

Bacterial biofilms and the pathophysiology of chronic rhinosinusitis

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Purpose of review

To review the evidence for the presence of bacterial biofilms in chronic rhinosinusitis (CRS) and mechanisms by which they may contribute to the chronic inflammation characteristic of this disease. Lastly, to provide an overview of the current and potential future treatments for bacterial biofilms in CRS.

Recent findings

Advances in the techniques for identifying biofilms have confirmed the presence of bacterial biofilms on the sinonasal mucosa of patients with CRS. The impact on mucosal inflammation of the polymicrobial or multiorganism milieu is not yet well understood. Numerous novel topical therapies for the treatment of bacterial biofilms in CRS have been suggested with some demonstrating clinical efficacy. Blocking of quorum sensing represents a potential future therapy for biofilm treatment in CRS and biofilm infection at large.

Summary

Biofilms represent an important influence on the pathophysiology of CRS. Further understanding of biofilm interactions and microbial organism behavior will provide us with future treatment modalities for this disease.

Keywords

biofilm therapy, biofilms, chronic rhinosinusitis, coagulase-negative staphylococci, endoscopic sinus surgery, nasal irrigation, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

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Introduction

The Center for Disease Control and Prevention (CDC) estimates that at least 65% of all human infections are caused by biofilms [1]. To date, research on biofilm disease in chronic rhinosinusitis (CRS) leads us to believe that biofilms contribute significantly to the chronic inflammation that is characteristic of this heterogeneous inflammatory disease. Given its significant impact on the quality of life of affected patients and the not insignificant cost to the health care system, a clear understanding of how biofilm disease is implicated in CRS is essential [2].

What is a biofilm?

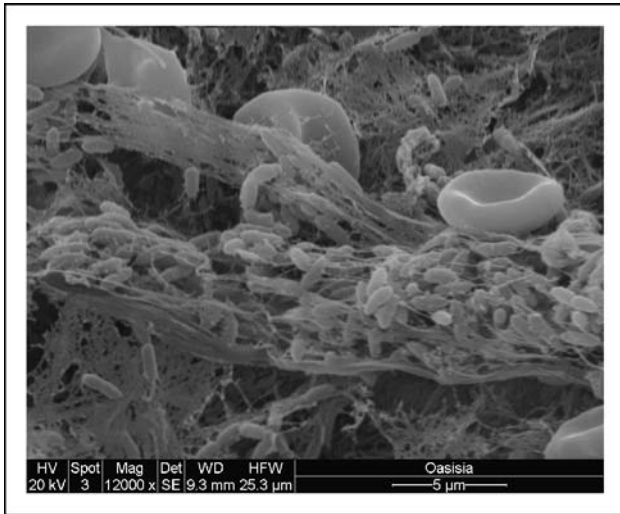
Our current understanding is that biofilm is the preferred state of existence of bacteria and that only 1% of all bacteria live in the planktonic form [3]. Biofilm is an organized community of bacteria adherent to an inert or living surface, embedded in a self-produced extracellular polymeric matrix (85% by volume) composed of a mixture of biopolymers, primarily polysaccharides, but also

containing protein and nucleic acid (see Fig. 1) [4,5]. When in this form, bacteria communicate with each other by quorum sensing using different classes of signaling molecules. Quorum sensing permits the integration and coordination of different steps of biofilm development including the recruitment of new bacteria, the production of matrix, gene expression and biofilm maturation [6]. Two quorum-sensing processes have been identified. The autoinducer-1 (AI-1) type is involved mainly in intraspecies quorum sensing and the AI-2 type is predominantly used in interspecies communication [7,8].

Biofilm formation occurs through several stages [4,9–12]. Biofilm formation is advantageous as it provides protection from both environmental (temperature, moisture, pH and ultraviolet light) and biological factors (e.g. phagocytosis, the host immune system). It also provides bacteria with the milieu to exchange genetic information essential for resistance to antimicrobial agents necessary for overall survival [4,12].

In general, bacteria in the form of a biofilm are resistant to antibiotic therapy. Although simplistic, this may be

Figure 1 Scanning electron microscopy image demonstrating rods in a biofilm on sinonasal mucosa from a surgical specimen obtained from a patient with chronic rhinosinusitis



Courtesy of Noam A. Cohen, MD, PhD, Jennifer Kofonow, MS, University of Pennsylvania, Philadelphia, Pennsylvania, USA.

because the biofilm functions as a physical barrier that prevents antibiotic penetration [13]. Other important mechanisms exist by which biofilms may limit antimicrobial efficacy [13–15]. The accumulation of waste products or oxygen and nutrient depletion within a biofilm can stimulate the general stress response (GSR) in bacteria, a low metabolic state where bacteria may remain indefinitely. When in the GSR, bacteria are much less susceptible to growth-dependent antimicrobial killing [16,17]. Another mechanism is the differentiation of bacterial species into a resistant phenotype due to osmotic stress and the down-regulation of transmembrane channels or antimicrobial target sites [13]. The expression of antimicrobial-resistant genes or the use of efflux pumps may also portend biofilm persistence [15]. Finally, quorum sensing may allow the sharing of genetic information within the bacterial community or for the induction, transcription and expression of target genes when signaled [14]. The dissemination of genetic information within a biofilm minimizes the metabolic cost to individual bacteria while facilitating the persistence of a large number of genes.

Evidence that biofilms exist in chronic rhinosinusitis

Multiple studies have confirmed the presence of bacterial biofilms in the sinonasal cavity of patients with CRS using operative samples [3,18,19,20*,21]. Bacterial biofilms have been detected by various methods in 30–100% of these samples. A recent prospective clinical study [22] of over 150 consecutive CRS patients with mucopuru-

Key points

- Bacterial biofilms are likely present in all patients with chronic rhinosinusitis (CRS) and have a significant role in the pathophysiology of the disease.
- Biofilm species may interact to become more virulent possibly leading to greater disease severity.
- Biofilm disease in CRS is very amenable to the use of topical treatments.
- Mucosal injury or incomplete treatment of a biofilm may result in greater biofilm growth and a resultant increase in mucosal inflammation.

lence identified a nearly 30% rate of bacteria with biofilm-forming capacity as assessed by an in-vitro biofilm formation assay. Bacteria identified in this study as well as the other CRS biofilm studies have generally found that 70% of the infections identified were secondary to *Pseudomonas aeruginosa* or *Staphylococcus aureus*, and they were often polymicrobial in nature. These results demonstrate that biofilms are present in most patients with CRS and that the bacteriology of these biofilms may not differ radically from the current paradigm for the bacteriology of CRS [23,24]. However, given our general understanding of the propensity of bacteria to form a biofilm, these findings are not completely unexpected.

Role for biofilms in chronic rhinosinusitis

The bacteriology of chronic rhinosinusitis has largely been studied using simple bacteriologic techniques that identify one to two planktonic organisms per sample. These studies have shaped our understanding of the bacterial influence on this heterogeneous inflammatory disease and our clinical understanding of CRS. In the development of CRS there are both bacterial and host factors which are permissive to this chronic inflammatory state.

Bacterial factors

Which bacteria are present may influence the evolution of CRS for an individual. The first study to explore the impact of biofilm-forming capacity of bacteria on persistence of CRS following ESS, used a semi-quantitative in-vitro technique with crystal violet [25]. It found that patients who were greater than 1 year after endoscopic sinus surgery (ESS) and had a poor evolution of their disease, grew biofilm isolates for *S. aureus* or *P. aeruginosa*. In contrast, patients with a favorable clinical evolution grew neither of these biofilms. Of interest, the growth of coagulase-negative staphylococcus (CNS) biofilms was not associated with a poor clinical disease evolution. A later study by Psaltis *et al.* [26] supported these findings when they evaluated the impact of bacterial biofilms on postoperative symptoms and mucosal inflammation. In their study, patients with biofilms were found to have a

greater disease severity with regards to preoperative radiological score as well as more severe postoperative symptoms and poor mucosal disease control on serial examination. This indicates that patients with sinonasal mucosal biofilms have CRS disease that is more difficult to control with current clinical strategies.

A recent study [27[•]] using molecular techniques has confirmed that the bacterial milieu in CRS is polymicrobial with anaerobic organisms predominating. To date, most bacteriology investigations in CRS consider a single organism as the predominant infectious cause of the disease. The implication(s) of a polymicrobial environment on mucosal inflammation has not yet been adequately researched to understand its impact on CRS pathophysiology.

However, the interaction among organisms in this milieu is of significant clinical interest. *S. aureus* has been recently shown to physically interact with the hyphae of candida when grown together *in vitro* to create a dual-species biofilm [28^{••}]. Of greater interest is that not only may the *S. aureus* interaction with fungal hyphae provide a method for epithelial penetration but also the species interaction resulted in a change in *S. aureus* virulence factor expression. This interaction may therefore facilitate a clinical infection by *S. aureus* by increasing its virulence through a yet unrecognized mechanism. Fungal biofilms have been identified in patients with CRS [20[•]]. These findings lend to the theory of a role for fungal organisms in the pathophysiology of CRS, potentially as a facilitator of *S. aureus* infection given these in-vitro results. A mechanism by which *S. aureus* may lead to inflammation in CRS is via the release of superantigen toxin causing a nonspecific T-cell activation [29]. The impact of organism interaction with, for example, fungal species on superantigen production by *S. aureus* remains to be determined. Similarly, what impact this interaction could have on the cytokine profile and therefore the clinical manifestations of the disease have also not been determined. Certainly, a better understanding the mucosal milieu will lead to advances for the treatment of CRS.

Host factors

Although the identification of bacterial biofilms in a high percentage of patients with CRS is now clearly confirmed in the literature, there is adequate evidence that bacterial biofilms are also implicated in the persistence of CRS. However, the relationship between bacterial biofilm and the underlying mucosal host inflammatory response is poorly understood.

S. aureus is present in about 50% of patients with CRS. Colonization by this organism as a biofilm may permit continued exotoxin production while being protected in the biofilm structure from both exogenous treatments

and host defense. In addition, the change of the bacterial phenotype from the planktonic to the biofilm form may upregulate *S. aureus* toxin production. Lastly, other virulence factors of *S. aureus* possess diverse biological properties, including disruption of epithelial membrane and deactivation of antibacterial peptides [30].

Toll-like receptors (TLRs) are the key players in innate immunity and they have been recognized to play a significant role in the pathogenesis of CRS. TLR2 is responsible for the recognition of Gram-positive bacteria. Sinonasal mucosa from patients with early recurrence following surgery has a significant lower expression level of TLR2 than control patients or surgery-responsive patients [31]. *In vitro*, *S. aureus* has also been demonstrated to suppress innate mucosal immunity by down regulating TLR expression [32]. Therefore, alteration of mucosal immunity by an organism may facilitate bacterial attachment and lead to subsequent biofilm formation. It has recently been shown that in the presence of biofilm disease, in a steroid-naïve CRS patient, there is a skewed predominantly T_H1 mucosal inflammatory response [33[•]]. This could result from the host immune response to planktonic organisms released from the bacterial biofilm. However, other means by which the biofilm may contribute to this inflammatory response is under investigation.

Altered innate mucosal immunity is potentially a major predisposing factor for the development of CRS. As such, the gene products of an individual may be a major contributing factor for mucosal bacterial colonization. Polymorphisms in several genes implicated in innate immunity have been associated with severe CRS [34,35[•],36–38]. Therefore, an altered response of such genes may permit bacteria at the epithelial surface to initiate the inflammatory response that is characteristic of CRS.

Treatments for bacterial biofilms in chronic rhinosinusitis

Bacterial biofilms are generally resistant to antimicrobial therapy at levels attainable by oral or intravenous administration. What is needed are novel biofilm disrupting therapies that are nontoxic to the mucosa and/or therapies that interfere with quorum sensing thereby preventing the proliferation of biofilm disease. Current treatments generally include ventilation of sinus cavity using endoscopic sinus surgery, antimicrobial strategies, biofilm-disrupting strategies and limited therapies that target quorum sensing.

Topical antibiotics

Topical antibiotics maximize the effective concentration while minimizing systemic absorption. Desrosiers *et al.*

[39] examined the in-vitro activity of moxifloxacin on *S. aureus* biofilm isolates from patients with poor disease control following ESS for CRS. Minimal inhibitory concentration (MIC) levels of moxifloxacin had no effect on mature biofilms. Concentrations of 1000 times the MIC were required to effect a log 2–2.5 reduction (greater than 99% reduction) in the number of viable bacteria. Despite this reduction, viable bacteria remained and could use the available biofilm matrix as a means for re-infection. Similar findings have resulted from a study of *P. aeruginosa* biofilms in a rabbit model treated with topical tobramycin. It demonstrated that the antibiotic was ineffective at eradicating the biofilm and that sub-therapeutic treatment of a bacterial biofilm may lead to further biofilm proliferation [40].

Mupirocin is a topical agent that has shown some promise against both planktonic and *S. aureus* biofilm infections. After Ha *et al.* [41] determined that mupirocin has in-vitro activity at concentrations of 125 µg/ml or less against *S. aureus* biofilms, it was then trialed in an in-vivo prospective pilot study [42] using twice daily 0.05% mupirocin irrigations for 3 weeks. Importantly, there was an improvement for both subjective CRS symptoms and objective ratings of mucosal inflammation in the study population [42].

There are examples of other novel agents that have antibiofilm properties. For instance, tea tree oil (TTO) has long been investigated as an antistaphylococcal agent. There is now in-vitro evidence to support the antibacterial and antibiofilm effects of TTO against *S. aureus* [43]. Another antibacterial agent is manuka honey which has been used clinically for the treatment of skin wounds. Alandjani *et al.* [44] have shown *in vitro* that it is an extremely effective agent for the eradication of both *S. aureus* (methicillin-sensitive and methicillin-resistant) and *P. aeruginosa* in either the planktonic or biofilm forms. Further study of the histological effect of manuka honey on the nasal respiratory mucosa of rabbits revealed no histological evidence of inflammation, epithelial injury or significant morphological changes [45^{*}]. However, manuka honey has yet to be shown to be efficacious as a treatment in patients with CRS.

Biofilm-disrupting strategies

Surfactants are amphipathic molecules that decrease the surface tension or inter-facial tension between two surfaces. This could lead to the disruption of a biofilm in the setting of CRS. Baby shampoo is nonionic surfactant and it was used in a prospective study of 18 postendoscopic sinus surgery patients who were suffering from recalcitrant CRS. Patients irrigated twice daily with a 1% solution for 4 weeks [46]. The most significant improvement was the subjective patient rating of postnasal drip and thickened mucus. However, more than 10% of the

patients could not complete the study due to treatment side-effects. The results do suggest that further development of surfactant-based therapies may be of interest for CRS.

The orthopedic literature has shown that the use of an irrigation device that creates a shear force while delivering a nontoxic, water-soluble surfactant is more effective for biofilm eradication than either method on its own [47,48]. As an extension of this model, Desrosiers *et al.* [49] compared the effects of both saline delivered with hydrodynamic force and citric acid with caprylyl sulfo-betaine (zwitterionic surfactant) (CAZS) delivered hydrodynamically against *S. aureus* and *P. aeruginosa* biofilms *in vitro*. Hydrodynamic CAZS achieved the greatest reduction, decreasing colony-forming unit (CFU) counts from control values by 3.9 logs for *S. aureus* and 5.2 logs for *P. aeruginosa*. However, subsequent application of this substance to sinus mucosa in an animal model led to significant ciliotoxicity [50]. Similarly, delayed analysis of sinus mucosa from a sheep sinus biofilm model showed that at 8 days after treatment with CAZS using a hydrodebrider, the sinus mucosa was found to have significantly more biofilm disease [51]. Therefore, less than complete treatment of a biofilm and/or further mucosal insult may in fact stimulate proliferation of that bacterial biofilm thereby increasing mucosal inflammation and clinical disease severity.

Ultrasound is effective in disrupting bacterial biofilms in the laboratory setting; however there are a few studies in this field. Young *et al.* [52] investigated the short-term effectiveness of therapeutic pulsed ultrasound on 22 patients who failed maximum medical treatment for CRS, 2–3 days per week for six sessions. Two were unable to complete the study protocol, 18 of 20 patients had a subjective improvement while two patients noted worsening of the symptoms [52]. The detrimental effect of ultrasound on biofilms may be due to starvation-stress or a paucity of cross-linking cations in the biofilm [53].

Interference with quorum sensing

Blocking quorum sensing is an antivirulence approach that inhibits the detrimental effects of a biofilm without placing selection pressure on a target organism thereby lessening the risk for the emergence of resistance. Although this field is relatively new, there are several published studies targeting quorum sensing. For example, for *P. aeruginosa* biofilms, disabling the signaling molecule acyl homoserine lactone (las AHL) leads to a flat, unstructured biofilm and makes *P. aeruginosa* more susceptible to disruption by sodium dodecylsulphate (SDS) [54]. Activation of the accessory gene regulator (agr) quorum-sensing system of *S. aureus* is necessary for the detachment and propagation of bacteria from a mature biofilm. Inactivating this system may potentially

prevent the spread of *S. aureus* although agr repression is needed for biofilm formation [55].

Conclusion

Most bacteria exist in the highly organized form of a biofilm. Bacterial biofilms represent one of many possible etiologies for the occurrence and persistence of inflammation in CRS. Both the synergistic effect of multiple organisms and the efficacious treatment of biofilms are areas of intense investigation. Further understanding of these factors will lead to improvements in the care of patients with CRS.

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