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Identification of adenoid biofilms in chronic rhinosinusitis

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Summary

Objective: To compare the percent mucosal surface area of adenoids removed from children with chronic rhinosinusitis (CRS) and those with obstructive sleep apnea (OSA) infected with biofilms.
Design: Comparative micro-anatomic investigation of adenoid mucosa using scanning electron microscopy from patients with CRS and OSA.
Subjects: 4 females and 12 males ranging from 3 months to 10 years of age.
Results: Adenoids removed from patients with CRS had dense mature biofilms covering the mucosal surface. More specifically, adenoids removed from patients with CRS had an average of 94.9% of their mucosal surface covered with mature biofilms vs. an average of 1.9% coverage on the adenoids removed from patients with OSA. These differences were statistically significant at the $p < 0.001$ level.
Conclusions: It is well established that adenoidectomy is useful in the treatment of CRS resistant to antibiotics. Adenoids removed from patients with CRS had almost their entire mucosal surface covered with biofilms vs. scant coverage for patients with OSA ($p < 0.001$). Decreased metabolic activity, decreased growth rate, and transmission of resistance genes all contribute to the antibiotic resistant nature of the biofilms. These metabolically sessile communities shed planktonic microorganisms on an intermittent basis. Therefore biofilms in the nasopharynx of children with CRS may act as a chronic reservoir for bacterial pathogens resistant to standard antibiotics. Also, the mechanical debridement of the nasopharyngeal biofilms may explain the observed clinical benefit associated with adenoidectomy in this subset of pediatric patients.

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1. Introduction

Chronic rhinosinusitis (CRS) in the pediatric population is a complex disease with a considerable impact upon the United States economy. Most otolaryngologists agree that the cornerstone of treating CRS is a prolonged course of a broad-spectrum, beta lactamase stable oral antibiotic. Although the direct cost of pediatric chronic sinusitis is unknown, it is estimated that more than $2 billion is spent annually on the purchase of over-the-counter medications for the treatment of CRS [1]. When combined with the cost of antibiotic therapy, the public health impact is enormous.

Chronic sinusitis is defined as an infectious process of the paranasal sinuses lasting for longer than 3 months. Nasal discharge is most often purulent, but may be minimal or absent in CRS. Throat clearing may be more prominent secondary to discharge from the posterior ethmoid sinuses. A cough may be present in the daytime and worsen at night. The child may suffer from sleep impairment, poor appetite and poor school performance.

The pathogens observed in CRS are not the same well defined set of organisms which cause acute sinusitis. Studies have shown that anaerobes are cultured from the paranasal sinuses at rates ranging from 2% to 100% [2]. Overall, bacteriology is difficult to ascertain in CRS as many organisms are recovered in low densities. As no concrete subset of organisms exists to target with oral antibiotics, CRS is a difficult disease to treat. Although broad spectrum, beta lactamase stable oral antibiotics are often used to target pathogens, CRS typically does not respond with permanent or sustained improvement to antibiotic therapy. Due to the wide variety of both aerobic and anaerobic organisms cultured from the paranasal sinuses, there is no current consensus as to treatment length, organism coverage, or antibiotics that are most effective [3]. It is generally believed that high dose antibiotics should be given for a minimum of 3 weeks in the treatment of CRS.

Alternatives to oral antibiotic therapy include functional endoscopic sinus surgery (FESS) and adenoidectomies. Although pediatric FESS is widely used for the treatment of refractory CRS, with success rates ranging from 80% to 93% [4], concerns exist over possible interference with facial skeletal growth and further scarring of the drainage pathways [5—7]. Recent literature suggests that adenoidectomies may be beneficial to patients with a diagnosis of CRS. In a study by Vandenberg and Heatley, 58% of patients demonstrated a near or complete resolution of the symptoms of CRS [8]. The adenoidectomy both eliminates nasal airway obstruction, and removes a nidus for chronic bacterial infection.

The presence of bacterial biofilms has been identified in our lab on adenoidal tissue removed from children with a diagnosis of recurrent acute otitis media (RAOM) [9]. Outside the human body, biofilms are found on mineral surfaces, living and dead plant or animal matter, polymers, ceramics and metal alloys. The process of biofilm formation occurs when individual cells adhere and coalesce to various surfaces. Exopolysaccharides (EPS) are produced, resulting in an EPS matrix which forms much of the volume of a biofilm community. Microchannels form through the hydrated EPS matrix, resulting in connections between microbes and periodic shedding of planktonic cells [10,11]. The individual planktonic cells may then multiply, disperse and infect the host.

Scanning electron microscopy was used to identify the biofilms in this study and in previous studies by our laboratory. Using techniques gathered from previous authors and from the industrial waste industry, it was easy to distinguish those surfaces coated with biofilms from those of a barren adenoid mucosa. Individual planktonic cocci and bacilli could be observed in the background EPS matrix with these techniques.

We feel that the identification of adenoid biofilms in patients with chronic rhinosinusitis might provide new information regarding the pathogenesis of CRS. This article reflects qualitative data gathered from the adenoids removed from children with CRS. The patients chosen for this study only represent an initial sampling of pediatric patients. As such, this paper is intended only as a descriptive study and is not intended to be statistical in nature.

2. Materials and methods

2.1. Sample collection

IRB approval for the study was obtained from Wayne State University. All samples were obtained from adenoidectomies performed for chronic rhinosinusitis by the authors at Children's Hospital of Michigan, St. Joseph Mercy's Hospital and Lahser Ambulatory Surgery Center. In some cases of OSA, both the adenoids and tonsils were removed. The ages of the patients ranged from 3 months to 10 years. There were 4 male patients and 12 female patients. Seven samples were collected from patients with chronic rhinosinusitis as their indication for surgery. Chronic rhinosinusitis was defined and documented in the medical chart as duration of sinonasal symptoms of 12 weeks duration with a failure to respond to a minimum 4-week course of an oral beta-lactamase stable antibiotic. Those subjects chosen as OSA controls had documented ade-

noid hypertrophy, with or without tonsillar hypertrophy. These subjects also were without an episode of antibiotic treatment pharyngitis/tonsillitis in the 6 months prior to adenoidectomy.

2.2. SEM preparation and fixation

All samples were prepared for scanning electron microscopy utilizing the following methodology. Tissue was initially fixed for 1 h in 2.5% glutaraldehyde in 0.1 M Sorensen's phosphate buffer (pH 7.4). Two rinses of 10 min each were then carried out using 0.1 M Sorensen's buffer. Next, the samples were treated with 1% osmium tetroxide for 1 h. After this, the tissue was dehydrated with successively greater concentrations of ethanol as follows: 30% for 15 min, 50% for 15 min, 70% for 15 min, 90% for 15 min, 100% for 15 min (this last dehydration step was repeated). Finally, the tissue was washed with HMDS (hexamethyldisilavane, Electron Microscopy Sciences) four times for 15 min. A few drops of HMDS were then placed on the samples and they were left to dry overnight in a hood. Samples were then mounted and gold sputter coated in final preparation for imaging.

2.3. SEM Imaging

Imaging was done at our SEM laboratory using an AMRY 1000B scanning electron microscope. The senior author was present at all imaging sessions. Biofilm architecture consistent with the extant literature was easily distinguishable from the barren surface of regions devoid of biofilms. We utilized the definition of Chole and Faddis for biofilm architecture—that of dense accumulations of bacteria within an amorphous matrix [12,13]. Although the previous study used transmission electron microscopy, we compared our SEM images to existing SEM biofilm images from other work and found unity [14]. Examination of the SEM images demonstrated uniform biofilm architecture comprising most of the adenoid surface. Although the appearance of a mucopus-covered adenoid versus a biofilm-covered adenoid has not been delineated in the literature, none of our samples had mucopurulent drainage at the time of surgery.

Furthermore, defining biofilm SEM appearance strictly as the presence of microcolonies embedded in a microfilament EPS matrix assists in this distinction in the present study. Images revealed varying morphology of bacteria including rods and cocci; similar to the work of Chole et al. These bacteria were embedded in a uniform, amorphous background substance that persisted despite solvent fixation and processing [12,13].

Table 1 Patient demographics, diagnosis, and presence or absence of biofilms

Age/gender	Diagnosis	Presence of biofilms
2 years, M	CRS	+
2 years, M	CRS	+
2 years, M	CRS	+
6 years, M	CRS	+
3 years, F	CRS	+
3 years, F	CRS	+
10 years, M	CRS	+
9 months, M	OSA	–
13 months, M	OSA	–
16 months, M	OSA	–
5 years, M	OSA	–
3 years, F	OSA	–
4 years, M	OSA	–
5 years, F	OSA	–
5 years, M	OSA	–
3 months, M	OSA	–

3. Results

Table 1 displays patient demographics and diagnoses along with their respective presence of biofilms. The presence of biofilms is defined as a dense covering of nearly the entire mucosal surface of the adenoid with the cellular microarchitecture previously mentioned. The absence of biofilms was defined as no discernable accumulations of bacteria in an amorphous matrix on the adenoid.

Adenoids removed from patients with OSA served as controls in this study. Fig. 1 is a high power (2000×) SEM micrograph of an adenoid surface comprising dense biofilm architecture. Fig. 2 (1500×) is a high power image of a barren adenoid surface from a patient with obstructive sleep apnea lacking biofilms.

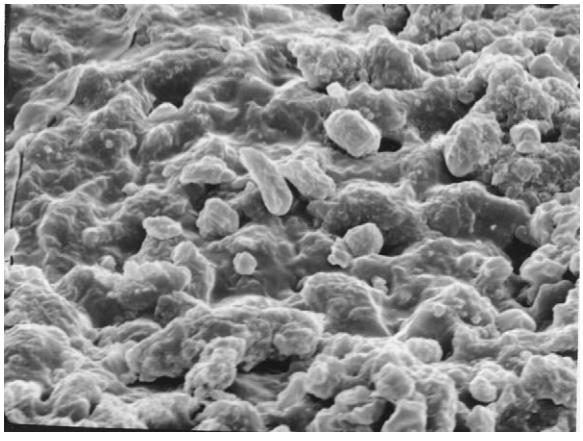

Fig. 1 High power (2000×) SEM image of biofilm architecture highlighting dense spherical colonies embedded in EPS matrix.

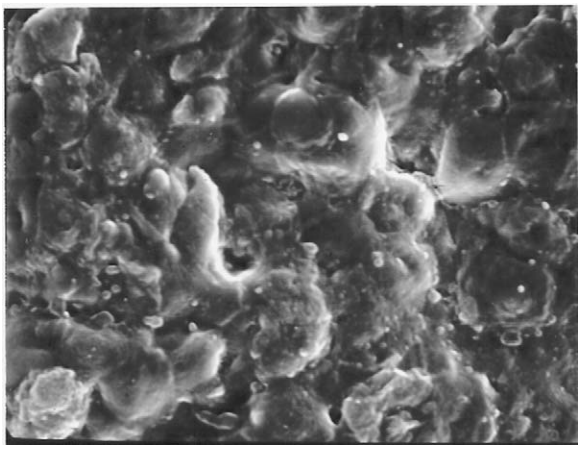

Fig. 2 High power (2000×) SEM image of adenoid mucosa devoid of biofilm architecture taken from an OSA patient.

4. Discussion

Biofilms have a profound impact on human pathogenesis. For example, *Streptococci* in dental plaque biofilms are associated with cavity formation and periodontal disease. If given access to the bloodstream, the organisms may weak havoc on a variety of indwelling medical devices such as catheters, synthetic joints, artificial heart valves and pacemakers. Once an infection is present, it usually persists until the biofilm-colonized surface is removed from the body. The EPS matrix affords the pathogens a form of ''identity protection'' from host mechanisms of opsonization and phagocytosis. The biofilms themselves have been shown to result in a state of chronic inflammation causing collateral damage to tissue not involved in the microbial infection [10,11,15]. Although biofilm infections are rarely fatal and are often traced to bacteria ubiquitous in water, air, soil or skin, they certainly can compromise quality of life.

Biofilms are characterized by decreased metabolic activity, decreased growth rate, and expression and transmission of resistance genes [16]. When planktonic organisms are shed from the biofilm, antibiotics may temporarily reverse the symptoms. However, recurrence is inevitable due to the large reservoir of bacteria. It is estimated that greater than 60% of all microbial infections are caused by biofilms [16].

One possible explanation of antibiotic resistance is incomplete penetration of the antimicrobials into the biofilm due to their inactivation during the diffusion process. Studies also describe altered chemical microenvironments within biofilms. Local accumulation of acidic waste products may interfere with the activity of antibiotics. Furthermore, this change in microenvironment may alter the growth patterns of the bacteria themselves, lending credence to the theory that bacteria form a spore-like state. Resistance has been shown to exist in newly formed biofilms too thin to pose a barrier to the penetration of antibiotic. This idea is also supported by the fact that most bacteria in biofilms are killed by antimicrobials and the surviving population may consist of less than 1% of the original colony [15].

Adenoidectomy has been shown to be an effective strategy in the management of CRS. It is interesting to note that the benefit of adenoidectomy seems unrelated to the size of the adenoid [14,17]. Therefore, it seems that the most likely benefit of adenoidectomy is the elimination of a nasopharyngeal source for re-infection of the sinus mucosa. A study conducted in our lab by Adappa and Coticchia [18] examined clinical improvements in pediatric patients undergoing concurrent adenoidectomy as well as a prolonged course of IV antibiotic therapy for CRS. Long term follow up (defined as greater than 6 months from the conclusion of IV therapy) revealed 91% of patients to have complete amelioration of clinical symptoms of CRS. This study offers further support for the treatment of CRS to be aimed at multiple bacterial sources.

We identified mature confluent biofilms covering almost the entire mucosal surface of adenoids removed from patients with CRS. The unique morphology of mucosal biofilms has previously been identified in both tonsillar tissue and animal models of acute otitis media [13,19]. In contrast, adenoids removed from patients with OSA had no obvious biofilm formation and relatively bland mucosal surfaces. A review of the morphological studies on normal adenoid tissue was consistent with our control images [20—23].

Previous investigators have demonstrated that microorganisms that exist in biofilms have unique antimicrobial resistance patterns [15,24—26]. This mechanism may allow sinus pathogens to persist in the nasopharynx of children with CRS despite the frequent antibiotic courses administered. Other investigators have demonstrated that biofilms periodically shed planktonic organisms [10,11]. Shedding of sinus pathogens from established biofilms in sinusitis prone children may be an important mechanism in the development of CRS.

5. Conclusion

Our identification of dense biofilm structures on adenoids of children with CRS suggests that biofilm production may be a virulence factor for organisms that are responsible for the development of CRS.

This is an important step in supporting the notion of an adenoidectomy's role in mechanical debridement of sinus pathogens. The differences found on SEM examination are extremely interesting. Additional investigation comparing surface density analysis of adenoids removed for OSA and CRS is currently underway in our lab.

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