



REVIEW ARTICLE

Biofilms in chronic rhinosinusitis: Pathophysiology and therapeutic strategies



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Abstract *Background:* There is increasing evidence that biofilms are critical to the pathophysiology of chronic infections including chronic rhinosinusitis (CRS). Until relatively recently, our understanding of biofilms was limited. Recent advances in methods for biofilm identification and molecular biology have offered new insights into the role of biofilms in CRS. With these insights, investigators have begun to investigate novel therapeutic strategies that may disrupt or eradicate biofilms in CRS.

Objective: This review seeks to explore the evidence implicating biofilms in CRS, discuss potential anti-biofilm therapeutic strategies, and suggest future directions for research.

Results: The existing evidence strongly supports the role of biofilms in the pathogenesis of CRS. Several anti-biofilm therapies have been investigated for use in CRS and these are at variable stages of development. Generally, these strategies: 1) neutralize biofilm microbes; 2) disperse existing biofilms; or 3) disrupt quorum sensing. Several of the most promising anti-biofilm therapeutic strategies are reviewed.

Conclusions: A better understanding of biofilm function and their contribution to the CRS disease process will be pivotal to the development of novel treatments that may augment and, potentially, redefine the CRS treatment paradigm. There is tremendous potential for future research. Copyright © 2016 Chinese Medical Association. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-SA license (<http://creativecommons.org/licenses/by-nc-sa/4.0/>).

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The etiology of chronic rhinosinusitis is multifactorial. The interaction between many systemic, local host, and environmental factors contribute to sinus inflammation and to the pathophysiology of disease. Systemic factors include genetic diseases such as cystic fibrosis, conditions causing immunodeficiency, autoimmune disease, idiopathic conditions such as Samter's triad (aspirin exacerbated respiratory disease), and acid reflux. Local host factors include sinonasal anatomic abnormalities, iatrogenic conditions such as scarring from prior sinus surgery, neoplasm, or the presence of a foreign body, among others. Possible environmental factors include the presence of biofilms and bacterial infection, as well as fungal infection, allergy, environmental pollutants, and smoking.

Over the last 15 years, increasing evidence has implicated biofilms in more than 65% of chronic infections in humans. Biofilms may also complicate infection management as they contribute to the development of antibiotic resistance and the inconsistency of culture results in certain illnesses.¹ In otolaryngology, the controversial role of biofilms in chronic rhinosinusitis has been the focus of recent research. This review seeks to explore the evidence implicating biofilms in chronic rhinosinusitis, discuss potential anti-biofilm therapeutic strategies, and suggest future directions for research.

Defining biofilms

Bacteria exist in two distinct forms: biofilm and planktonic. Biofilm is the preferred state in which an estimated 99% of bacteria exist. The bacteria that constitute a biofilm display several critical differences in regard to growth dynamics and genetic expression relative to their planktonic counterparts.^{2,3}

The development of a microbial biofilm is a complex process. Initially, sessile planktonic bacteria adhere to a surface and form microcolonies.⁴ Initial attachment is formed by weak van der Waals forces and may involve bacterial flagella.⁵ The expression of cell adhesion structures, including pili, is upregulated to allow for stronger, permanent interactions.⁶ Once attached, bacteria begin to proliferate and secrete an extracellular matrix composed of polysaccharides, nucleic acids, and proteins. This matrix protects the biofilm against harmful factors in the environment.

As the biofilm expands, the concentrations of several signaling molecules increase and lead to alterations in intracellular signaling. For example, cyclic di-guanosine monophosphate (c-di-GMP) serves to activate biofilm maturation through modulation of cell-to-cell adhesion, quorum sensing, metabolic regulation, stress response, and the phenotypic shift from the planktonic to biofilm state.⁷ Once mature, bacteria within the biofilm transcribe DNA in a synchronized manner exhibiting the characteristics of a single multicellular organism that colonizes host tissues. Biofilms then spread by releasing free floating planktonic bacteria from the most distal sites of the structure.⁸ These shed bacteria can then adhere to distant sites in the host (Fig. 1).⁹

Quorum sensing

Quorum sensing refers to the ability of bacterial cells to communicate. It is a cell density-dependent signal transduction process that allows for rapid coordination of behavior to stimulating factors in the environment. Through quorum sensing, biofilms can adapt to fluctuating levels of nutrients, competitive microorganisms, and toxic materials.¹¹ Communication is mediated by small signal molecules called autoinducers (AIs) secreted by bacteria into the extracellular environment where they bind to specific bacterial cell membrane receptors. This binding initiates a signal transduction cascade modulating the expression of specific genes with downstream regulatory effectors.⁴ Three quorum sensing pathways have been identified. AI-1 refers to acyl-homoserine lactone (AHL) molecules which are produced by gram-negative bacteria only.¹² AI-2 molecules function in both gram positive and negative communication.¹³ AI-3 is the least understood pathway but appears to involve molecules that influence the histidine kinase-linked receptor QseC.¹⁴

Antimicrobial resistance

Biofilms harbor a 10–1000 fold higher resistance to antimicrobials than planktonic bacteria.¹⁵ This resistance is a manifestation of the physical barrier properties of the extracellular matrix, the induction of a bacterial stress response, and coordinated behavior of the biofilm through quorum sensing. Antibiotic resistance also involves enzymatic inactivation of antibiotics, modification of antimicrobial end-targets, and efflux pump-mediated exclusion of antibiotics.⁴ Bacterial gene transference is also enhanced in biofilms—for example, transference of the CTX-M-15 gene confers cephalosporin resistance to *Klebsiella pneumoniae* biofilms.¹⁶

The extracellular matrix that encapsulates biofilms creates a physical diffusion barrier.^{17,18} The viscosity of the outer slime layer prevents the diffusion of antibiotics into the deeper layers of the biofilm. Negatively charged components of the extracellular matrix repel positively charged antibiotics such as aminoglycosides.¹⁹ The extracellular matrix also harbors enzymes, such as beta-lactamases, which act to destroy the structural integrity of antibiotics.⁴

The spatial organization of biofilms and the induction of a bacterial stress response are closely related. Mature biofilms are structured in a stacked, multilayered topography where cells in the deeper layers are subject to a different environment than those in the more superficial layers.²⁰ These different microenvironments alter gene expression and metabolic activity leading to heterogeneity within the community.¹² Interior regions of the biofilm are also, typically, starved of nutrients and oxygen suppressing metabolism and growth rate as part of the bacterial stress response. Because antibiotics are generally more effective against fast growing cells, the stress response functions to increase antimicrobial resistance.^{21,22}

'Persister cells' which lie in a dormant state within the deep interior of a biofilm likely contribute to the recalcitrant nature of biofilm-mediated infections.²³ The formation of this subpopulation may be triggered by the bacterial

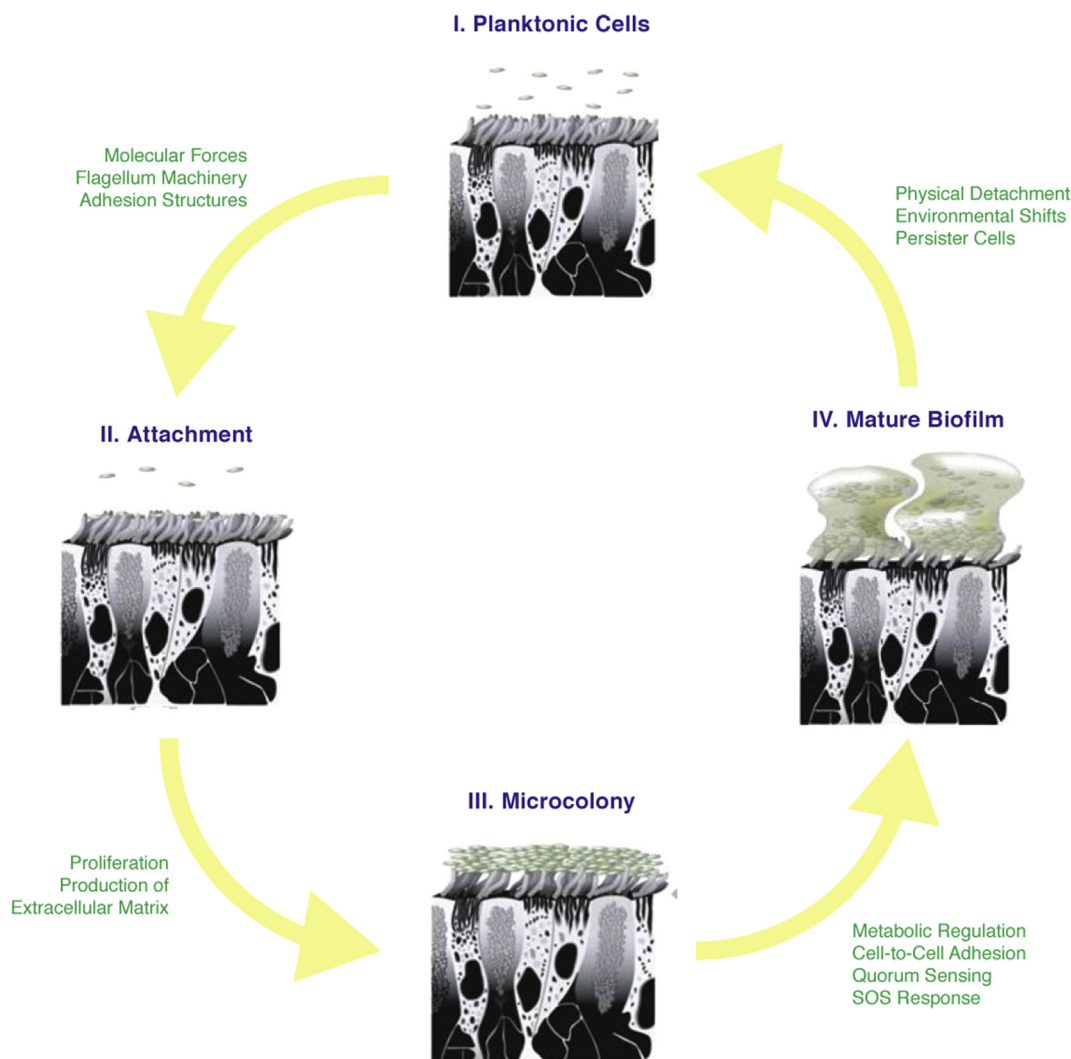


Fig. 1 The biofilm life cycle.¹⁰

stress response and when the biofilm enters the mid-exponential growth phase.^{23,24} Persister cells are able to endure high concentrations of antibiotics and promote infection by relocating to other host sites and forming new biofilms harboring the same resistant phenotype as the original population (Fig. 2).²⁵

Biofilm-induced pathogenesis

Biofilms have been implicated in otitis media with effusion,²⁶ cholesteatoma,²⁷ and chronic tonsillitis.²⁸ The contribution of biofilms to the pathogenesis of chronic rhinosinusitis remains less clear. Parsek and Singh developed specific diagnostic criteria for biofilm-associated infections in 2003 (Table 1).²⁹

Early research identified biofilm structures on the mucosal surfaces of patients with CRS.^{31–33} However, subsequent studies noted the absence of biofilms in diseased patients and their presence on the nasal mucosa of healthy controls.^{34–36} This variability highlights the complex multifactorial pathophysiology of CRS.⁹ The

seemingly contradictory results noted might be partially attributable to differences in the biofilm detection methods used (Table 2).

Several modalities exist for biofilm detection including scanning electron microscopy (SEM), transmission electron microscopy (TEM), fluorescence *in situ* hybridization (FISH), and confocal laser scanning microscopy (CLSM). Early biofilm detection studies used nonspecific techniques such as SEM and TEM, which allowed for ultrastructural analysis but did not allow for species identification.³⁹ FISH-based studies use universal bacterial probes or species-specific primers.^{40,41} FISH helped demonstrate that *ex vivo* nasal swab culture, used in early studies for biofilm species identification, was unreliable as it frequently sampled planktonic rather than biofilm bacteria.^{42–44} The main drawback of FISH is the need to presumptively identify the organism which is then probed, possibly overlooking pathogenic microbes.⁴⁵ CLSM has emerged as the optimal biofilm detection approach in CRS.^{46,47} CLSM allows for deep scanning of mucosal tissue, and species identification through concurrent FISH, LIVE/DEAD BacLight, or through the use of immunofluorescent markers.^{39,41,48}

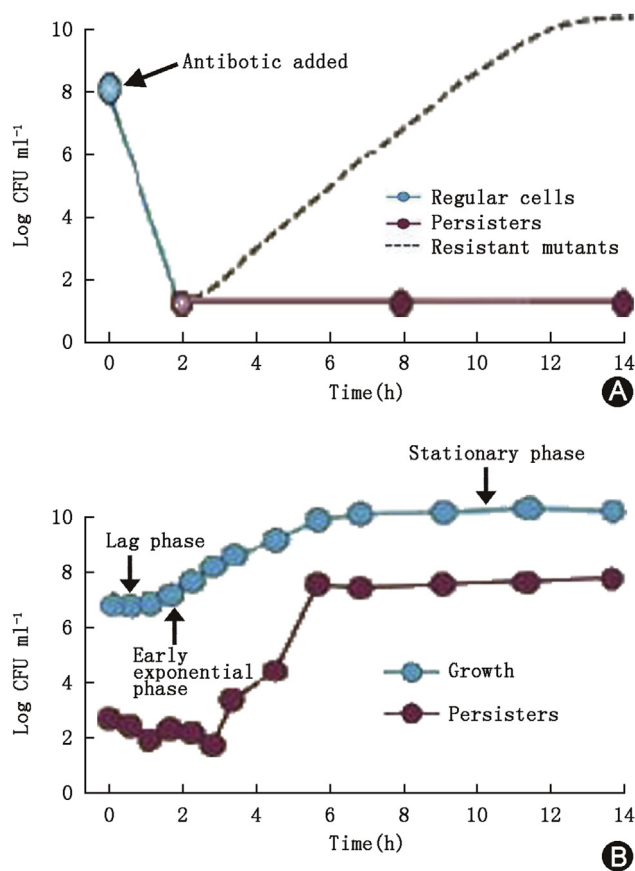


Fig. 2 Formation of persister cells.²³ A: Administration of antibiotic to a biofilm population resulting in cell death with the continued survival of a subpopulation of persister cells and resistant microbes. B: Frequency of isolation of persister cells as a function of the growth phase of the biofilm culture.

A complex polymicrobial community of both bacteria and fungi exists within biofilms.^{36,39} Foreman et al demonstrated that *Staphylococcus aureus* was identified in 50% of biofilms from CRS patients, with *Pseudomonas aeruginosa* and *Haemophilus influenza* identified in 22% and 28% of cultures, respectively.³⁹ Other bacterial species such as *Streptococcus viridans*, coagulase-negative staphylococci, *Enterococcus faecalis*, and *S. viridans* form biofilms in CRS patients.⁴⁹ Anaerobes including *Propionibacterium* and *Corynebacterium* have been visualized.^{44,45} The degree to which individual bacteria contribute to CRS disease pathogenesis remains unclear.

Study	Year	Detection method	CRS (%)	Control (%)
Ramadan et al ³⁴	2005	SEM	5/5 (100)	N/A
Sanclement et al ³²	2005	SEM, TEM	24/30 (80)	0/4 (0)
Sanderson et al ³⁵	2006	FISH	14/18 (78)	2/5 (40)
Psaltis et al ³⁷	2007	CSLM	17/38 (45)	0/9 (0)
Healy et al ³⁶	2008	FISH, epifluorescence microscopy	9/11 (82)	2/3 (67)
Bezzera et al ³⁸	2011	SEM	24/33 (73)	13/27 (48)

In 2008, twenty-five years after Katzenstein et al⁵⁰ first reported fungus in the sinuses of CRS patients, Healy et al reported the coexistence of fungi and bacterial biofilms.³⁶ In 2009, a study by Foreman et al⁵¹ found fungal biofilms in 11/50 (22%) of CRS patients with 7 of these cases demonstrating concomitant infection with *S. aureus*. Boase et al⁵² demonstrated that fungal inoculation of obstructed frontal sinuses in sheep led to robust biofilm formation, but only in the setting of *S. aureus* co-inoculation. Indeed, symbiotic interactions between bacteria and fungus may augment biofilm survival by enhancing the transfer of antimicrobial resistance traits, assisting surface adherence, and improving the protective effect of the extracellular matrix.⁵³ To illustrate, *Staphylococcus* biofilms, when grown with *Candida albicans*, exhibit increased antimicrobial resistance and enhanced growth.⁵⁴ Bacteria and fungi may also share molecular signaling. *C. albicans* secretes farnesol increasing the secretion of toxins by *Pseudomonas* species.⁵⁵ Microbial synergism is corroborated in clinical studies. In one study, sinonasal mucosal antifungal IgE levels were significantly elevated in the presence of *S. aureus*.³⁹ These findings suggest that *S. aureus* may either facilitate fungal growth or may, perhaps, cause mucosal injury allowing for fungal colonization and establishment of a chronic inflammatory state.

The potential significance of intracellular bacterial uptake has garnered significant attention. *S. aureus* is able to alter its phenotype following internalization into epithelial cells, adopting a more virulent phenotype and developing increased antibiotic resistance.^{56,57} When this concept is

Table 1 Diagnostic criteria for biofilm-associated infections.³⁰

Pathogenic bacteria must be associated with a surface
Direct examination of infected tissue must demonstrate aggregated cell clusters encased in a matrix, which may be of bacterial or host origin
Infection must be confined to a particular site of a host
Recalcitrance to antibiotic treatment despite demonstrated susceptibility of planktonic bacteria on sensitivity testing
Culture-negative results in the setting of a clinically documented high suspicion for infection
Evidence of ineffective host clearance as demonstrated by the presence of biofilm colonies in discrete areas in host tissue associated with host inflammatory cells

extrapolated to CRS, it suggests that intracellular *Staphylococci* may play a role as a primary infectious agent and as a reservoir for reinfection.⁵⁸ In a prospective trial of CRS patients, Tan et al⁵⁹ demonstrated that the presence of intracellular bacteria in sinonasal tissue was associated with a significantly higher risk of late clinical and microbiological relapse, whereas the presence of biofilm on sinonasal mucosa without intracellular bacteria did not impact outcomes. Despite methodological limitations – specifically that clinical relapse was based on endoscopy and positive cultures instead of symptom scores – this study suggests that mucosal intracellular bacteria may promote disease recalcitrance.

Research suggests that the establishment of biofilms in the sinonasal mucosa and the resulting pathogenicity likely requires defects in adaptive and innate immunity. Innate immunity represents a first-line of defense against microbial infection and compromised function has been associated with biofilm formation. Biofilm formation has been associated with the down-regulation of antibacterial peptides such as lactoferrin and MUC7 in the nasal mucosa, and the down-regulation of toll-like receptors that recognize gram-positive bacteria.^{60–62} SEM studies indicate that the mucosa of CRS patients with biofilm-positive disease is markedly damaged compared to biofilm-negative mucosa. The severity of damage ranges from morphologic changes such as disarrayed cilia to reduced ciliary beat frequency to the complete absence of cilia.^{34,63–66} The resultant mucociliary impairment further promotes bacterial persistence.

The adaptive immune system consists of lymphocytes that identify and eliminate pathogens by recognition of surface antigens, allowing for the development of immunological memory. Hekiert et al demonstrated that biofilm presence correlated with a skewing of the adaptive immune response towards the T-helper 1 (Th1) response.⁶⁷ The Th1 response is associated with the activation of cytotoxic T cells and macrophages for direct attack on pathogens. In this study, the levels of interferon-gamma, granulocyte colony-stimulating factor, macrophage inflammatory protein-1 beta, and neutrophils in the sinonasal mucosa were significantly elevated. A larger study several years later, however, demonstrated an association between *S. aureus* biofilms and a T-helper 2 (Th2) skewing of the adaptive response—more typically associated with hypersensitivity and allergic responses.⁶⁸ Thus, the role of the T-helper response in the context of biofilm colonization remains unclear. There is increasing evidence that the presence of biofilms alters chemokine production, which may potentiate CRS. The production of interleukin-5, interleukin-6, and eosinophilic cationic protein is increased in the setting of biofilms.^{68,69} This milieu of inflammatory cytokines may lead to mucosal inflammation, and osteitis of the underlying bone.⁷⁰ The correlation, if any, between chemokine production, immunopathologic changes, and resultant symptoms of CRS has yet to be firmly elucidated.

Although consensus has yet to be reached, the concept of biofilm-induced CRS has been supported by a number of peer reviewed publications.^(20–24) Together, these provide evidence that the expression of cytokines and cell surface proteins can be modulated in local mucosa secondary to the presence of bacterial biofilms. There is also evidence suggesting that the osteitic bone underlying

biofilm-infected mucosa can stimulate the production of inflammatory cytokines and may act as a “depot” for these pro-inflammatory mediators.¹⁰

Clinical implications

Bacterial biofilms are likely a key modulator of the refractory nature of CRS. In 2006, Bendouah et al⁷¹ demonstrated that *P. aeruginosa* and *S. aureus* biofilms were associated with poor clinical improvement following surgical intervention. Prince et al⁴² showed that patients with recalcitrant CRS after FESS were more likely to harbor biofilm-forming bacteria. Psaltis et al⁷² demonstrated that CRS patients with bacterial biofilms had worse pre-operative imaging scores and, at a median of 8 months follow-up, these patients were more likely to have ongoing post-operative symptoms relative to patients without biofilms. A follow-up prospective trial confirmed these findings utilizing validated subjective and objective measures, and also showed that biofilm-positive patients had statistically worse sinus symptoms, and required extra post-operative visits and multiple antibiotic treatments.⁷³ This conclusion requires further investigation as these results have been variably replicable in other studies.⁷⁴

Recent studies have demonstrated that individual biofilm species are associated with disease phenotypes. Foreman and Wormald⁷⁵ first showed that *S. aureus* biofilms are associated with severe, surgically recalcitrant disease in a small retrospective study. A later prospective, blinded study reinforced these findings.⁷⁶ Those patients with *S. aureus* biofilms had worse objective symptom scores, worse nasal endoscopy scores, worse quality of life outcomes, and required more post-operative visits when compared to patients with other biofilms. These types of clinical findings have prompted the search for effective anti-biofilm therapies.

Treatment strategies

Biofilm eradication strategies are increasingly important due to the paucity of antimicrobials in development by the pharmaceutical industry, and the concurrent propagation of antimicrobial resistance. Therapeutic avenues for the elimination of biofilms include:

- 1) Antimicrobial neutralization
- 2) Dispersion of existing biofilms
- 3) Disruption of quorum sensing

Antimicrobial neutralization

The extracellular matrix expressed by biofilms protects the bacteria within from host immune defense mechanisms and prevents entry of antimicrobial agents. Furthermore, within a biofilm, bacteria evolve reduced antibiotic susceptibility via heritable resistance mechanisms including adaptive mutations and horizontal gene transfer.⁷⁷ Consequently, the eradication of biofilms using traditional antibiotics is challenging.

Interestingly the paucity of novel antibiotics has prompted further investigation of existing agents. Macrolides typically exert their antimicrobial effect through the inhibition of bacterial protein synthesis through reversible inhibition of the 50S bacterial ribosomal subunit. Macrolides have been shown to, potentially, harbor an anti-biofilm effect through their inhibition of the production of key molecules involved in quorum sensing.^{78,79} Though the exact mechanism is unclear, it has been proposed that macrolides may indirectly interfere with an unidentified protein critical to the transcription of an autoinducer synthase.

Wallwork et al⁷⁹ evaluated a prospective cohort of 64 CRS patients in a double-blind, randomized, placebo-controlled trial. Patients received either 12 weeks of roxithromycin therapy or placebo, and clinical outcomes were assessed by the Sinonasal Outcome Test-20 (SNOT-20) and a Likert-type scale. There was a statistically significant decrease in SNOT-20 by 0.4 points 12 weeks after therapy, and a 0.7-point drop in the patient response scale. No statistically significant decreases in patient response were noted after 12 weeks calling into question the long-term benefits of macrolide therapy. Critically, this type of study does delineate between benefits related to the direct antimicrobial action of macrolides versus the anti-biofilm effect, and does not account for the anti-inflammatory effect of macrolides in CRS.^{80,81}

The discrepancy between observed *in vitro* effects and clinically significant benefit in the context of macrolide usage is highlighted by a recent meta-analysis, which concluded that there was limited evidence to support the use of long-term macrolide therapy for CRS.⁸² Although the meta-analysis demonstrated a statistical benefit to long-term macrolide therapy, the less than 1 point changes in SNOT scores are less likely to be clinically significant.⁸² Further study will be necessary to determine whether the *in vitro* anti-biofilm effect of macrolides can be harnessed as either a monotherapy or, perhaps, in combination with other treatments to effect lasting clinical improvement in CRS patients.

Topical antibiotics have long been a subject of research focus due to their ability to deliver significantly higher doses of antibiotic to the sinonasal mucosal surface with limited systemic absorption. A study by Ha et al,⁸³ in which strains of *S. aureus* susceptible to various agents were allowed to form biofilms *in vitro* were then subject to increased concentrations of topical antimicrobials, found that topical mupirocin could reduce *S. aureus* biofilm mass by more than 90%, while topical ciprofloxacin and vancomycin were both largely ineffective. In the clinical context, Jervis-Bardy et al⁸⁴ demonstrated the short-term efficacy of mupirocin sinonasal rinses against *S. aureus* infection in surgically recalcitrant CRS in a randomized controlled clinical trial of 25 patients after FESS. Patients were randomized to one month of twice daily nasal saline rinses with 0.05% mupirocin or one month of twice daily nasal saline rinses with oral Augmentin. At one month, negative cultures were noted in 89% of patients in the mupirocin group compared to 0% in the placebo group. Mupirocin group patients were also found to have a statistically significant reduction in Lund–Kennedy endoscopic scores relative to the control arm. Interestingly, this microbiologic benefit did not translate into symptom score improvements relative

to the pre-treatment baseline. Furthermore, re-assessment of the mupirocin treated cohort at 2–6 months post-treatment demonstrated that 83.3% of participants developed positive cultures for *S. aureus* with a return of Lund–Kennedy scores to baseline.⁸⁴ The implication of this finding is that the observed clinical benefits of mupirocin therapy are, generally, short-lived. Indeed, subsequent studies demonstrate that mupirocin rinses have a microbiologic failure rate of 75% over time.

There have been efforts to develop novel non-antibiotic antimicrobial agents. One such example, N,N-dichloro-2,2-dimethyltaurine (NVC-422), is a synthetic and stable form of N,N-dichlorotaurine, a compound generated during the phagocytic antimicrobial oxidative burst. NVC-422 exerts a broad-spectrum effect with demonstrated efficacy against *S. aureus*, including methicillin-resistant strains, *S. pneumoniae*, *Escherichia coli*, *Candida* species, and viruses such as herpes simplex and adenovirus.⁸⁵ Singhal et al⁸⁵ report that there was no development of resistance against NVC-422 after serial passages of the targeted microbe. After establishment of *S. aureus* biofilms in the frontal sinuses of sheep, two sinus irrigations with NVC-422 induced a dose-dependent reduction in biomass relative to untreated control sinuses [(0.11 ± 0.11) μm³/μm² in 0.5% NVC-422 compared to (2.01 ± 2.7) μm³/μm² in control].⁸⁵ There have been no further publications relating to this agent.

Manuka honey is a natural product of New Zealand whose main active ingredient, methylglyoxal (MGO), has a high phenol content which is known to be bactericidal. Manuka honey has demonstrated significant bactericidal effect against planktonic and biofilm forms of *S. aureus* and *P. aeruginosa*, eliminating 82% of methicillin-sensitive *S. aureus*, 63% of MRSA biofilms, and 91% of *P. aeruginosa* biofilms.⁸⁶ Studies conducted in sheep models of CRS revealed statistically significant reductions in biofilm biomass compared to saline flushes at concentrations of 1.8 mg/mL MGO (0.676 ± 0.079) μm³/μm² vs. (0.114 ± 0.033) μm³/μm², *P* = 0.001 and 3.6 mg/mL (0.608 ± 0.101) μm³/μm² vs. (0.141 ± 0.039) μm³/μm², *P* = 0.001.⁸⁷ Animals given MGO alone were noted to have more toxic effects, including severe sinus inflammation and metaplasia of respiratory epithelium, compared to animals treated with MGO in the presence of Manuka honey suggesting natural anti-inflammatory properties in other components of the honey.^{86,87} Clinical trials investigating the efficacy of Manuka honey in patients with CRS are awaited.

Following the use of antibiotics, there is an observed decrease in the diversity of the sinus microbiome with increases in non-commensal pathologic species known to form biofilms. In order to replete the microbiome, researchers have investigated *Lactobacillus* species, which have been found to be in high concentrations on healthy mucosa and in low concentration after antibiotic use in the setting of CRS.^{88,89} The presence of *L. fermentum* in cultures with *S. aureus* and *P. aeruginosa* led to a 40%–50% dispersion of biofilms and marked inhibition of bacterial growth rate. After inoculation of mammalian cells *in vitro* with *S. aureus* and *P. aeruginosa*, mucosal cell viability was shown to decrease by 50%–60%. In contrast, the presence of *L. fermentum* in addition to both pathogenic bacterial strains, increased mucosal cell viability by 80–95%. Early *in vitro* studies are promising suggesting that *Lactobacillus*

species may inhibit the growth, cytotoxicity and biofilm formation of several *S. aureus* and *P. aeruginosa* strains.^{9,90} In the clinical setting, Mukerji et al⁹¹ evaluated the use of probiotics as an adjunctive treatment for CRS. In this prospective, placebo-controlled trial, 77 patients were randomized to receive either oral probiotic *L. rhamnosus* or oral placebo treatment for 4 weeks. No significant difference in SNOT-20 scores was seen.

Antimicrobial photodynamic therapy (aPDT) is a novel non-antibiotic therapy that causes microbe destruction by causing perforation of cell membranes in the presence of photo reactive dyes. Activation of these compounds by laser light generates oxygen free radicals that disrupt the bacterial cell membrane, permitting the entry of dye into the cells where it can cause lethal cellular damage. This therapy showed promise in eradicating planktonic bacteria and significantly reduced biofilm biomass by over 99.9% *in vitro*.⁹² Biel et al⁹³ examined aPDT's effect on biofilms in a plastic model of the human maxillary sinus cavity. Their experiments revealed a 5 log reduction in *P. aeruginosa* biofilms and a 3.1 log reduction in MRSA biofilms after a single treatment. An earlier study by the same group demonstrated that aPDT does not appear to damage cultured human respiratory epithelial cells.⁹⁴ The clinical applicability of aPDT is under investigation.

Corticosteroids have been shown to enhance some functions of the mucosal innate immune system including increased production of complement and acute phase proteins.⁹⁵ A recent study found that high concentrations of fluticasone (400 µg/200 µL), budesonide (750–2000 µg/200 µL) and mometasone (200–400 µg/200 µL) directly reduced biofilm biomass by up to 99% *in vitro*.⁹⁶ This brings to light new mechanisms of action of intranasal steroids against biofilms and warrants further study.

Dispersion of existing biofilms

Another tactic for biofilm eradication is the use of surfactants to disrupt biofilm integrity. Chiu et al⁹⁷ explored the use of baby shampoo as an anti-biofilm detergent. Eighteen patients who underwent FESS were instructed to irrigate their sinonasal cavities with 1% baby shampoo in saline for 4 weeks post-operatively. At 4 weeks, the authors observed a 46.6% improvement in patient SNOT-22 scores and a 63% improvement in olfaction with a significant decrease in mucus thickness and post-nasal drainage.⁹⁷ However, 10% of patients reported intolerable side effects. Furthermore, although shampoo rinses inhibited biofilm growth, this therapy failed to eradicate biofilm.

A subsequent randomized controlled trial by Farag et al⁹⁸ involved 44 patients with CRS randomized to receive post-FESS baby shampoo rinses or hypertonic saline rinses. Both treatment arms demonstrated similar improvements in symptoms and olfactory thresholds. However, 52% of the shampoo group had significant side effects with 20% of the cohort withdrawing from the study compared to only 5% of patients reporting side effects in the hypertonic saline group. Specifically, baby shampoo irrigations may cause headaches and nasal burning making use as a therapy less viable.^{98,99}

Citric acid/Zwitterionic surfactant (CAZS) is a novel surfactant consisting of citric acid, which chelates calcium

in the calcium ion bridges integral to biofilm structural integrity. The zwitterionic surfactant is then able to detach the biofilm from the mucosal surface and force it into solution. Desrosiers et al¹⁰⁰ demonstrated *in vitro* that CAZS was successful in reducing *S. aureus* and *P. aeruginosa* colony forming units. CAZS induced statistically significant reductions in *S. aureus* and *P. aeruginosa* biofilm biomass, which was similar to the reduction achieved through hydrodynamic delivery of saline (2.5 and 2.9 log reduction vs. 2.3 and 2.4 log reduction, respectively, $P < 0.002$). However, the greatest reduction in biofilms was observed with hydrodynamic delivery of CAZS (3.9 and 5.2 log reduction in *S. aureus* and *P. aeruginosa* biomass, respectively, $P = 0.001$).¹⁰⁰ Of some concern, subsequent *in vivo* studies by Tamashiro et al¹⁰¹ showed CAZS to be toxic to cilia in a rabbit model, temporarily neutralizing mucociliary clearance. The investigators observed deciliation of 80%–85% 1–3 days after treatment with 96.25% recovery 6 days after stopping CAZS, leaving the sinuses more susceptible to infection in that time frame. SinuSurf, another detergent specifically developed for nasal use, showed dramatic anti-biofilm effects *in vitro*, but was taken off the market due to toxic effects.¹⁰² Thus far, despite a significant effect anti-biofilm effect, the benefits of detergent agents may be outweighed by toxicity.

Targeting enzymes and polysaccharides necessary for formation of biofilms represents a potential therapeutic strategy. Poly-*N*-acetylglucosamine (PNAG) is a polysaccharide produced by *S. aureus* that is critical to the formation of the biofilm matrix. This polysaccharide helps confer biofilm resistance to host immune peptides by forming a charge barrier preventing interaction with bacterial proteins. Dispersin B is an enzyme that degrades PNAG and may be used to target biofilms and disrupt their structure.¹⁰³ Izano et al¹⁰⁴ demonstrated both the inhibition of *S. aureus* biofilm formation and the detachment of preformed biofilms *in vitro* before and after treatment with Dispersin B.

Extracellular DNA functions to stabilize the biofilm matrix, transfer plasmids carrying resistance-conferring genes, and promote biofilm adhesion to surfaces. A novel bacterial deoxyribonuclease, NucB, produced by the marine bacterium *Bacillus licheniformis*, potentially degrades extracellular DNA.⁴³ Inoculation of FESS-derived biofilm cultures with NucB induced complete eradication of biofilms originating from nuclease-producing bacteria (*S. aureus*, *S. anginosus* group, *S. lugdunensis*, *S. salivarius*), and induced a 33% reduction in biofilms consisting of non-nuclease producing strains (*Corynebacterium* species, *Moraxella catarrhalis*). NucB was not effective against planktonic bacteria, exerting its effect exclusively on the biofilm forms. The role of NucB in eradicating CRS biofilms is under investigation.^{43,105}

Interruption of quorum sensing

One of the most novel anti-biofilm strategies involves the interruption of quorum sensing molecules secreted by bacteria. Lee et al¹⁰⁶ identified the bitter taste receptor, T2R38, as a key stimulator of nitric oxide production, subsequently leading to activation of the innate immune

response against biofilms. T2R38 is activated in response to AHL quorum-sensing molecules secreted by *P. aeruginosa* and other gram-negative bacteria. AHL inhibition leads to unstructured *P. aeruginosa* biofilms that are more susceptible to surfactants and antimicrobials. Genetic variations in T2R38 were linked to a decreased ability to clear and kill bacteria in the upper respiratory tract and were also correlated to an increased susceptibility to gram-negative sinonasal infections. Further studies have indicated T2R38 as a risk factor for CRS that necessitates FESS. Adappa et al.¹⁰⁷ compared genotypes of T2R38 in patients with CRS undergoing primary FESS ($n = 70$) to the general population ($n = 347$) and found a significantly higher frequency of the non-functional genotype in the patient cohort ($\chi^2(2) = 6.526, P = 0.0383$). Evaluation of T2R38 pathway agonists as an anti-biofilm therapy is anticipated.

In the context of *Pseudomonas* biofilms, macrolide antibiotics have been shown to reduce the expression of the *las* and *rhl* quorum sensing systems. Each system includes a gene for a transcriptional activator, *lasR* and *rhIR*, and a gene for an autoinducer synthase, *lasI* and *rhII*, which are necessary for the production of autoinducer molecules.⁷⁸ In one study, the expression of elastase and rhamnolipid – two extracellular virulence factors whose production is regulated by the *las* and *rhl* quorum sensing systems, respectively – were measured in the presence of azithromycin. Expression of these virulence factors negatively correlates with autoinducer levels in *Pseudomonas*. Azithromycin therapy induced an 80% reduction in *lasI* and a 50% decline in *rhII* levels suggesting suppression of quorum sensing. It is unknown whether these interesting *in vitro* results will translate to an anti-biofilm effect in the complex biologic milieu of CRS patients.⁷⁸

Recently, paraoxonases (PONs) have been shown to play an important role against biofilm formation.¹⁰⁸ PONs are enzymes expressed by the liver and kidney with the ability to degrade lactones including AHL. *In vitro*, PONs inactivate AHL and decrease *P. aeruginosa* biofilm growth.¹⁰⁹ In a new experimental model with *Drosophila melanogaster*, PON transgenic flies show increased survival following infection with *P. aeruginosa* and *Serratia marcescens*, both AHL sensing bacteria.¹¹⁰ This understanding of innate immunity as a defense against biofilms has become increasingly important and raises the possibility of modulating the innate immune system to better respond against biofilm.

Conclusion

CRS involves a complex interplay of infectious, inflammatory, and host factors. Biofilms are the preferred state of bacterial existence. Several novel therapies directed against biofilms are in use or in development. However, the efficacy of these agents and their potential integration into the armamentarium of strategies directed against CRS will largely be reliant on better establishing the role of biofilms in the pathogenesis of chronic rhinosinusitis. With this understanding, a focus of several ongoing studies around the world, treatment strategies tailored towards the specific causes of CRS in individual patients may be developed.

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