The effect of bacterial biofilms on post-sinus surgical outcomes

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ABSTRACT

Background: Although the existence of biofilms on the sinus mucosa of patients with chronic rhinosinusitis (CRS) is now well established, the role that these structures play remains unclear. It is thought that biofilms may contribute to the recalcitrant and persistent nature that characterizes CRS, but little research exists documenting the effect that they have on postoperative mucosal outcomes. This article presents a retrospective analysis of sinus surgical patients and correlates the presence of biofilms with mucosal outcomes. This study was performed to evaluate the role that bacterial biofilms have on post-sinus surgical outcomes.

Methods: A retrospective analysis of prospectively collected data was performed on 40 patients undergoing endoscopic sinus surgery (ESS) for CRS. Preoperative demographic, clinical, and radiologic data were recorded from each patient and, intraoperatively, sinus culture specimens and mucosal samples were obtained for microbiological and microscopic examination. Biofilm determination was performed using confocal scanning laser microscopy. Postoperatively, patients were followed up for a minimum of 8 months with endoscopic evaluation of their sinonasal mucosa. The presence of ongoing symptoms was recorded also.

Results: Bacterial biofilms were found in 20 (50%) of the 40 CRS patients. Patients with biofilms had significantly worse preoperative radiological scores and, postoperatively, had statistically worse postoperative symptoms and mucosal outcomes. The only other factor that was statistically related to an unfavorable outcome was the presence of fungus at the time of surgery. In this study the presence of polyposis, eosinophilic mucin, or pus was not related to poor outcomes.

Conclusion: This retrospective study showed that bacterial biofilms and fungus were correlated with the persistence of postoperative symptoms and mucosal inflammation after sinus surgery for CRS. This provides evidence that biofilms indeed may play an active role in perpetuating inflammation in CRS patients and may explain the recurrent and resistant nature of this disease. Therapies targeted at removing biofilms may be important in the management of recalcitrant CRS.

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Key words: Bacterial biofilms, chronic rhinosinusitis, postsurgical outcomes, fungus, pathogenesis, polyposis

Until recently, many chronic diseases including chronic rhinosinusitis (CRS) and otitis media with effusion have been thought to have non bacterial etiologies. This was largely because of their low bacterial culture yield rates, their lack of response to antibiotic therapy, and the lack of correlation often noted by clinicians between bacteriological findings and macroscopic/clinical features of patients with such conditions. The discovery of bacteria existing in an alternate biofilm form has led many researchers to revisit the pathogenesis of these conditions and reexamine the possible role that bacteria may play in these diseases.

In the past few years, it has become increasingly evident that biofilms exist on the mucosa of CRS patients. Research groups have used various imaging modalities, including scanning and transmission electron microscopy,1–4 and fluorescent in situ hybridization5 to visualize these structures. More recently, our department described the use of the confocal scanning laser microscope (CSLM) to observe biofilms in their natural state on fresh sinus mucosal specimens. Using this noninvasive and nondestructive modality we documented these three-dimensional structures in ~50% of patients undergoing endoscopic sinus surgery (ESS) for CRS. Despite the mounting evidence that biofilms exist in CRS patients, the exact role they play in the pathogenesis of this condition still remains unclear. This article represents a continuation of our previous work examining biofilm status in patients with CRS. We present pre- and postoperative data examining the role that biofilms and other factors may have on postoperative outcomes.

MATERIALS AND METHODS

Study Design

The study design involved a retrospective analysis of prospectively collected data in a tertiary rhinological setting. The subject population consisted of 43 consecutive patients undergoing ESS for CRS. The diagnosis of CRS was made according to the criteria set out by the Rhinosinusitis Task Force and endorsed by the American Academy of Otolaryngology. Patients included in the study were immunocompetent, had received standardized preoperative medical therapy, and had not used steroid or antibiotic medications in the week preceding surgery.

Data Collection/Preoperative Investigations

Clinical data, including demographical information, relevant medical and surgical history, asthma, allergy, and smoking status were all recorded. Each patient was asked specifi-
cally to indicate the severity (score of 0–5) of the following five sinusitis symptoms: nasal obstruction, rhinorrhea, postnasal drip, headache/facial pain, and loss of smell. Radioallergosorbent testing (RAST) to four common allergens and total IgE levels also were performed in some individuals before surgery if there was evidence of mucus on pre- or intraoperative endoscopy. All patients underwent preoperative computerized tomography (CT) scanning and were staged and evaluated blindly according to the Lund-McKay CT scoring system.

Intraoperative Assessment/Subclassification of CRS
At the time of surgery, the surgeon recorded the presence of mucin, pus, and polyps. If macroscopic pus or fungal mucin were present, swabs were sent for microscopy, culture, and sensitivity. The characteristic eosinophilic mucus (EM) collected from the sinuses at surgery was confirmed by histology. Patients defined as fungal positive if either fungal stains or cultures from the EM were positive, as previously published.7 Patients were then classified as follows:

- Chronic Rhinosinusitis. No EM at the time of surgery and negative culture and staining for fungus.
- Allergic Fungal Sinusitis. Presence of EM determined by histology, fungal allergy, and positive fungal stain or culture, characteristic CT findings.
- Nonallergic Fungal Eosinophilic Sinusitis. Presence of EM determined by histology, positive fungal stain, or fungal culture and negative fungal allergy.
- Nonallergic, Nonfungal Eosinophilic Sinusitis. Presence of EM determined by histology, negative fungal stain, and culture and negative fungal allergy.

Biofilm Analysis
Two random mucosal samples, ranging from 5 to 10 mm², were taken from the middle meatus and ethmoid cavity and stored immediately in Dulbecco's modified eagle medium (Gibco, Invitrogen Corp., Grand Island, NY) on ice. The samples were then transferred to Adelaide Microscopy, a CSLM imaging facility, for immediate biofilm analysis. Standardized tissue preparation and microscopic analysis for biofilms was performed as documented in our previously published work. Biofilm analysis was performed by authors (A.J.P and K.R.H.) who were not present at the time of the procedure and thus were blinded as to the operative findings of the surgeon.

Postoperative Follow-Up and Outcome Assessment
Postoperatively, all patients were subjected to standardized management and followed up for a minimum of 8 months. Patients were assessed clinically for the presence of ongoing symptoms and nasal endoscopy was used to document the state of their mucosa. Patients were asked to report any symptoms that persisted for >8 months postoperatively. If they reported any symptoms, however mild, they were classified as having ongoing symptoms. The postoperative appearance of the sinus mucosa and ostia was recorded and classified as being normal or as having ongoing inflammation. The later option was chosen if there was any evidence of inflammation, edema, pus, mucin, or significant crusting. The size of the sinus ostia was recorded also. All follow-up and endoscopy was performed by P-J.W., who was blinded as to the biofilm status of the patient.

Statistical Analysis
Statistical analysis was performed using SPSS (r) 10.1.0 (SPSS, Inc., Chicago, IL). All data were considered nonparametric and were characterized by median ± interquartile range. Analysis of the 40 patients was performed per protocol. For all statistical tests used p = 0.05 was considered significant. Differences were analyzed using the following statistical tests: a chi-square or Fisher’s exact test for dichotomous data, the Mann-Whitney U test for two-way independent samples, the Kruskal-Wallis test for multiple independent samples, and Spearman rank correlation for two independent ordinal samples. Effect size is presented with all p values where appropriate as well as odds ratio (OR) and 95% confidence intervals (CIs) for the OR.

RESULTS
Demographics/Patient Factors
Of the 43 patients initially enrolled in the study, 3 were lost to follow-up. The male to female distribution of the remaining 40 patients was 29 to 11, with a mean age of 53.7 ± 15.9 years (range, 21–85 years) and mean follow-up of 11 ± 1.9 months (range, 8–14 months). The median preoperative Lund-Mackay score was 17 (range, 3–24) and the median preoperative symptom score was 16 of a maximal 25 (range, 8–22). Twenty-eight (60%) patients had visible polyps at initial examination and 24 (60%) patients had undergone previous sinus surgery.

Intraoperative Findings/Biofilm Status
Patients were subclassified according to histopathological examination of biopsied sinonasal mucosa; 19 (47.5%) patients were subcategorized as chronic bacterial rhinosinusitis showing no evidence of fungus or EM on histopathology or culture. The remaining 21 patients (52.5%) showed that EM were subcategorized as follows according to their fungal and allergy status: allergic fungal sinusitis (AFS), n = 8; nonallergic fungal eosinophilic sinusitis (NAFES), n = 3; nonallergic nonfungal eosinophilic sinusitis (NANFES), n = 10.

Fifty percent of the CRS patients enrolled in the study showed evidence of biofilms on CSLM. Table 1 summarizes the pre-, peri-, and postoperative findings for each subclassification of CRS. However, it must be stated that the small number of patients in each group make a meaningful comparison between the four different subcategories of CRS difficult.

Postoperative Outcome
The presence of ongoing symptoms and the postoperative appearance of the sinus and its overlying mucosa were both used as outcome measures. Twenty-one (52.5%) of the patients enrolled in this study showed evidence of ongoing mucosal disease. No attempt had been made to grade the severity of the postoperative inflammation and for the purpose of this study any abnormality of the mucosa, however mild, was recorded as unfavorable. Of the patients with persisting inflammation, nine had ongoing active infection with
<table>
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<tr>
<th>Table 1 Preoperative, Intraoperative, and Postoperative Findings</th>
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<tr>
<td><strong>CRS</strong></td>
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<td>(n = 19)</td>
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<tr>
<td>Symptom score</td>
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<td>Median (range)</td>
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<tr>
<td>Lund-Mckay median (range)</td>
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<td>More than one previous ESS procedure</td>
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<td>Mean no. of ESS procedures (±STD)</td>
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<td>EM</td>
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<td>Pus</td>
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<td>Fungus*</td>
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<td>Biofilm positive</td>
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<td>Mucosal inflammation</td>
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<td>Symptoms</td>
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<td>+ve Bacterial culture</td>
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<td>+ve Fungal culture</td>
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*By silver stains or fungal culture.
pus visualized in at least one of their sinuses, six had fungal recurrence with associated mucosal inflammation; two showed mucosal edema with associated secretions, three showed mild mucosal edema only, and one had anterior adhesions with associated mucosal edema. Seventeen (42.5%) of the patients followed up in the study informed the surgeon that they still experienced symptoms at least 8 months postsurgery, despite the majority of them describing a subjective improvement from their preoperative condition. Table 1 summarizes the postoperative findings.

### Statistical Analysis

**Biofilms and Planktonic Bacteria Cultures.** Table 2 compares the clinical, operative, and postoperative findings of biofilm and nonbiofilm patients. Patients with biofilms (n = 20) had similar preoperative symptom scores to patients without biofilms (p = 0.76; Table 2) but worse preoperative CT scores (median = 18.5 and interquartile range = 17-22 versus median = 14.5 and interquartile range = 14-18; p = 0.013; Mann-Whitney U value = 108).

There was a very strong statistical relationship between unfavorable mucosal outcomes and the presence of ongoing symptoms (Fisher's exact test, p < 0.0001 and OR = 117.7 with 95% CI = 6.046-2292) with biofilm patients more likely to have ongoing postoperative symptoms (Fisher's exact test, p = 0.01 and OR = 7.43 with 95% CI = 1.78-31.1) as well as endoscopic evidence of ongoing mucosal inflammation (Fisher's exact test, p = 0.01 and OR = 7.00 with 95% CI = 1.74-28.18).

**Presence of Pus Intraoperatively.** Patients with frank pus visible in the sinuses at the time of surgery had similar symptom scores (p = 0.23) and CT (p = 0.24) scores as those without pus. Visible pus in the sinner did not correlate with the positive bacterial cultures (p = 1.1) or presence of biofilms (p = 0.76) and did not have a statistically significant relationship to ongoing postoperative symptoms (p = 0.52) or mucosal inflammation (p = 0.53).

**Polyp Status**

Patients with polyps (n = 28) had similar preoperative symptom scores to patients without visible polyposis (p = 0.26); however, they had significantly worse preoperative CT scores (median = 17.50 and interquartile range = 14.5-21.5 versus median = 13.50 and interquartile range = 11.0-17.5; p = 0.005; Mann-Whitney U value = 73.00). Regarding postoperative outcome, patients with polyposis did not differ significantly from patients without polyps in terms of postoperative mucosal healing (p = 1.00) or the presence of ongoing symptoms (p = 0.74).

One of the more clinically relevant findings in this study was found in the nonpolyp group. In this group, those patients with biofilms had an extremely high likelihood of poor mucosal outcomes (Fisher's exact test, p = 0.0047 and OR = 56.33 with 95% CI = 1.916-1656) and, conversely, those patients without biofilms had good mucosal outcomes (Fisher's exact test, p = 0.0047 and OR = 56.33 with 95% CI = 1.916-1656).

**Fungal Status.** Eleven of the 40 (27.5%) patients studied showed the presence of fungus either on staining or culture. Patients with fungus had similar preoperative symptom scores (p = 0.30) and CT scores (p = 0.16) as patients without fungus. In terms of postoperative outcomes patients with fungus fared statistically worse with regards to the presence of symptoms (Fisher's exact test, p = 0.0305 and OR = 5.925 with 95% CI = 1.267-27.72) as well as evidence of ongoing mucosal inflammation (Fisher's exact test, p = 0.034 and OR = 6.375 with 95% CI = 1.163-34.95). When subgroup analysis was performed on the data, there was no significant difference between the subgroups of AFS, AFS-like, CRS, NAFS, and NAFS in terms of both the persistence of symptoms (p = 0.154) and the mucosal outcomes (p = 0.152).

**Eosinophilic Mucus.** Patients with EM (n = 21) had higher symptom scores than patients without (median = 18.0, interquartile range = 15.5-19.0 compared with a median = 15.0 and interquartile range = 12.0-17.0; p = 0.015). They also showed a trend toward worse CT scores; however, this was not statistically significant (p = 0.07). There was no significant difference between EM+ and EM− patients in terms of the presence of ongoing postoperative symptoms (p = 0.11) or postoperative mucosal outcomes (p = 0.11).

**Previous Sinus Surgery.** Twenty-four (60%) of the patients enrolled in the study had undergone at least one previous ESS procedure. Patients who had undergone previous ESSs reported significantly higher symptoms scores preoperatively (median = 17 and interquartile range = 15-19 versus median = 15 and interquartile range = 13-16.5; p = 0.033 Mann-Whitney U value = 114.5). Postoperatively, revision cases were more likely to report ongoing symptoms (Fisher's exact test, p = 0.0047).

### Table 2: Comparison of biofilm and nonbiofilm patients

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<thead>
<tr>
<th></th>
<th>Biofilm Visualized (n = 20)</th>
<th>No Biofilm Visualized (n = 20)</th>
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<tbody>
<tr>
<td>Age (mean ± STD)</td>
<td>53.7 ± 16</td>
<td>50.6 ± 16.2</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>15:5</td>
<td>14:6</td>
</tr>
<tr>
<td>Preoperative symptom score</td>
<td>17 (13.5-18.5)</td>
<td>16 (13.5-18.0)</td>
</tr>
<tr>
<td>Score out of 25</td>
<td>18.5 (17.0-22.0)</td>
<td>14.5 (14.0-18.0)</td>
</tr>
<tr>
<td>Lund-McKay score</td>
<td>2.57</td>
<td>1.78</td>
</tr>
<tr>
<td>Average no. of previous ESS</td>
<td>13 (65%)</td>
<td>4 (20%)</td>
</tr>
<tr>
<td>Presence of postoperative symptoms</td>
<td>15 (75%)</td>
<td>6 (30%)</td>
</tr>
<tr>
<td>Ongoing postoperative inflammation</td>
<td>10/11 (91%)</td>
<td>9/18 (50%)</td>
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test, p = 0.0002 and OR = 30.00 with 95% CI = 3.339–269.5) and have evidence of ongoing inflammation (p = 0.009 and OR = 7.286 with 95% CI = 1.737–30.57) than patients undergoing their first ESS procedure. Also of note was that there appeared to be no significant preponderance of biofilms in the revision cases.

CT Score. There was no correlation between preoperative CT score and symptom score (Spearman’s rank correlation, 0.153; p = 0.346). However, when preoperative CT score was segmented by those patients who had resolution of symptoms versus those who did not, there was a significant difference (p = 0.004; Mann Whitney U value = 91.0). Patients with good mucosal outcomes had significantly lower Lund-McKay scores than those with poor mucosal outcomes (U = 93.0; p = 0.004).

DISCUSSION

The success rate of functional ESS (FESS) is extremely variable. Although some studies claim resolution or improvement of symptoms in up to 83–92% of patients,5,10 the majority of this research is of low-level evidence.11 A recent systematic review of the literature by the Cochrane Collaboration Group concluded that based on the evidence available, FESS does not confer any additional benefits to that obtained by medical treatment of CRS. To date, multiple studies have been performed addressing possible outcome predictors after FESS, including demographic differences, smoking, presence of nasal polyposis, and disease severity.10,13–15 This retrospective study found that of all the variables analyzed, only the presence of biofilms or fungus were related to poorer outcomes at 8 months follow-up.

The existence of biofilms on the mucosa of patients with CRS is now well established.12,4,5,11 Using CSLM, biofilms were shown in 50% of the group studied. Patients with biofilms were found to have significantly higher Lund-McKay scores than those without; however, no significant difference in symptom scores was found between the two groups. The lack of correlation between symptom and radiological scores indicated in this study is in concordance with previous studies.18,19 This study found a strong correlation between the intraoperative presence of biofilms and endoscopic evidence of ongoing mucosal inflammation. This finding is supported by Bendouah et al.,20 who recently showed a correlation between the in vitro biofilm-producing capacity of Pseudomonas aeruginosa and Staphylococcus aureus and unfavorable evolution after ESS. The persistent inflammation observed in the biofilm patients is perhaps a consequence of the extreme difficulty observed in removing biofilms, which irreversibly attach to mucosal surfaces and have inherently resistant phenotypes. Residual bacterial macrocolonies left behind after surgery possibly could serve as a nidus for biofilm regeneration and act as a stimuli for an ongoing inflammatory response. The role of biofilms in perpetuating inflammation is extremely evident in patients who showed no evidence of polyposis preoperatively. In this subset of patients, a very strong correlation was seen between mucosal outcome and biofilm status (Fisher’s exact test two-sided P value = 0.005), with every biofilm-positive patient showing ongoing inflammation at 8 months. Conversely, nonpolypos patients without biofilms showed an almost universally good postoperative mucosal outcome.

Bacterial cultures were performed only on 29/40 patients. The decision to culture was based on the visualization of frank pus as well as the surgeon’s own index of suspicion. Forty-five percent of patients had visible pus at the time of surgery. Again, as shown in our 2005 study, there was no statistical relationship between pus and Lund-McKay scores.21 Pus also was not shown to correlate with the presence of either planktonic or biofilm bacteria. Nineteen of 29 (66%) of the bacterial swabs taken yielded a positive culture result, with the culturing of planktonic bacteria positively related to the presence of biofilms. This finding is consistent with current research that suggests that although bacteria existing in biofilms are very difficult to culture on standard agar plates because of their lower growth and metabolic rates, biofilms may function as a source of planktonic cells through high cell yield and detachment.22 Nevertheless, the results obtained in our study need to be interpreted with caution as 7 of the 20 biofilm patients included in this study did not show evidence of pus at the time of surgery and, consequently, did not have bacterial swabs. Regarding mucosal outcome, unlike biofilms, the presence of planktonic bacteria at the time of surgery was not related to ongoing postoperative inflammation. This may reflect the distinct genotypic differences and greater inherent resistance to medical and surgical treatment exhibited by bacteria existing in a biofilm form.

Other than biofilms, the presence of fungus was the only other factor found to have a statistically significant association with an unfavourable outcome. To date, the prevalence and impact of fungus in patients with CRS has been a point of much debate. In 1999, Ponikau et al.23 identified fungus in nearly all surgical patients and in 100% of normal individuals with CRS. Their study concluded fungus to be the sole etiological agent behind all forms of rhinosinusitis and as a consequence led them to suggest redefining CRS as "eosinophilic fungal rhinosinusitis." Although innovative, this viewpoint has never been widely accepted. Many other have acknowledged the importance of fungus in some types of CRS but believe its pathogenesis to be multifactorial. Our study's sinus fungal culture rate of 28% is very similar to that observed in a large study conducted by our department in 2003 and resembles the experience of many departments around the world. Despite this, researchers such as Ponikau et al.25 believe that this low yield rate is more reflective of the low sensitivity of the fungal detection methods currently used. With regards to prognosis, this retrospective study showed that 9/11 (80%) patients in whom fungus were isolated had evidence of ongoing mucosal inflammation at a minimum of 8 months follow-up. This is in keeping with the experience of many clinicians who report fungal sinusitis, in particular AFSS, to be a difficult to treat condition with extremely high recurrence rates.26 Another interesting finding of this study was the presence of bacterial biofilms in 63% (7/11) of fungal-positive patients. This coexistence of bacteria and fungus within biofilms has already been well documented. More recently, specific microbial surface molecules called adhesins have been identified as the likely mediators for the coadhesion and aggregation of mixed microbial communities within these biofilms and on mucous surfaces.27 With regards to polyposis, our study reinforces the findings of other studies that show CRS patients with nasal polyps
have worse preoperative CT scores.\textsuperscript{15,28} This is thought to be caused by largely the nature of the polyoid mucosa, which not only makes the appearance of the sinus CT scans worse, but may act to obstruct drainage pathways leading to reten-
tion of sinus secretions and/or pus.\textsuperscript{18} Interestingly, unlike the majority of previous research, our study did not establish a relationship between polyposis and adverse postoperative endoscopic outcomes. The reason for this remains unclear but may be caused by partly the fact this study did not grade mucosal outcomes in an ordinal manner, but rather scored it as being either normal or abnormal.

As with any retrospective analysis, this study has its limitations. First, it is acknowledged that the patient follow-up was relatively short and thus conclusions on long-term mucosal outcomes cannot be made. Second, this study relies on a symptom scoring method that has not been validated against the more widely accepted Visual Analogue Score or Chronic Sinusitis Survey scoring systems. Finally, when assessing postoperative outcomes, we used nominal reporting measures related to mucosal status and symptoms. These measures not only lack the robust features of an ordinal or continuous scale, but in this particular study also may be inherently biased toward categorizing patients as having unfavorable outcomes. To address the three main limitations of this study, a comprehensive prospective blinded study will be initiated to verify the current study’s findings. It is hoped that this study will also incorporate fluorescent in situ hybridization as a detection modality to specifically identify which bacteria predominate in the biofilms found in the mucosa of CRS patients.

**CONCLUSION**

This study is one of the first studies to examine the effect that biofilms have on post-ESS outcomes. It provides evidence that biofilms may play an active role in the pathogenesis of CRS, particularly refractory cases. Blinded prospective studies are required to further examine the relationship between CRS and biofilms and provide a higher level evidence of any significant relationship between the two.

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