited, including anatomic factors leading to ostial obstruction, ciliary dysfunction, bacterial or fungal infection, superantigen stimulation of the immune system, allergy, and immune deficiency. What is currently evident is that CRS is an inflammatory disease, and that the etiology for persistent inflammation in an individual patient is often indiscernible.

Although bacterial involvement in CRS has long been suspected, researchers have questioned this role because of the high rates of negative cultures and the absence of a lasting response to antibiotics. The science of bacterial biofilms offers novel insight into the disease by furnishing what many perceive as the "missing link" between bacterial presence and inflammation in CRS. The recognition of bacterial biofilm as a recognized, and perhaps preferred, form of bacterial existence and the evidence for its implication in chronic infections throughout the body is now insurmountable [7]. Contributing to this is the growing evidence for the presence of bacterial biofilms in CRS.

Previous evidence

The first report supporting the presence of bacterial biofilms in CRS in the English literature emerged in 2004 [31]. Using scanning electron microscopy, the authors demonstrated that structures consistent with bacterial biofilms were present on the biopsied mucosa of a limited number of patients who were clinically nonresponsive to medical treatment for CRS. These findings were later confirmed with work that identified biofilms on the mucosa of patients with CRS at the time of endoscopic sinus surgery (ESS) using electron microscopy [32].

Subsequently, in a rabbit animal model of sinusitis using *P. aeruginosa*, investigators showed that this organism could form a biofilm in an anatomically obstructed

model of sinusitis. In the control sinuses that were obstructed, but in which no organism was introduced, no biofilm growth was observed and mucosal histology appeared normal using scanning electron microscopy [33]. A recent prospective study of 38 patients with CRS undergoing ESS demonstrated a bacterial biofilm in 44% of the patients, whereas no bacterial biofilms were observed in the nine control patients using confocal scanning laser microscopy [34•]. Unfortunately, the biofilm stain used in this study did not allow for assessment of the organisms involved.

To date, the bacteria commonly implicated in patients with CRS have been identified to exist in the form of a biofilm. In numerous studies of the bacteriology of CRS done with conventional culture methods, *Staphylococcus aureus* has been implicated as one of the most common bacteria to colonize the paranasal sinuses in both asymptomatic postoperative and operative patients with CRS [35,36]. As such, this organism has been demonstrated to occur in the form of a biofilm in patients with CRS, as have coagulase-negative *Staphylococcus (CNS)*, *P. aeruginosa*, and *H. influenzae* [37–40].

However, the current thinking on bacteriology may need to be revised. In one thought-provoking study of bacterial biofilms in CRS, researchers used confocal scanning laser microscopy with fluorescent in-situ hybridization using specific molecular probes and identified *Streptococcus pneumoniae*, *H. influenzae*, *S. aureus*, and *P. aeruginosa* in mucosal biopsies from individuals undergoing ESS for CRS [40]. Bacterial biofilm was identified in 14 of 18 CRS patients but in only 2 of 5 controls. Although biofilms containing either pure or mixed isolates of all species assessed were present, surprisingly, the principal pathogen identified was *H. influenzae*, previously not believed to play a role in chronic sinus infection. This is also surprising because it was not recovered in any of the simultaneously performed conventional sinus cultures. A caveat is that *H. influenzae* was also recovered in 2 of 5 of the asymptomatic control specimens, reinforcing the importance of other factors such as host susceptibility to the development and persistence of inflammation in CRS [40].

Rather convincingly, these studies demonstrate that bacterial biofilms are present in patients with CRS, and potentially in a significant proportion of the CRS population. In addition, they suggest that the bacteriology of these bacterial biofilms may differ radically from previous descriptions of conventional cultures, potentially implicating *H. influenzae* as an important pathogen in CRS, a heretofore unsuspected finding.

Taken together, these studies suggest that the presence of a bacterial biofilm may be contributing to the poor response to medical treatment for certain patients with CRS. In such instances, a bacterial biofilm may serve as a constant nidus for infection of the paranasal sinuses, resulting in the prolonged inflammatory response of the affected respiratory mucosa. However, it remains to be clarified which organisms are involved and the exact mechanisms by which a bacterial biofilm may perpetuate the inflammation seen in CRS.

Clinical relevance

Although bacterial biofilms have now incontrovertibly been demonstrated to be present on the sinus mucosa of individuals with CRS, their role in the development and persistence of the disease is only beginning to be established. To date, only two studies exploring the functional importance of biofilms have been performed.

Our group studied the biofilm-forming capacity of bacteria strains recovered from individuals at least 1 year after ESS [41•]. In this study, isolates for *S. aureus*, *P. aeruginosa*, and *CNS* were obtained from unselected individuals following ESS, and an in vitro technique using crystal violet was used to semi-quantitatively determine biofilm-forming capacity of these isolates. Biofilm-forming capacity was noted for a number of isolates of *CNS, S. aureus*, and *P. aeruginosa*. Interestingly, only patients who had a poor outcome postoperatively demonstrated in vitro biofilm-producing capacity with either *P. aeruginosa* or *S. aureus*, whereas patients with these organisms that did not have this biofilm-forming capacity had a more favorable outcome. The presence of a *CNS* biofilm did not predispose patients to an unfavorable postoperative course. This study suggests that the persistence of pathogenic organisms via biofilm, and not the presence of the biofilm itself, is influencing disease.

Psaltis et al. [42] assessed the functional impact of the presence of detectable biofilm. They retrospectively assessed the postoperative outcome of individuals undergoing ESS for chronic sinusitis with and without nasal polyposis, evaluated according to the presence of bacterial biofilm in surgical specimens. Using confocal scanning laser microscopy and a fluorescent cell-permeant stain, they identified bacterial biofilms in 20 of 40 (50%) patients. Forging the link between the presence of biofilm and severity of disease, they noted that biofilm detection on mucosal biopsy was associated with a worse preoperative radiological score, and worse postoperative symptoms and mucosal outcomes at follow-up [42].

The mechanism by which biofilms influence the severity of sinus disease is not clear; nevertheless there is support for the concept that certain pathogenic bacteria in the form of biofilms are related to the persistence of CRS. Therefore, bacterial biofilms are an important avenue for further research and their modulation is a potential therapeutic target.

Clinical applications

Identification of biofilms

What remains particularly problematic for the clinician is the identification of a bacterial biofilm given the current lack of clinical tests. Clinicians have relied upon electron microscopy with mucosal biopsy specimens to detect bac-

terial biofilms. Confocal scanning laser microscopy may be the most sensitive and specific of such instruments for detecting a biofilm, whereas mis-estimates may occur with the use of either scanning or transmission electron microscopy [43]. A more widely applicable test for the presence of a bacterial biofilm may be an in vitro assay, which does not rely on expensive technology. Such an assay technique has been successfully used to detect *CNS*, *P. aeruginosa*, and *S. aureus* biofilms [39]. The detection of a bacterial biofilm in a particular patient may prove important for predicting the likely clinical course and determining appropriate targeted therapy. Development of a clinically applicable biofilm detection method remains a priority for research. In the future, aside from patient prognosis, one would hope that an easily performed clinical test for biofilm detection could also be paired with specific biofilm therapies that could ameliorate the prognosis for patients affected by these bacteria.

Treatment of Biofilms

Since the discovery of biofilms, it has been recognized that infections caused by these bacteria are generally resistant to standard forms of antimicrobial therapy. However, there are numerous potential ways to eradicate a bacterial biofilm. These include ventilating the sinus cavity, killing the pathogenic bacteria, interfering with the quorum sensing of biofilms, and interfering with the biofilm structure either mechanically or chemically. We focus here on the local chemical and mechanical treatments of biofilms for CRS. Given the enhanced access to the sinus mucosa via the now-patent sinus ostia after ESS, topical therapy may represent a privileged avenue for the management of biofilms in patients after ESS. Although the optimal therapy remains to be determined, several theoretical concepts and in vitro results may help guide research in this area.

Saline

Given that the polymers of the extracellular polymeric substance of bacterial biofilms are water soluble, a biofilm should disperse into solution when immersed in saline. However, in a *P. aeruginosa* biofilm, due to the calcium-ion bridging that cross-links mannuronic/guluronic acid, this dissolution is prevented, thus rendering treatment with simple saline irrigations inefficacious [44••].

Antimicrobials

Because biofilms are not eradicated by standard antimicrobial therapy, they can persist to cause an intense inflammatory response in patients with refractory CRS. Topical treatment with an antimicrobial may be effective; however, the concentration may need to be adjusted. Using an in vivo rabbit model of maxillary sinusitis, it has been shown that *P. aeruginosa* biofilms are not eradicated with either topical tobramycin or saline treatments [45]. In another in vitro study, although moxifloxacin at levels approaching minimal inhibitory concentration attainable with oral antibiotic therapy had no effect on biofilms, high concentrations of 1000 times minimal inhibitory concentration of moxifloxacin effected a 2.0- to 2.5-log reduction (or > 99% reduction) in the number of viable bacteria in vitro in a mature *S. aureus* biofilm [46].

In a recent report, mupirocin, but not ciprofloxacin or vancomycin, was shown to result in a reduction of biofilm-forming *S. aureus* in clinical isolates. Mupirocin reduced biofilm mass by greater than 90% at concentrations of 125 μg/mL or less in all *S. aureus* isolates. This suggests that topical irrigations with this agent may have a role against *S. aureus* biofilm disease in the clinical setting [47].

Modification of these formulations may be required to enhance effectiveness. In other studies, the addition of agents to the antimicrobial treatments of in vitro *P. aeruginosa* biofilms such as arginine [48], asiatic acid, corosolic acid [49], and electrical currents [50] have resulted in increased antimicrobial efficacy.

Mechanical treatment

Much of the medical literature on mechanical treatments for eradicating bacterial biofilms emanates from orthopedic research. Hip implant infections, most commonly by skin organisms, were identified as particularly problematic because the original implant must be removed, followed by a delayed re-implantation procedure. Thus, methods for treating and preventing biofilm-mediated infections are extremely important, especially given the increasing use of biomedical implants in orthopedic surgery consequent to an aging population [51].

Early investigations identified chemical treatments that were effective at producing a logarithmic reduction in the biofilm load of *Staphylococcus* species and *P. aeruginosa* on implant devices, which were subsequently improved upon by the addition of a mechanical pressure irrigation system [52]. Studies using in vivo models then showed that certain chemicals, when used for mechanical irrigation, may have a greater impact on infections caused by one organism but not another. For example, Castile soap has demonstrated better efficacy for the treatment of *P. aeruginosa* infection, whereas benzalkonium chloride had a greater benefit in wounds containing *S. aureus* [53]. In general, the use of a surfactant irrigation solution rather than an antibiotic or saline has been shown to have greater efficacy in treating these infections in an orthopedic model because they disrupt the bonds of the organism to the infected surface [54].

This work in orthopedics suggested that an effective biofilm-removal method should include two principal components: 1) a device that delivers the solution with pressure (a shear force); and 2) a nontoxic, water-soluble, low-viscosity chemical solution (surfactant) capable of breaking the chemical bonds of the biofilm in question. To treat CRS, a pressure irrigation system using a mixture of sterile water, citric acid, and capry-

lyl sulfobetaine (zwitterionic surfactant) has been used in vitro to assess its effect at reducing the biomass of human sinus bacterial biofilms [44••]. The citric acid/zwitterionic surfactant (CAZS) works because the citric acid sequesters the calcium ion of the *P. aeruginosa* extracellular polymeric substance structure, thereby allowing the surfactant to bring the polymer into solution. When tested on the biofilm mass of *S. aureus* and *P. aeruginosa*, the use of saline irrigation alone produced a 2-log reduction in colony-forming units (CFU), whereas the use of CAZS irrigation produced 3.9-log (*S. aureus*) and 5.2-log (*P. aeruginosa*) reductions in CFU. Similarly, when administered as a static solution, CAZS was more effective than the other tested substances because it produced a 2.5-log to 2.9-log reduction in CFU (300×–800×) [44••]. These extremely encouraging results for the treatment of bacterial biofilms need to be verified in the affected patient population before widespread clinical application of this topical therapy.

However, although early reports are encouraging, no topical antimicrobial therapy has been shown to be safe or effective for eradicating biofilms in CRS in human sinus disease, and these in vitro or animal studies should not be extrapolated directly into treatment of patients.

Conclusions

The identification of bacterial biofilms in the upper respiratory tract has been a significant development in understanding the pathophysiology of chronic infectious illnesses of this region. Although our comprehension of the exact mechanisms by which these organisms cause infection and inflammation are yet to be identified, future directions for investigation in CRS include the development of rapid clinical tests that facilitate the diagnosis of infection by a bacterial biofilm. Similarly, the eradication or control of these infections depends upon the production of efficacious topical treatments, systemic biofilm signal-manipulating therapies, or possibly even host immune-response modulation.

Disclosures

No potential conflict of interest relevant to this article was reported for Dr. Kilty.

Dr. Desrosiers is a consultant on biofilms to MedtronicXomed (Jacksonville, FL).

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