Clinical Characteristics of Biofilms in Patients with Chronic Rhinosinusitis: A Prospective Case–Control Study

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Abstract Recent studies have indicated that biofilms are involved in the pathogenesis of recurrent and recalcitrant chronic rhinosinusitis (CRS). The present study was performed to evaluate the presence of biofilms and to evaluate the relationships between the presence of biofilms and clinical features of CRS. A total of 33 patients were included in this study. Maxillary sinus mucosa from 26 CRS patients and the ethmoid mucosa from 7 patients undergoing septoplasty were collected. Biofilms were evaluated by scanning electron microscopy. Preoperative symptom scores, preoperative and intraoperative nasendoscopic findings, and postoperative healing period were compared between the groups. Biofilms were detected in 14 (42.4 %) of the 33 patients. Biofilms were present in 13 (50 %) of the 26 patients in the CRS group, but in only one (14.3 %) of the seven patients in the control group. There were no significant differences in preoperative symptom scores or preoperative nasal Lund–Kennedy endoscopic scores between the groups. However, the average Lund–Mackay and intraoperative sinus Lund–Kennedy endoscopic scores were significantly higher in biofilm-positive than biofilm-negative patients ($P < 0.05$). In the follow-up period, the healing time was significantly longer in biofilm-positive than biofilm-negative patients ($P < 0.05$). This

study suggested that the presence of bacterial biofilms may contribute to the pathogenesis of CRS and the clinical characteristics of CRS patients after endoscopic sinus surgery.

Keywords Chronic rhinosinusitis (CRS) · Biofilm · Scanning electron microscopy (SEM) · Endoscopic sinus surgery

Introduction

Chronic rhinosinusitis (CRS) is a very common disease that sometimes requires long-term treatment with antibiotics. CRS may require repeated operations because of the lack of responsiveness to medical treatment. The pathogenetic factors for CRS are bacteria, allergy, fungus, superantigens, and biofilms [1]. Biofilms are aggregates of microorganisms in which cells adhere to each other on the surface of living or nonliving substrates. The bacteria in biofilms are known to be 1,000 times more resistant to antibiotics than the floating form of bacteria [2]. After forming biofilms, bacteria penetrate host defenses, thus decreasing antibiotic susceptibility, and releases floating bacteria to new sites [3]. Nevertheless, the precise roles of biofilms in the recurrence or exacerbation of CRS have not yet been fully elucidated. Biofilms have been suggested to be one of the factors contributing to recurrent recalcitrant or persistent CRS. However, most studies have focused on the presence of biofilms in the mucosa of patients suffering from CRS without evaluating symptoms, status of mucosa, or clinical features [4–6].

The present study was performed to evaluate the presence of biofilms and to examine the relationships between the presence of biofilms and clinical features of CRS.

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Materials and Methods

Patients

A total of 33 patients were included in this study. The patients were divided into two groups: patients who underwent endoscopic sinus surgery for CRS (CRS group, $n = 26$) and those who underwent septoplasty (control group, $n = 7$). The mean age of the patients was 44.3 ± 23.2 years. The male to female ratio was 23:10. This study protocol was approved by the Institutional Review Board of Gachon University Gil Medical Center.

Methods

Mucosal specimens were obtained from the maxillary sinus mucosa in the CRS group and healthy ethmoid mucosa in the control group. After getting agreement author obtained healthy ethmoid mucosa from anterior ethmoid sinus with cutting forcep after uncinectomy in control group undergoing septoplasty. Scanning electron microscopy (SEM) were performed to detect biofilms. Biofilm positivity was defined as positive results.

SEM

Each nasal mucosa specimen was washed with 1 M phosphate buffered saline (PBS) and fixed for more than 12 h with 2.5 % glutaraldehyde buffer. The specimens were cut into section 5 μ m thick, washed again with 0.1 M PBS, and then mounted with 1 % OsO₄ for 2 h for the secondary fixation process. After fixation, the sections were dehydrated with alcohol and dried with 100 % isoamyl acetate until the critical point. Sections were examined by SEM after sputter coating with white gold. Biofilm positivity was defined as the presence of three-dimensional structures with bacterial colonies according to the diagnostic criteria of Mladina et al. [5]. Round-shaped biofilms with bacterial colonies were seen as a polymorphous three-dimensional structures with a polysaccharide matrix and water channels.

Pre- and Intra-operative Findings

The patients with CRS underwent systemic examination and paranasal sinus computed tomography (PNS CT). The CT scans were interpreted according to the Lund–MacKay scoring system [7]. Each patient was assessed preoperatively to evaluate the symptom scores of five symptoms; i.e., nasal obstruction, loss of smell, rhinorrhea, headache/facial pain, and postnasal drip, using a visual analog scale. The preoperative state of the nasal mucosa was evaluated using Lund–Kennedy endoscopy score. The mucosal condition of the maxillary sinus was intraoperatively evaluated

according to the Lund–Kennedy endoscopy score [8]. We standardized treatment for all study patients. All patients took antibiotics and antihistamine for 3 weeks and did not take any steroids (oral or nasal spray). The follow-up period until complete resolution of endoscopic findings was evaluated after surgery. Mucosal swelling, discharge, or significant crust formation was defined as current inflammation. No mucosal swelling or little serous discharge was defined as complete resolution.

Statistical Analysis

The Lund–MacKay score and healing time were analyzed with the Mann–Whitney *U* test. The other factors were analyzed by Fisher's exact test. In all analyses, $P < 0.05$ was taken to indicate statistical significance.

Results

Positive Result for Biofilm

Biofilms were detected in 14 (42.4 %) of the 33 patients on SEM. Biofilms were present in 13 (50 %) of the 26 patients in the CRS group, but in only one (14.3 %) of the seven patients in the control group (Table 1; Fig. 1).

Pre- and Post-operative Findings

The average preoperative symptom scores were 36.5 ± 5.88 points in biofilm-positive patients and 34.78 ± 5.82 points in biofilm-negative patients; this difference was not statistically significant ($P > 0.05$). The average Lund–MacKay scores were 18.07 ± 3.24 points in biofilm-positive patients and 10.18 ± 2.48 points the biofilm-negative patients, and the difference was statistically significant ($P < 0.05$).

The preoperative nasal Lund–Kennedy endoscopic scores were 5.83 ± 3.16 points in the biofilm-positive group and 5.43 ± 3.13 points in the biofilm-negative group ($P > 0.05$).

The intraoperative sinus Lund–Kennedy endoscopic scores were 5.16 ± 3.11 points in the biofilm-positive group and 3.85 ± 2.41 points in the biofilm-negative group ($P < 0.05$). With regard to follow-up findings, the time until complete resolution was significantly longer in the biofilm-positive patients (18.2 ± 5.3 weeks) than in the biofilm-negative patients (12.1 ± 4.9 weeks) ($P < 0.05$) (Fig. 2; Table 2).

Discussion

The results of this study indicated that the presence of biofilms in CRS patients was correlated with a higher

Table 1 Findings of biofilms in 33 patients using all kinds of diagnostic tools

	CRS group (<i>n</i> = 26)		Control group (<i>n</i> = 7)	
	Biofilm(+) group	Biofilm(−) group	Biofilm(+) group	Biofilm(−) group
SEM	13	13	1	6

CRS chronic rhinosinusitis, SEM scanning electron microscopy

Fig. 1 Scanning electron microscopy. The pictures show normal nasal mucosa without biofilms (**a, b**). The pictures show biofilms with bacterial colonies (white and black arrows, **c, d**). (**a, c** $\times 200$, **b, d** $\times 2,000$)

Lund–Mackay score, high intraoperative sinus Lund–Kennedy endoscopic score, and longer time until complete resolution. Many studies have indicated that biofilms play important roles in the pathogenesis of CRS and affect the outcomes of endoscopic sinus surgery [9–14].

Many authors reported that CRS with nasal polyps (CRScNP) and CRS without nasal polyps (CRSwNP) are two different disease entities. Eosinophilic inflammation is known to be dominant in CRSwNP, while neutrophilic inflammation is dominant in CRScNP [15, 16]. However, many studies have suggested that biofilm formation is not related to the presence of nasal polyps [17, 18]. Biofilms are known to be affected by Th1 infection. Therefore, the biofilm may not influence the development of nasal polyps. In this study, we did not separate subjects according to the presence of nasal polyps.

The prevalence of biofilms in CRS patients has been reported to range from 28.6 to 60 % [19, 20]. Cryer first detected biofilms in CRS patients by SEM [21]. They defined the tower-shaped formations comprised of aggregates of matrix-encased bacteria as the morphological hallmark of biofilms. Ramadan et al. [22] also reported evidence of biofilms in specimens obtained from all of five

patients by SEM and transmission electron microscopy (TEM). The morphological criteria for biofilms by SEM are water channels, 3-D structure, and matrix-embedded spherical or elliptical bodies within the bacteria ranging from 0.05 to 5.0 μm . Ferguson reported a biofilm detection rate of 50 % by TEM [23]. SEM can detect the 3-D structure, glycocalyx, and water channels of biofilms, while TEM can detect evidence of bacteria [24]. In contrast to electron microscopy, Confocal Scanning Laser Microscope (CSLM) better preserves the structure of a biofilm. The matrix can be detected using the double-staining technique in combination with CSLM, which allows simultaneous imaging of the structural elements of a mucosal biofilm [25]. There is as yet no standard method for detecting biofilms, and so biofilm detection rates vary according to the detection method used.

In this study, biofilms were also found in one (14.3 %) of the seven patients in the control group, suggesting that biofilms may be normal bacterial colonies. Some investigators also found biofilms on the nasal mucosae of normal controls [4]. Mladina et al. found bacterial biofilms on healthy sinus mucosa and suggested that bacterial biofilms could be normal respiratory mucosal blankets containing a

Fig. 2 Nasal endoscopic findings of CRS patients with biofilm. The pictures show mucosal swelling of the maxillary sinus with gelatinous yellowish discharge

Table 2 Characteristics of chronic rhinosinusitis patients according to the presence of biofilms

	Biofilm(+) group (n = 13)	Biofilm(−) group (n = 13)	p value
Age (mean ± SD)	42.2 ± 22.3	40.1 ± 20.4	
Gender (M:F)	8:5	7:6	
Symptom score (mean ± SD)	36.5 ± 5.88	34.78 ± 5.82	0.330
Lund–Mckay score (mean ± SD)	18.07 ± 3.24	10.18 ± 2.48	<0.001
Preoperative nasal Lund–Kennedy endoscopic score (mean ± SD)	5.83 ± 3.16	5.43 ± 3.13	0.250
Intraoperative sinus Lund–Kennedy endoscopic score (mean ± SD)	5.16 ± 3.11	3.85 ± 2.41	<0.05
Complete resolution (week, mean ± SD)	18.2 ± 5.3	12.1 ± 4.9	<0.05

large number of bacteria and part of the mucociliary system [26]. The role of biofilm in chronic sinusitis is not yet known. It remains unclear under what conditions the bacteria are extruded out through the tight mucopolysaccharide membrane of the biofilm, and whether the bacteria released from the biofilm can induce inflammation of the sinus mucosa. The floating bacteria act as the pathogens of sinusitis. However, many studies have indicated that the floating bacteria show no correlation with biofilm bacteria. As *Staphylococcus aureus* is present in the normal flora of the nasal cavity, biofilms may represent one type of normal nasal cavity flora. Therefore, it is important to determine the conditions and mechanisms by which biofilm attachment is activated in the sinus mucosa.

In this study, the Lund–MacKay score was significantly higher in biofilm-positive than biofilm-negative patients. These results are consistent with previous reports [10, 11]. However, the CT findings do not guarantee predicting the presence of biofilm. In addition to the preoperative nasal Lund–Kennedy endoscopic score, the intraoperative mucosal findings of the maxillary sinus were related to the presence of biofilms. The sinus Lund–Kennedy endoscopic score was significantly higher in the biofilm-positive group than the biofilm-negative group. This result is consistent with that reported by Psaltis et al. [10] This may be due to the sustained inflammatory process caused by the presence of biofilms. Most previous studies were conducted in ethmoid sinus mucosa. However, we used the maxillary sinus mucosa, which

shows the greatest incidence of recurrent and persistent sinusitis after ESS. Endoscopic findings in the maxillary sinus mucosa are usually poorer than those in the ethmoid and frontal sinus after surgery. The reason for the poor prognosis of the maxillary sinus is not yet known. We postulated that the structural antigavity effect may result in persistence of inflammation due to the presence of discharge. Therefore, biofilms may form more easily in the maxillary sinus.

In this study, the time until complete resolution was investigated based on endoscopic findings. The results suggested that the inflammatory process is of longer duration when biofilms are present. Similar to our study, Hochstim et al. [27] reported that the presence of bacterial biofilms is strongly associated with persistent mucosal inflammation after endoscopic sinus surgery. Some authors reported that the Lund–Kennedy scores 6 and 12 months after surgery were significantly higher in biofilm-positive patients than in biofilm-negative patients. They also reported that bacterial biofilms affect prognosis [11, 28]. Some authors reported poorer postoperative CT scores, postoperative symptoms, mucosal outcome, extra postoperative visits, and multiple antibiotic therapy in biofilm-positive patients [10, 13].

Bacterial biofilms are difficult to remove. CRS patients with bacterial biofilms often show failure of medical or surgical intervention. Biofilms are composed of bacteria within expolymeric matrix, and so can avoid the host defense system and exhibit reduced susceptibility to

antibiotics [29]. The penetration of antibiotics into biofilm is significantly reduced, and bacteria in the biofilm are exposed to the drug in gradually increasing doses. This gradual exposure can result in stress-induced metabolic or transcriptional changes in the bacteria and increased resistance to antibiotic therapy [30]. Some authors have reported multiple microbes in biofilms, and different biofilm species are associated with differences in disease severity and postoperative results [31, 32]. Many different treatments for biofilm-positive mucosa, such as use of baby shampoo, manuka honey, and mechanical debridement, have been reported [33, 34]. In chronic recurrent and persistent sinusitis, the bacteria protruding from the biofilm matrix may be responsible for the persistence of inflammation. However, whether the bacteria that are continuously extruded from the biofilm matrix or previous floating bacteria are responsible for resistance to treatment is not known. Although endoscopic sinus surgery cannot eliminate the biofilm completely, it may reduce their prevalence [35]. Host factors, viral infection, or inadequate removal of mucosa may influence the reactivation of biofilm in the sinus mucosa. Another possible treatment for biofilm is low-dose macrolides. Low-dose clarithromycin therapy has been shown to alter the structure of biofilms [36]. Further studies are needed to develop methods for treatment that block the information pathway between bacteria and gene transcription, which controls the attachment and formation of biofilms. In addition, further research is required to determine the factors that affect biofilm formation and to identify the host factors associated with chronic sinusitis, such as cytokines or chemokines.

Conclusion

Our results suggest that bacterial biofilms on the sinus mucosa of CRS patients may be related to higher Lund–Mackay scores, more severe intraoperative mucosal findings, and prolonged healing time. Further studies are needed to ascertain the clinical factors influenced by biofilms in CRS patients and to identify the factors that affect the formation and treatment of biofilms.

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