

Role of Bacterial and Fungal Biofilms in Chronic Rhinosinusitis

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Abstract Chronic rhinosinusitis (CRS) is a very common condition that remains poorly understood from a pathogenic standpoint. Recent interest has been sparked by a potential role for biofilms in this process, with a significant body of evidence implicating them in inciting sinonasal inflammation. Biofilms are clearly present on the sinus mucosa of CRS patients, and their presence there is associated with severe disease characteristics and surgical recalcitrance. We are beginning to understand the importance of the species within these biofilms, but there may be other as-yet-unidentified factors at play in influencing disease outcomes. Recent exciting research has emerged documenting the immune response to the presence of biofilms—research that will ultimately solidify the nature and extent of the contribution of biofilms in CRS pathogenesis. Future research should focus on evidence-based antibiofilm treatments with reference to efficacy and timing of treatment.

Keywords Bacterial · Fungal · Biofilms · Chronic rhinosinusitis · Microbes · Sinonasal inflammation

Introduction

Chronic rhinosinusitis (CRS) is a chronic inflammatory disorder of the sinonasal tract that places a significant quality-of-life burden on its sufferers and socioeconomic

stress on the wider community [1, 2]. Somewhat surprisingly given its high prevalence, estimated to be in the order of 16% of the adult population [3–5], the underlying pathogenic mechanisms that result in its unique characteristics remain poorly understood. CRS is increasingly thought to represent multiple distinct disease processes that up until now have been grouped together under a wider umbrella term. A significant effort is currently being dedicated to improving the subdivision of this disease both clinically and with specific biomarkers, which may homogenize patient groups more clearly and subsequently guide more focused etiologic research [6]. However, this has yet to yield meaningful disease differentiation that would allow improved understanding of the initiating and perpetuating mechanisms of each individual disease presentation.

Over the past 20 years, a wide range of intrinsic and extrinsic factors have been proposed to contribute to the onset of inflammation in CRS [7–9]. However, none have been definitively implicated. Ultimately, a complex interaction between the host's defective local immune defenses and multiple extrinsic, environmental agents is likely to explain how the chronic inflammatory milieu of this disease becomes established (Fig. 1) [10]. Among the extrinsic factors that have been proposed, the role of microbes remains particularly attractive [11]. Bacteria and fungi have long been linked to the development of CRS, but the nature of their interaction with the host remains largely unknown. A significant microbiological advance in recent times has been our understanding of the biofilm mode of microbial growth and how this contributes to a wide range of chronic disease processes [12]. The application of the biofilm paradigm to the etiopathogenesis of CRS is certainly compelling. The resulting literature addressing this hypothesis is the subject of the review presented herein.

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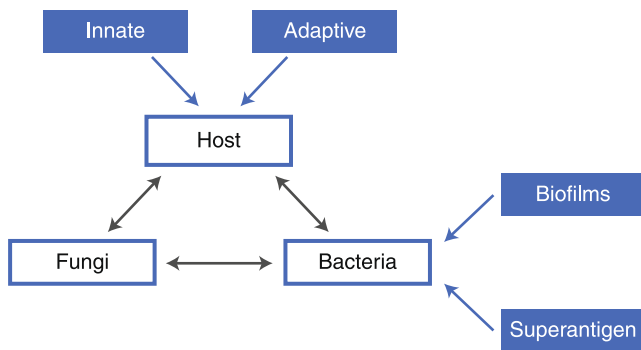


Fig. 1 Chronic rhinosinusitis (CRS) pathogenesis. CRS most likely results from a complex interaction between the host and environment. Defects in innate and adaptive immune function coupled with environmental triggers, including microbes, establish a cycle of chronic sinonasal inflammation

What Is a Biofilm? Structure and Function of Microbial Communities

Bacteria are known to exist in two genotypically and phenotypically distinct forms. The vast majority of bacteria can adopt a planktonic or biofilm form, depending on environmental conditions. This was first recognized in the late 17th century by the Dutch lens maker Anton van Leeuwenhoek, who observed both forms under high-powered magnification [13]. Unfortunately, these observations were largely overlooked, and our traditional understanding of bacterial disease mechanisms was largely derived from the subsequent work of Robert Koch during the 18th century [14]. His seminal work formed the basis of modern microbiology, in which the causal relationship between microbes and disease has centered on fulfillment of Koch's four postulates.

Despite the medical community's focus on planktonic-related infections, it is actually the biofilm form that predominates in nature (99% of bacteria exist in this form), presumably because of the survival advantage conferred by its protected "bacterial community" environment [15]. With time, the role biofilms play in a wide range of chronic infections in the human host is becoming recognized, such that it is now proposed that more than 65% of bacterial diseases are caused by bacteria existing in the biofilm form [16]. A biofilm is defined as "a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription" [17]. Basic science research has taught us a great deal about the structure and function of biofilms, as well as their fundamental differentiation from planktonic clones. Bacteria progress through a well-described life cycle, during which

they acquire the genetic modifications and structural qualities that ultimately define the biofilm [13].

A mature biofilm forms as a highly specialized configuration composed of towers and water channels, surrounded by a slime-like matrix. All these features have functional relevance for the biofilm's survival and virulence [18]. The potential disease-producing mechanisms of bacteria existing in this form are varied and still being uncovered. Among other things, cell detachment, endotoxin or exotoxin production, and resistance to host immune attack are all virulence mechanisms adopted by biofilm bacteria [17]. In contrast to the planktonic infections with which we are more familiar, biofilms are highly capable of evoking sustained responses from the host immune system, producing a persistent infection that might best be considered "tenacious survival" rather than aggressive virulence [19]. This results in a markedly different disease presentation when compared with acute infections mediated by bacteria existing solely in the planktonic form.

Biofilms have pervaded almost every aspect of clinical medicine, from their role in implantable prosthesis-related infections to chronic inflammatory diseases of host tissues. The clinical characteristics of biofilm-mediated disease are now well-understood [12]. Chronic diseases with a relapsing and remitting course, variable culture rates, and extreme antibiotic resistance typify diseases now thought to have an underlying biofilm etiology. Within the specialty of otolaryngology, otitis media with effusion [20], cholesteatoma [21], and chronic tonsillitis [22] are all associated with biofilms, along with infections of prosthetic devices such as cochlear implants [23], tympanostomy tubes [24], and tracheostomy tubes [25]. CRS sufferers typically report these hallmark features when assessed in the clinic. It is not surprising then that the biofilm hypothesis has been applied to CRS, and a detailed investigation of their involvement in this disease undertaken.

Are Sinonasal Biofilms Pathological? Healthy Versus Diseased Mucosa

The early research linking biofilms to CRS focused on morphologic identification of biofilm structures on the mucosal surface of CRS patients [26–29]. The premise was that the presence of biofilms in disease, yet absence in health could link them to the diseased sinus state present in CRS. To date, this remains a contentious issue that has not been definitively answered. The opposing arguments contend that biofilms cause disease by inciting a chronic inflammatory state or are opportunistic in colonizing the fertile, inflamed mucosa and thus are only a marker of preexisting sinonasal inflammation. This question remains unanswered (Table 1).

Although the majority of studies have failed to identify biofilms in controls, there have been clear reports of this

Table 1 Literature review of biofilm presence^a

Study	Modality	CRS	Controls
Cryer et al. [26]	SEM	4/16 (25%)	Not applicable
Sanclement et al. [27]	SEM/TEM	24/30 (80%)	0/4 (0%)
Ferguson and Stolz [28]	TEM	2/4 (50%)	Not applicable
Sanderson et al. [33]	FISH	14/18 (78%)	2/5 (40%)
Psaltis et al. [29]	BacLight/CLSM	17/38 (44%)	0/9 (0%)
Healy et al. [32]	FISH	12/13 (92%)	3/3 (100%)
Galli et al. [55]	SEM	10/24 (41.7%)	0/20 (0%)
Hekiart et al. [57]	SEM	17/60 (28%)	Not applicable
Foreman et al. [36]	FISH/CLSM	36/50 (72%)	0/10 (0%)
Singhal et al. [48]	BacLight/CLSM	36/51 (71%)	0/5 (0%)
Bezerra et al. [30]	SEM	24/33 (73%)	13/27 (48%)
Mladina et al. [31]	SEM	Not applicable	62/65 (95%)

^a The rate of biofilm prevalence in CRS patients varies but is never 100%, regardless of detection technique, reinforcing the multifactorial nature of this disease. The identification of biofilms in the nondiseased sinus is more contentious, with many studies not finding biofilm-like structures in controls. This is contradicted by a number of other reports

CLSM confocal laser scanning microscopy; CRS chronic rhinosinusitis; FISH fluorescence in situ hybridization; SEM scanning electron microscopy; TEM transmission electron microscopy

occurrence, including a recent large series (Table 1) [30–33]. The potential explanations for this include the utilized imaging modality, site of control tissue sampling, tissue preparation methodology, unrecognized sinonasal inflammation in supposed controls, along with variable definitions used to report a biofilm. The selection of controls and imaging modality is particularly important. Studies with biofilms present in the control patients have tended to use patients undergoing septoplasty for nasal obstruction [30]. From the outset, these patients do not have normal sinonasal cavities. Furthermore, this approach samples the nasal cavity rather than the sinuses per se. It is debatable whether the sinonasal tract is a single functional entity or if the nasal cavities and sinuses behave differently in terms of immunologic tolerance and subsequent colonizing of bacterial flora. In a sheep model of sinusitis, Ha et al. [34] elegantly outlined the pitfalls of using electron microscopy for biofilm detection (Table 2), highlighting the low sensitivity of transmission electron microscopy and inferior specificity of scanning electron microscopy (SEM). Unfortunately, SEM continues to be a commonly used biofilm detection modality in studies of CRS patients. This is mainly due to its accessibility in many laboratories, coupled with familiarity of the techniques involved. These factors may well influence the reported results.

What Microbes Comprise Sinus Biofilms? The Evolution of Imaging and Understanding

Once the presence of biofilms on sinus mucosa was established, it became clear that a more detailed understanding

of biofilm characteristics, including bacterial species identification, was needed to provide insights into the role biofilms might play in this disease. The early biofilm detection studies in CRS patients utilized nonspecific imaging techniques, and while they significantly advanced our understanding of the morphologic features of biofilms, their nonspecificity did not allow identification of the individual bacterial species that comprised the biofilms [26, 28, 29, 35].

Subsequent studies utilized fluorescence in situ hybridization (FISH), a technique that can identify species within the biofilms of CRS patients [32, 33, 36]. Authors from two separate institutions reported a dominant role for *Haemophilus influenzae* and *Staphylococcus aureus*. Sanderson et al. [33] and Healy et al. [32] identified *H. influenzae* biofilms in 14 of 18 (78%) and 8 of 11 (73%) CRS patients, respectively, along with identifying *H. influenzae* biofilms in controls. However, they reported a relative paucity of *S. aureus* biofilms in these patients and none in controls. Conversely, our department found *S. aureus* to be the most common biofilm-forming organism in our population (25 of 50, 50%) of CRS sufferers, but we were unable to identify biofilms of any species in the control group [36]. Other authors have since corroborated the predominance of *S. aureus* [37]. Interestingly, more than half of our biofilm-positive patients had polymicrobial flora present, and this did include fungal biofilms (11 of 50, 22%). As a result of these studies, it appears that other species, such as *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*, are less commonly found in the biofilm form in this disease and therefore are less likely to play a pathogenic role in CRS.

Table 2 Current biofilm imaging modalities^a

Modality	Advantages	Disadvantages
SEM	High-quality imaging Well-established technique in most laboratories	Small tissue sample Requires fixation, dehydration, critical point dehydration Difficult to differentiate bacteria from surface mucus Cannot image the 3-dimensional biofilm structure
TEM	Enables cross-sectional imaging	Poor sensitivity due to ultrathin sectioning
BacLight LIVE/DEAD	Uses CLSM for 3-dimensional imaging Rapid tissue preparation No fixative/dehydrating agents	No species information
FISH	Uses CLSM for 3-dimensional imaging Enables species identification	Limited number of species can be identified
Molecular diagnostics	High sensitivity May identify new species	No differentiation of biofilm vs planktonic phenotype

^a All techniques have advantages and disadvantages, as summarized. Ultimately, researchers should select a modality based on their research aims. Molecular diagnostics may well be the future of microbial detection in the sinuses, but this technique cannot provide phenotypic information about the microbes in question

CLSM confocal laser scanning microscopy; FISH fluorescence in situ hybridization; SEM scanning electron microscopy; TEM transmission electron microscopy

When using FISH as a diagnostic modality, researchers need to presumptively identify the organisms for which they will probe (Table 2). Furthermore, due to technical constraints, only a limited number of probes can be applied to the tissue in question. Thus, it is possible that potentially important microbes involved in CRS initiation may be overlooked using a technique such as FISH. The selection of FISH probes can only be based on previous microbiological data and personal experience of the investigators, thus limiting the potential for development of new theories on the role of bacteria in CRS by continuing to propagate our traditional bacterial understanding. Newer molecular-based technology may significantly advance our knowledge of CRS microbial involvement by circumventing this technical limitation of FISH. Using techniques such as multi-locus base composition analysis, recent studies have identified a relative abundance of anaerobic species that have not been traditionally associated with CRS, including *Propionibacterium* spp, *Diaphorobacter* spp, *Peptoniphilus* spp, and *Corynebacterium* spp [38•, 39]. The actual role of these species as primary pathogens, synergistic mediators, or mere innocent bystanders remains to be clarified. In addition, molecular diagnostic studies also supported the relative importance of *S. aureus*, confirming a prevalence of 50% in CRS patients (in line with FISH studies from our department [36, 40]), and downplayed the potential role *H. influenzae* plays in this disease [38•]. The frequency correlation for bacterial species between molecular techniques and FISH has been directly corroborated by recent work from our department. Molecular analysis of the microbial

community in CRS patients suggested a *S. aureus* prevalence of 61%. FISH analysis of *S. aureus* biofilms in the same patient cohort had a sensitivity of 78% and specificity of 93% compared with molecular analysis (unpublished data).

As it stands, far less is known about the characterization of fungal biofilms in CRS. To date, there has only been investigation of fungal biofilms using a pan-fungal FISH probe. Although we are making some gains in our bacterial understanding and know that fungal biofilms exist in CRS, attempts to characterize fungal biofilms have yet to be reported. Of all the proposed etiologic agents in CRS, fungi remain perhaps the most controversial [41]. Their ubiquity and relatively low culture rate make it difficult to assess their pathologic significance. Application of the biofilm paradigm and biofilm investigative techniques to the role fungi play in CRS may ultimately clarify some of the current controversies. It is clear from a sinus explant model that fungi can form a highly coordinated network of hyphae, cross-linking each other and generally arranging themselves in a parallel-packed manner, which enhances biofilm strength, and produce an exopolysaccharide matrix with internal channels on the sinus mucosal surface [42]. This description is consistent with fungal biofilms in other areas of the literature. Fungal species data from molecular studies will be available shortly and may guide future research endeavors. Development and utilization of both in vitro and animal in vivo models for the investigation of fungal biofilms in this disease will assist in expanding our knowledge of fungal biofilms as well as their association and interaction with their bacterial colleagues.

Is There Microbial Synergism on Sinus Mucosa? The Interplay between Bacteria and Fungi in Biofilms

Healy et al. [32] first reported a coexistence of fungi and bacterial biofilms in their study of 11 CRS patients. Seven of 11 (64%) patients had fungal elements within bacterial biofilms. Hochstim et al. [43] reported similar findings, with 3 of the 15 biofilm-positive patients in their study demonstrating detectable fungal elements. Our group has demonstrated robust fungal biofilms in 11 of 50 (22%) of CRS patients, with a significant association between fungal biofilms and *S. aureus* biofilms (7 of 11, 64%) [36]. This may be mere coincidence given the high rate of *S. aureus* biofilms in the study; however, more recent evidence from both a sheep model of sinusitis and human studies suggests this result has pathogenic implications in CRS.

In a sheep model of biofilm-associated sinusitis, 30 sheep were sensitized intraperitoneally to common CRS antigens *Alternaria alternata* and *Aspergillus fumigatus* and then had these species inoculated in their obstructed frontal sinus [44]. Fungal biofilm formation could not be demonstrated in this arm of the study. However, when the fungus was co-inoculated with *S. aureus*, again in sensitized sheep, robust biofilm formation occurred within the frontal sinus. This hints at a primary pathogenic effect of *S. aureus* biofilms and suggests that fungal biofilms require the *S. aureus* biofilm matrix to establish biofilm growth or perhaps require a mucosal injury to provide a suitable environment to form a biofilm.

More recent human research builds on a synergism between *S. aureus* and fungi by observing microbe-specific mucosal IgE patterns. For both *S. aureus* and fungi, organism-specific IgE extracted from the mucosa of CRS with nasal polyp patients was significantly higher when these organisms were detected on the mucosal surface. This suggests that these biofilms are interacting with the host adaptive immune system and supports a role of biofilm in perpetuating chronic inflammation in these patients. Additionally, it was observed that the mucosal antifungal IgE levels were also significantly elevated in the presence of *S. aureus* organisms. This suggests that *S. aureus* organisms, or perhaps secreted superantigen, are capable of enhancing mucosal allergic sensitivities in CRS with nasal polyp patients (unpublished data). Again, this implies an interaction between *S. aureus* and both colonizing fungi and inhaled fungal allergens that may be of pathogenic importance in the development of chronic sinus inflammation. This requires further research.

Different Biofilms, Different Diseases? Clinical Implications of Biofilm Composition

Clearly understanding the clinical relevance of bacterial and fungal biofilms in CRS is the ultimate goal of current

biofilm research. All sinus surgeons are confronted with a heterogeneous patient group with a diverse disease spectrum. This inevitably influences the success of surgery on the paranasal sinuses—some patients will have complete disease resolution, whereas others will experience persisting symptoms and objective evidence of ongoing mucosal inflammatory disease. Preoperative or intraoperative identification of patients who are at more risk of having worse outcomes may enable evidence-based implementation of early-intervention protocols with targeted therapy for these patients. We would propose that patients with *S. aureus* biofilms represent one such group at high risk of surgical recalcitrance and may benefit from a more aggressive approach to postoperative antimicrobial and anti-inflammatory therapy. This view is supported by recent literature addressing the influence of different biofilm-forming species on postoperative outcomes [45, 46].

The deleterious effect of biofilms (regardless of biofilm species makeup) was well-documented in both retrospective and prospective studies published from our department [47, 48] and by other investigators [49–51]. More recently, this work has been extrapolated to further characterize the impact of different biofilm species on postsurgical outcomes, building on the early in vitro work of Bendouah et al. [52]. *S. aureus* has become clearly associated with more severe disease presentation, based on subjective and objective measures, coupled with significantly poorer postsurgical progression. In a retrospective study of 24 medically recalcitrant CRS patients, *S. aureus* biofilm-positive patients presented with higher symptom scores and Lund-Mackay CT scores [45]. Their disease was less responsive to surgery, with 70% of this group having persisting evidence of disease at the end of the 11-month follow-up period. This group was in contrast to the patients who only had *H. influenzae* biofilms present on their mucosa. These patients had surgically responsive disease, behaving more like the biofilm-negative patients of previous clinical studies. These results were reinforced in a prospectively performed study of 39 CRS patients observed for 12 months with validated, ordinal subjective and objective outcome measures. *S. aureus* was identified as the nemesis of the endoscopic sinus surgeon [46]. Importantly, when present on its own or within a polymicrobial biofilm, the adverse impact of *S. aureus* biofilms was maintained, suggesting that *S. aureus* plays a dominant role within polymicrobial biofilms. As a consequence of poorer outcomes, the socioeconomic impact of this group also becomes significant. Singhal et al. [46, 48] highlighted that this group required a significantly greater number of physician visits during the postoperative period to manage their disease. No doubt this would also result in increased medication use and possibly further imaging, placing further burden on the health system and highlighting the need to find solutions for this particularly severe group of CRS sufferers.

How Do Biofilms Cause Disease? Biofilm–Host Interactions

The observation of biofilms on the surface of CRS patients and their association with adverse disease characteristics circumstantially implicates them in the disease. As previously outlined, the characteristics of CRS would be consistent with a biofilm-mediated disease. Nevertheless, without demonstrating their interaction with the host's immune system, disease causality cannot be assumed. A significant recent advance in this field of research has been to investigate specifically the interaction of biofilms as an external disease trigger with the local immune response. In summary, this research suggests that multiple defects in host immune function are associated with biofilm presence on the mucosa, strengthening the argument that biofilms contribute to chronic sinus inflammation.

First, lactoferrin, an abundant antimicrobial peptide present in airway secretions that also prevents bacteria attachment and aggregation, and MUC7, the only known glycoprotein with antibacterial properties, are downregulated in biofilm-associated CRS [53, 54], potentially blunting the host's innate bacteria-clearing mechanisms and facilitating surface attachment of the bacteria. Bacterial attachment, the first step in the biofilm life cycle, is then further facilitated by overexpression of sialic acid glycoproteins as well as the sialyltransferase gene responsible for building sialic acid residues, which leaves the host vulnerable to bacterial attachment by providing more adherence targets [54]. Further adherence targets promote bacterial aggregation and increase the likelihood of reaching the bacterial quorum required to facilitate biofilm formation.

Once established, it is clear that biofilms induce significant change in the mucociliary blanket. SEM studies have demonstrated that in contrast to their biofilm-negative counterparts, CRS patients with biofilms present on their mucosa have marked destruction of the epithelial layer with complete absence of cilia [35, 49, 55]. The resultant mucociliary impairment is then likely to promote further bacterial adherence and prevent bacterial clearance by the host's immune system. Interestingly, when bacterial biofilms are observed in areas of epithelial damage, there is a consistent elevation in T-lymphocyte and macrophage numbers, indicating a local inflammatory response [56]. If the epithelial layer has not been disrupted by the biofilm, this leukoattractant effect is not observed, further highlighting the significance of epithelial damage in the pathogenesis of biofilm-associated CRS.

It is now also evident that biofilms are associated with alterations in the host's adaptive immune function. In a study of 19 CRS patients, Hekiart et al. [57] found that the presence of biofilms was associated with a T-helper type 1 (Th1) skewing of the adaptive immune response, although biofilm species were not identified. Conversely, in a larger

series of 53 CRS patients, *S. aureus* biofilms were associated with a Th2 skewing of the adaptive response, with this subgroup of CRS patients demonstrating significantly elevated interleukin-5, interleukin-6, and eosinophilic cationic protein [58•]. Of further interest was the observation that this could occur both dependent on and independent of the staphylococcal superantigen pathway, which has been well-described previously in the literature [59–61]. While *S. aureus* biofilms may be a reservoir for continual superantigen release into the sinuses, that the association with the Th2 response was seen to be independent of this pathway and furthermore appeared to produce a different skewing of the cytokine profile suggested an independent and as-yet-undescribed interaction between biofilms and the adaptive immune system of the host. Further emphasizing the importance of biofilm species characterization, this effect was lost when *H. influenzae* biofilms were assessed in the same way. Reviewing the adaptive immune response of biofilms in similar diseases, such as chronic periodontitis, in which the Th2 bias and species-specific observations also hold true, further validates these results [62, 63].

The multifactorial nature of CRS etiology is highlighted in the previous paragraph with the correlation of two proposed agents—biofilms and superantigens—in the same patient. Could there be other environmental but nonmicrobial influences at play in this disease? The proinflammatory effect of cigarette smoke exposure has been demonstrated consistently in the lower airway and more recently in the upper airway [64]. It is known that smoke exposure results in generation of oxygen free radicals and induces adaptive immune cytokines. Tying this in with the biofilm hypothesis, it was recently demonstrated that in an in vitro model, repetitive exposure to whole tobacco smoke induces biofilm formation. In the clinical setting, bacteria isolated from patients with tobacco exposure form robust biofilms when exposed to smoke in the laboratory setting [65•]. Furthermore, with smoke withdrawal, the bacteria revert to a non-biofilm phenotype. Thus, it appears that nonmicrobial environmental factors might be contributory to biofilm establishment in the paranasal sinuses and the subsequent onset of chronic sinonasal inflammation. Whether this truly represents a direct effect on the microbial environment or whether it is an extension of the known effects cigarette smoke exposure has on both the respiratory epithelium and local immune function remains to be elucidated.

Where To From Here? Future Directions in Biofilm Research

Significant progress has been made in defining the role of bacterial and fungal biofilms in CRS since their first report in 2004 [26]. The bacterial species are well-delineated, and

the impact biofilms have on the clinical course of this disease has also been clearly defined. Further research is required to solidify a direct causal relationship between microbe and disease. The use of confocal laser scanning microscopy (CLSM) with or without species-specific probes is well-established as the current optimal biofilm detection approach in CRS, and for now, future research efforts should be conducted using this modality [34, 40]. We believe this would help clarify at least some of the inconsistencies that exist in the literature to date. With this being noted, an inherent limitation of this approach is the need for sinus mucosal biopsies. Intraoperatively, this is routine, but in the preoperative and postoperative periods, the determination of a patient's biofilm status is rendered technically and ethically difficult [66]. Noninvasive biofilm detection methods should be a goal for future research. This will facilitate the testing and adoption of evidence-based early-intervention protocols, which will inevitably be developed in the coming years, with the aim of improving patient outcomes.

While bacterial species within a biofilm appear to be an important predictor of disease severity, the impact of biofilm volume or biomass remains undetermined [51]. This is partly due to difficulties in accurately quantifying the biomass present in each patient. It is clear that biofilms can be patchy in their distribution across the mucosa, and with relatively small samples taken for biofilm analysis, these may not truly represent the state of the entire mucosa. We have trialed FISH for this purpose [67] but currently believe the use of BacLight LIVE/DEAD staining with COMSTAT2 software [68] for image analysis is the most accurate for quantifying biofilm biomass within the sinuses [69]. This technique has been validated in our department, and it remains to be seen what impact this line of research will have on our understanding of the clinical relevance of biofilms in CRS.

Many antibiofilm treatments have been trialed in the literature in a range of in vitro, animal in vivo, and human studies. A discussion of all these is beyond the scope of this review. Suffice to say, however, that the demonstrated clinical improvement of patients treated with such modalities provides further weight to biofilms playing a role in this disease. Future research undoubtedly will uncover more directed and more efficacious treatment options to eradicate sinonasal biofilms. As the detection, characterization, immune responses, and clinical implications of biofilms in CRS become clearer, there will ultimately be a need to develop new agents to add to our medical armamentarium. Only then can we hope to improve the outcomes of our most recalcitrant patients.

Conclusions

The application of the biofilm paradigm to CRS is a recent and attractive hypothesis to explain the development of

chronic sinonasal inflammation. A large body of research has accumulated in recent years documenting the prevalence, species composition, clinical relevance, and immunologic implications of biofilms, in addition to the trial of potential eradication strategies. Further research is still required to solidify some of the hypotheses generated thus far; however, ultimately, a switch in focus toward developing targeted, efficacious antibiofilm treatments and determining the optimal timing and delivery will be key to improving patient outcomes in this debilitating disease.

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Drs. Foreman, Boase, and Psaltis reported no potential conflicts of interest relevant to this article.

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