**Staphylococcus aureus** Biofilms: Nemesis of Endoscopic Sinus Surgery

Deepti Singhal, MS; Andrew Foreman, BMBS (Hons); Josh-Jervis Bardy, MBBS; Peter-John Wormald, MD

**Objectives/Hypothesis:** Chronic rhinosinusitis (CRS) patients with biofilms have persistent postoperative symptoms, ongoing mucosal inflammation, and recurrent infections. Recent evidence suggests that biofilms of differing species confer varying disease profiles in CRS patients. We aimed to prospectively investigate the effects of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and fungal biofilms on outcomes following endoscopic sinus surgery (ESS).

**Study Design:** Prospective blinded study.

**Methods:** In this prospective blinded study, 39 patients undergoing ESS for CRS assessed their symptoms preoperatively using internationally accepted standardized symptom scoring systems and quality-of-life measures (10-point visual analog scale, Sino-Nasal Outcome Test-20, global severity of CRS). Their sinonasal mucosa was graded (Lund–Kennedy scale) and extent of radiologic disease on computed tomography scans scored (Lund-McKay scale). Random sinonasal tissue samples were assessed for different bacterial species forming biofilms by using fluorescent in-situ hybridization and confocal laser microscopy. For 12 months after surgery, CRS symptoms, quality of life, and objective evidence of persisting disease were assessed by using the preoperative tools.

**Results:** Different bacterial species combinations were found in 30 of 39 patients; 60% of these 30 biofilms were polymicrobial biofilms and 70% had *S. aureus* biofilms. Preoperative nasendoscopy and radiologic disease severity were significantly worse in patients with multiple biofilms (*P* = .02 and *P* = .01, respectively), and they had worse postsurgery mucosal outcomes on endoscopy (*P* = .01) requiring significantly more postoperative visits (*P* = .04). Those with *S. aureus* biofilms progressed poorly with their symptom scores and quality-of-life outcomes, with significant differences in nasendoscopy scores (*P* = .007).

**Conclusions:** *S. aureus* biofilms play a dominant role in negatively affecting outcomes of ESS with persisting postoperative symptoms, ongoing mucosal inflammation, and infections.

**Key Words:** Chronic rhinosinusitis, *Staphylococcus aureus*, biofilms, postsurgical outcomes, confocal laser microscopy, endoscopic sinus surgery, Sino-Nasal Outcome Test-20, nasendoscopy.

**Level of Evidence:** 1c.

**INTRODUCTION**

Chronic rhinosinusitis (CRS) is recurring, persistent inflammation of the sinonasal tissues and is known to cause significant physical symptoms, negatively affect quality of life, and substantially impair daily functioning. Although most patients do well after endoscopic sinus surgery (ESS), in some patients it continues to be a recalcitrant condition. Biofilms have been shown to negatively affect treatment outcomes in CRS patients. The presence of biofilms on the mucosa of CRS patients is associated with more severe disease preoperatively, persistent postoperative symptoms, ongoing mucosal inflammation, and infections following ESS. However, these studies used the BacLight Viability Probe for diagnosis, and this technique precludes species identification. Bendoah et al. detected the biofilm-forming capacity of bacteria and correlated that with dichotomous postsurgical outcome. They found that *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms were associated with a more unfavorable surgical outcome.

Fluorescence in situ hybridization (FISH) techniques have now been applied with species-specific probes to identify some of the bacterial species forming biofilms in CRS patients. A retrospective analysis of CRS patients in whom biofilm-forming organisms were known demonstrated that different biofilm species display different disease characteristics. *Haemophilus influenzae* biofilms were found in patients with mild disease, whereas *S. aureus* biofilms were associated with a more severe surgically recalcitrant disease profile. But the results of the study were limited, as it was a retrospective review of a relatively small number of patients and used nonvalidated symptom scoring methods and nominal reporting of postoperative endoscopic outcomes.
Thus a prospective, blinded study of CRS patients undergoing ESS was conducted using internationally accepted, standardized symptom, radiologic, and endoscopic scoring systems to more conclusively report on the different disease and treatment outcomes seen with different biofilm species.

MATERIALS AND METHODS

Study Design

A prospective, blinded study of patients undergoing ESS for CRS in a tertiary rhinology clinic was conducted. Approval was obtained from the ethics committee, and informed consent was obtained from all patients. The 39 recruited patients fulfilled the criteria for CRS diagnosis as per the Rhinosinusitis Task Force definition for the disease and were considered for ESS after a poor response to maximal medical therapy. Patients younger than 18 years and those with ciliary dysmotility or immunocompromised conditions as well as any patients taking steroids or antibiotics in the 3 weeks before their surgery were excluded from the study.

Preoperative Data Collection

Demographic and clinical data (age, sex, medical and surgical history, allergies, and previous ESS procedures) were recorded. Before undergoing ESS, patients completed questionnaires documenting the severity of their rhinosinusitis. They graded their individual symptoms (nasal congestion/obstruction, nasal discharge, alteration in sense of smell, headache, facial pain/pressure, sneezing) and their combined symptom score using the widely accepted and validated 10-point visual analog scoring system (VAS). They assessed the effect of sinusitis on their quality of life using the Sino-Nasal-Outcome-Test-20 (SNOT-20) symptom inventory and the global assessment of rhinosinusitis symptom severity (GARS) 7-point VAS. The treating surgeon graded the patient’s sinonasal mucosa using the Lund–Kennedy scoring system. All patients underwent preoperative computed tomography, which was scored using the Lund–Mackay scoring system.

Perioperative Data Collection

Presence or absence of pus, polyps, and eosinophilic mucous at the time of the sinus procedure were documented, and swabs from the sinonasal areas of all patients were sent for microscopy and culture for isolation of possible bacteria or fungi. To ensure that all patients received the same standard of surgical care, all the endoscopic sinus procedures were performed by the senior surgeon only. Sinonasal tissue biopsies were sent for histopathologic evaluation, and two random mucosal samples were taken from either the osteomeatal region or the sinuses of each patient at the time of their surgery and transported on ice to our laboratory in Dulbecco's Modified Eagle's Medium (Gibco; Invitrogen Corp., Grand Island, NY); they were cryopreserved for delayed processing.

Tissue Analysis

Biofilm characterization was performed by an independent investigator (A.F.) who was blinded to the patient questionnaire responses and the operative findings of the surgeon. The specimens were processed and analyzed using a FISH protocol described previously, species-specific probes for S. aureus, H. influenzae, and P. aeruginosa, and a universal fungal probe. The hybridized slides were analyzed with a Leica TCS SP5 confocal laser scanning microscope (CLSM) (Leica Microsystems, Wetzel, Germany), and biofilms were identified as per the previously established biofilm definitions of FISH-CLSM protocol.

Postoperative Data Collection

The patients were all followed up after surgery by the operating surgeon, who was blinded to the biofilm status of the patients so that a standardized postoperative care was ensured for each patient. As per the standard postoperative care, the patients were assessed at 2 weeks, 6 weeks, 6 months, and 12 months. The patients also remained blinded to their biofilm results throughout the follow-up period; on each visit, the patients graded their symptoms and quality of life on the same scales as they had done preoperatively (i.e., VAS, SNOT-20, and GARS). Sinonasal mucosa was graded endoscopically at each visit by the same surgeon. Any deviation from the standardized postoperative surgical care was recorded.

Statistical Analysis

Statistical analysis was performed using Graph Pad Prism 5.0 software (Graph Pad Software Inc., San Diego, CA). All data were considered nonparametric; hence, median and interquartile ranges (IQR) are reported in the results. All statistical tests were considered to be significant at $P = .05$. Differences were analyzed by using the Fisher exact test for dichotomous data and the Mann-Whitney $U$ test for two-way independent samples.

For analysis of the data, the patients were divided first into biofilm-positive or biofilm-negative groups depending on the presence or absence of any biofilm on FISH-CLSM analysis. Patients with biofilms were further grouped, based on the number of species contained within their biofilm, into the unimicrobial-biofilm or polymicrobial-biofilm group. Patients with unimicrobial biofilms had evidence of only one bacterial species forming biofilms on FISH (i.e., S. aureus, P. aeruginosa, or H. influenzae) or fungal biofilms. Patients with polymicrobial biofilms had different combinations of S. aureus, P. aeruginosa, H. influenzae, or fungal biofilms. These polymicrobial biofilms were further divided into two groups: staphyloccocal polymicrobial biofilms or nonstaphyloccocal polymicrobial biofilms. The staphyloccocal biofilms had S. aureus biofilm detected with P. aeruginosa and/or H. influenzae and/or fungal biofilms. Nonstaphyloccocal polymicrobial biofilms had only combinations of P. aeruginosa and/or H. influenzae and/or fungal biofilms, with no S. aureus biofilms detected in analyzed tissue specimens.

RESULTS

Demographic Factors

Of the 39 patients who met the inclusion criteria and participated in the study, 19 were male (48.7%) and 20 were female (51.3%). Median age was 51.5 years (IQR: 37.3–57.7 years). More than three fourths of the study population had experienced sinusitis for more than 6 years, and 70% of them reported that they had symptoms “all the time.” Twenty-one patients had coexisting asthma, and six had a history of aspirin sensitivity. Approximately two thirds of the study population was nonsmokers, and only two subjects gave a current history of smoking.
Biofilm Status

With CLSM, 30 of the 39 (76.9%) patients in our study showed evidence of biofilms. A total of 50 biofilms were identified by FISH probes in these 30 patients in one of the various combinations. Eighteen (60%) of the 30 patients with biofilms had polymicrobial biofilms, and 15 of those 18 were polymicrobial biofilms with Staphylococcus aureus; the remaining three were other combinations of microbes forming biofilms without S. aureus. In the patients with staphyloccocal polymicrobial biofilms, five samples had P. aeruginosa biofilms with S. aureus biofilms, four had S. aureus and H. influenzae biofilms, three had S. aureus biofilms and fungal elements, three had S. aureus with both P. aeruginosa biofilms and fungal elements, two had S. aureus, P. aeruginosa, and H. influenzae biofilms, and one had S. aureus and H. influenzae biofilms with fungal elements. Of the three samples with nonstaphyloccocal polymicrobial biofilms, two had P. aeruginosa and H. influenzae biofilms, and one had H. influenzae biofilm with coexisting fungal elements. Unimicrobial biofilms were seen in 12 (40%) of the 30 biofilm-positive patients, with S. aureus biofilms being the most common unimicrobial biofilm. Six of the 12 unimicrobial-biofilm patients had S. aureus biofilms, four patients showed H. influenzae biofilms, and two had fungal biofilms only.

Preoperative Data Analysis

Preoperative severity of CRS. The preoperative subjective and objective measures of severity of sinusitis for the different patient groups in the study are compared in Table I. The preoperative subjective quality-of-life measures (GARS and SNOT-20) were comparably similar in the polymicrobial- and unimicrobial-biofilm groups, whereas the nasendoscopic and radiologic evidence of disease was significantly worse in patients with multiple biofilms ($P = .02$ and $P = .01$, respectively), and these patients also had higher symptom scores when compared with those patients with single-species biofilms ($P = .053$).

Previous surgery. Twenty-five of the 39 patients had undergone at least one previous ESS procedure. The average number of prior procedures was 2.2 (range, 0–9) for patients with polymicrobial biofilms and 1.2 (range, 0–6) for patients with unimicrobial biofilms. Patients who had $S$. aureus biofilms (alone or in combination) had undergone an average of 2 (range, 0–9) prior procedures in comparison to 1.3 (0–5) in patients with no $S$. aureus biofilms. However, these differences in numbers of procedures were not statistically significant.

Perioperative Data Analysis

Intraoperative swabs cultured 31 bacterial isolates from 28 of the 39 patients. Nonpathogenic respiratory flora was identified from eight of those, and the remaining 23 of the 31 cultures demonstrated pathogenic species. $S$. aureus was the most common isolate, being cultured in 11 samples. Other pathogens cultured included five cultures of Streptococcus pneumoniae, two of Escherichia coli, and one each of Proteus mirabilis, P. aeruginosa, Moraxella catarrhalis, Acinetobacter, and coagulase-negative Staphylococcus. There was no correlation between the bacteria isolated via culture and the species-specific biofilm identified via FISH, in keeping with the biofilm hypothesis of biofilm bacteria not being culturable via the conventional techniques. Eosinophilic mucus was identified on histology in 15 of the 39 patients, and nasal polyps were identified in 23 of the 39 patients with no specific correlation with any of the specific biofilm-forming species.

Postoperative Results

Follow-up visits. The patients were reviewed at 2 weeks, 6 weeks, 6 months, and 12 months as part of their postsurgical care. But based on patient needs or symptoms and/or the surgeon’s assessment, 25 of the 39 patients required extra visits apart from those specified. The median number of total visits and extra visits required by each of the patient subgroups are described in Table II. The patients with multiple bacterial biofilms required significantly more extra visits to the rhinology clinic as compared with the patients with single-species biofilms ($P = .04$). As seen from the data in Table II, patients with $S$. aureus biofilm by itself or in combination with other biofilms had come for more follow-up...
visits as compared with other subgroup of patients within their group.

**Symptom outcomes.** The VAS symptom scores did not show any dependence on the number of different bacterial biofilms found in the patients’ mucosa. In the unimicrobial-biofilm patients, the median total sinus symptom score of 34 preoperatively showed some initial improvement to 24.5 (IQR: 16–36) at 2 weeks and 18 (IQR: 8–35) at 6 weeks. It stabilized at that value at 6 months and showed marginal improvement to 13 (IQR: 8–18) at 12 months. The polymicrobial group had median total sinus symptom scores of 26.5 (IQR: 18.2–30.2) at 2 weeks, which improved to 11 (IQR: 7–16.5) at 6 weeks and again stabilized at 12 (IQR: 5–22.75) at 6 months and 13 (5–22.5) at 12 months. However, the symptom scores of the further biofilm subgroups progressed differently during the follow-up period, as shown in Figure 1. The patients with *S aureus* biofilms (either alone or in combination) progressed poorly with their symptom scores, and the VAS symptom scores were significantly different between unimicrobial *S aureus* and *H influenzae* biofilms (P = .01 on analysis of variance test).

**Nasendoscopy outcomes.** The polymicrobial biofilm patients had median Lund–Kennedy scores of 6 (IQR: 3.7–9.2) at 2 weeks, 4.5 (IQR: 2–11.5) at 6 weeks, 3 (IQR: 1–8) at 6 months, and 5.5 (2–8.2) at 12 months. In the same follow-up period, unimicrobial-biofilm patients had median nasendoscopy scores of 4 (IQR: 2–8.7), 1 (IQR: 0–7.5), 3 (IQR: 0–8), and 2 (IQR: 0–5). And these different scores were statistically significant between the two groups (P = .01). The difference in nasendoscopy scores between the different-species biofilm subgroups as shown in Figure 2 was also statistically very significant (P = .001 on analysis of variance test).

**Quality-of-life outcomes.** The median global assessment of severity of CRS score when compared between the different subgroups (Fig. 3) was significantly worse for the *S aureus* biofilms (P = .007), with the scores slowly creeping toward the preoperative baseline in the follow-up period. The median total SNOT-20 scores for the different subgroups described in Table III did not show statistical significance but did show that patients with *S aureus* biofilms (alone or in combination) had worse SNOT-20 scores when compared with other subgroup patients within their group.

**DISCUSSION**

This study is a prospective analysis of the impact of different biofilm species on the outcomes of ESS performed for medically recalcitrant CRS. It has shown that *S aureus* biofilms have a negative impact on postsurgical outcomes, both as a single-species biofilm and in combination with other species of biofilms.

Rhinologists are increasingly being faced with a subgroup of CRS patients whose disease process fails to

---

**TABLE II.**

<table>
<thead>
<tr>
<th>Biofilm Subgroup</th>
<th>Median No. of Follow-up Visits in First Year After ESS</th>
<th>Average No. of Extra Visits Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biofilm negative (n = 9)</td>
<td>4.5</td>
<td>1</td>
</tr>
<tr>
<td>Polymicrobial biofilms (n = 18)</td>
<td>6.0</td>
<td>2.1</td>
</tr>
<tr>
<td>With <em>S aureus</em> (n = 15)</td>
<td>6.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Without <em>S aureus</em> (n = 3)</td>
<td>6.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Microbial biofilms (n = 12)</td>
<td>4.5</td>
<td>0.9</td>
</tr>
<tr>
<td><em>S aureus</em> biofilm (n = 6)</td>
<td>5.0</td>
<td>1.5</td>
</tr>
<tr>
<td><em>H influenzae</em> biofilm (n = 4)</td>
<td>4.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Fungal biofilm (n = 2)</td>
<td>4.0</td>
<td>0</td>
</tr>
</tbody>
</table>

ESS = endoscopic sinus surgery; *S aureus* = *Staphylococcus aureus*; *H influenzae* = *Haemophilus influenzae*.
Fig. 3. Global assessment of rhinosinusitis severity comparison. H influenzae = Haemophilus influenzae; S aureus = Staphylococcus aureus; Polymicrob = polymicrobial; staph = Staphylococcus aureus; preop = preoperative.

improve despite maximal medical and surgical management. Factors like nasal polyposis, aspirin sensitivity, smoking, and acid regurgitation have been reported to affect post-ESS outcomes.\textsuperscript{13–16} This study confirms the deleterious role of \textit{S aureus} in influencing the outcome after ESS.\textsuperscript{3,4,6,7} \textit{S aureus}, \textit{H influenzae}, \textit{P aeruginosa}, and fungal biofilms have been characterized as the commonly occurring species-specific biofilms in different study populations.\textsuperscript{5–7,17,18} In our study, we used FISH probes specific for these species along with validated outcome measures to identify which species-specific biofilms are associated with poor post-treatment outcomes. However, as there is a limit to the number of species-specific FISH probes that can be used for biofilm detection, we were not able to identify some of the other clinically relevant species like \textit{S pneumoniae} and \textit{M catarrhalis} and define their disease outcomes.

CRS is typically a polymicrobial disease on the basis of standard culture techniques. In addition, biofilms in CRS are polymicrobial, and it appears that the number of species within a biofilm is also a significant predictor of disease progression in our study. The finding of multiple microbes forming biofilms on the sinonasal mucosa was associated with more severe disease preoperatively, as seen by higher symptom VAS and nasendoscopy scores as compared with patients with single-species biofilms. Postoperatively these patients had worse postsurgery mucosal outcomes on endoscopy requiring significantly more postoperative visits to the rhinology clinic.

\textit{S aureus} biofilms (either alone or in combination with other species) were identified in 21 (70%) of the 30 biofilm-positive patients, and this group progressed poorly with their subjective and objective post-ESS outcomes. \textit{S aureus} has been identified in different study populations as the most common bacteria isolated from patients with surgically recalcitrant disease\textsuperscript{19–23} and also the most common biofilm-forming organism found on intraoperative mucosal specimens.\textsuperscript{7} Our prospective analysis of patients displaying \textit{S aureus} biofilms on their sinonasal mucosa has provided direct evidence that these patients have worse VAS symptom scores, worse nasendoscopy scores, and worse quality of life outcomes after ESS in comparison with the patients with other microbial biofilms. These patients also required more follow-up visits as compared with the remaining biofilm patients, indicating that repeated infections and persistent disease prevail in this subgroup. The patients also had a tendency to have undergone more prior sinus surgeries, reflecting the more severe and recalcitrant nature of the disease when associated with \textit{S aureus} biofilm. This negative impact on post-treatment outcomes is more pronounced when the \textit{S aureus} biofilms occur as single-species biofilms and is still seen in a somewhat attenuated manner when they occur along with other microbial biofilms.

Further work done in our department (in press) has found a coexistence of \textit{S aureus} biofilms and superantigens in CRS patients; these findings have suggested the biofilms may be the source of superantigens. An eosinophilic, Th2 polarized immune response with increased levels of eosinophilic cationic protein and interleukin-5 in patients with \textit{S aureus} biofilms has also been observed, possibly providing a link in the pathogenesis of CRS. The biofilm phenotype exhibits resistance to this immune response, possibly protected by the surrounding EPS against phagocytosis and antibody and immune cells, which instead lead to collateral tissue damage caused through their cytotoxic, proteolytic, and proinflammatory actions and result in severe and sustained inflammation. Further investigation is required regarding the precise immune pathway so that possible treatments that target it can be devised to control the resulting inflammation.

The role of \textit{H influenzae} in CRS has recently been reinvigorated, with studies identifying \textit{H influenzae} biofilms commonly on the sinonasal mucosal specimens of CRS patients.\textsuperscript{7,17,18} The disease profile of patients with \textit{H influenzae} biofilms has been described as mild, with rapid resolution of signs and symptoms following

<table>
<thead>
<tr>
<th>TABLE III.</th>
<th>Sino-Nasal Outcome Test-20 Scores for the Different Species-Specific Subgroups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>Presurgery</td>
</tr>
<tr>
<td>Biofilm negative</td>
<td>29.0</td>
</tr>
<tr>
<td>Polymicrobial biofilms</td>
<td>32.5</td>
</tr>
<tr>
<td>With \textit{S aureus}</td>
<td>31.0</td>
</tr>
<tr>
<td>Without \textit{S aureus}</td>
<td>34.0</td>
</tr>
<tr>
<td>Microbial biofilms</td>
<td>32.5</td>
</tr>
<tr>
<td>\textit{S aureus} biofilm</td>
<td>35.5</td>
</tr>
<tr>
<td>\textit{H influenzae} biofilm</td>
<td>26.0</td>
</tr>
<tr>
<td>Fungal biofilm</td>
<td>32.5</td>
</tr>
</tbody>
</table>

\textsuperscript{SNOT-20} = Sino-Nasal Outcome Test-20; \textit{S aureus} = \textit{Staphylococcus aureus}; \textit{H influenzae} = \textit{Haemophilus influenzae}. 

Laryngoscope 121: July 2011  
Singhal et al.: \textit{Staphylococcus aureus} Biofilms in ESS  
1582
surgery. In our study, 12 patients had H influenzae biofilms, of which four were unimicrobial biofilms and eight were found in various combinations with other microbial biofilms. Patients with unimicrobial H influenzae biofilms had the least severity of disease preoperatively, with the lowest symptom and nasendoscopy scores. During the first 6 to 8 weeks, they had a rapid resolution of symptoms and mucosal signs on endoscopy, with continued marked improvement of quality-of-life outcome measures, similar to patients without biofilms. This significant improvement was not evident when H influenzae and S aureus biofilms were found together, indicating that its pathogenic effect may have been either weak from the start or overpowered by the refractory and recalcitrant pathogenic mechanisms surrounding S aureus biofilm. When H influenzae biofilms combined with P aeruginosa or fungal biofilms as a polymicrobial biofilm without S aureus, the disease pattern was once again milder and reflective of the possibly mild combined pathogenic effects of these bacterial biofilms. These two pieces of information further support the theory that S aureus plays a dominant role in CRS biofilms.

No unimicrobial P aeruginosa biofilms were seen in our study population; hence the disease profile associated with them could not be defined. Of the 10 P aeruginosa polymicrobial biofilms, eight were present with S aureus; thus, disease outcomes for P aeruginosa are difficult to differentiate from the staphylococcal biofilm. There were only two patients with single-species fungal biofilms, and the disease pattern in this very small population was mild, although the recovery of signs and symptoms was more protracted. The remaining four fungal biofilms coexisted with S aureus biofilms, and the patterns were again reflective of a staphylococcal biofilm. Thus, in this small study population, S aureus biofilms were spread through the biofilm subgroups, causing a loss of possible differences in the outcome measures and also indicating that S aureus biofilms play a dominant role in determining disease severity and guiding the postoperative course.

The study thus shows that different bacterial biofilms are associated with different disease progressions after ESS. A clinically relevant species-directed biofilm analysis will help to determine the possible at-risk group of patients and guide more directional research toward the elimination of those biofilms. This specific knowledge may help in developing novel and species-specific antibiotic treatments that may enable us to aggressively treat these patients and help improve the postaural outcomes of ESS.

CONCLUSION

S aureus biofilms play a dominant role in negatively affecting the outcomes of ESS with persistence of postoperative symptoms, ongoing mucosal inflammation, and infections. Patients with unimicrobial H influenzae biofilms have a milder disease pattern with a rapid resolution of symptoms and mucosal signs following ESS. Future studies evaluating therapeutic intervention specifically targeting S aureus biofilms are needed.

BIBLIOGRAPHY