Biofilm Surface Area in the Pediatric Nasopharynx

Chronic Rhinosinusitis vs Obstructive Sleep Apnea

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Objective: To compare the percentage of mucosal surface area of adenoids infected with biofilms removed from children with chronic rhinosinusitis (CRS) vs children with obstructive sleep apnea (OSA).

Design: Comparative microanatomical investigation of adenoid mucosa from patients with CRS and OSA using scanning electron microscopy.

Setting: University-affiliated hospitals and ambulatory surgery center.

Patients: Four girls and 12 boys ranging in age from 3 months to 10 years.

Main Outcome Measure: Measurements of biofilm coverage of the entire adenoidal surface.

Results: Adenoids removed from patients with CRS had dense mature biofilms covering the mucosal surface; they had a mean of 94.9% of their mucosal surface covered with mature biofilms, compared with a mean of 1.9% coverage on the adenoids removed from patients with OSA. This difference was statistically significant at \( P < 0.001 \).

Conclusions: Adenoids removed from patients with CRS had almost their entire mucosal surface covered with biofilms vs scant coverage for patients with OSA. Biofilms in the nasopharynx of children with CRS may act as a chronic reservoir for bacterial pathogens resistant to standard antibiotics. 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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CHRONIC RHINOSINUSITIS (CRS) in the pediatric population is a complex disease with a considerable effect on the US economy. The health care expenditure in the United States in 1996 for rhinosinusitis in the pediatric population was estimated to be $1.8 billion.1 Most otolaryngologists agree that the cornerstone of treating CRS is a prolonged course of a broad-spectrum \( \beta \)-lactamase stable oral antibiotic in an attempt to cover the varied pathogens that may be present in the sinuses. These are not the same well-defined set of organisms that may exist in acute sinusitis. Anaerobes are cultured from the paranasal sinuses of children diagnosed as having CRS at rates ranging from 2% to 100%.2

Alternatives to the standard course of oral antibiotic therapy include adenoidectomy, functional endoscopic sinus surgery, and long-term intravenous antibiotic therapy. Adappa and Coticchia3 achieved a 91% long-term success rate in treating 22 patients with CRS during a mean of 5 weeks of intravenous antibiotic therapy with concurrent adenoidectomy. Recent findings suggest that adenoidectomy by itself may provide benefits for patients with CRS. In a study by Vandenberg and Heatley,4 58% of children demonstrated near or complete symptom resolution of CRS after adenoidectomy. These authors suggest that adenoidectomy improves sinonasal symptoms by eliminating airway obstruction and secretion stasis, as well as by removing a nidus for chronic bacterial infection.

Biofilms are increasingly associated with chronic infectious processes. Using a simple light microscope, Antonie van Leeuwenhoek (1632-1723) was the first to describe the concept of microorganisms bound to a surface in the form of dental plaque.5 In the 1970s, there was a re-emergence of the notion of the bacterial biofilm when Characklis6 studied microbial slime in industrial water systems and noted its resistance to disinfectants. A transition in thinking has begun in that bacteria are no longer thought to exist singularly as mere planktonic organisms but rather as well-organized ecosystems even within a human host. These complex eco-
systems are well suited for conditions of environmental stress such as crowding and altered oxygen tension. It is believed that, at any given time, 99% of bacteria exist in the form of a hydrated matrix of polysaccharides and protein slime known as a biofilm. According to a recent public announcement from the National Institutes of Health, more than 60% of human bacterial infections involve biofilms. Different nosocomial infections related to the use of urinary catheters, orthopedic devices, prosthetic heart valves, and central venous catheters are associated with the adherence of biofilms to a surface. Infectious processes attributed to bacterial biofilms are increasingly difficult to treat secondary to resistance to antimicrobial therapy, and in the case of artificial heart valves and indwelling catheters, removal is the only means of eradication.

Biofilms are difficult to eradicate, and their formation on adenoid surfaces provides a mechanism for persistent infection seen in CRS. Biofilms form when individual planktonic bacteria coalesce and adhere to various surfaces via glycoconjugate moieties. The life cycle of biofilms can be divided into 3 parts, namely, attachment, growth, and detachment. During the attachment phase, the substrate must be adequate to be adsorbed for the bacteria to irreversibly attach to the surface. As the cells divide, an exopolysaccharide matrix is formed. The biofilm then begins to form towers and water channels through the matrix. These channels aid in the elimination of waste and contribute to a pH gradient within the matrix. Finally, the individual bacteria begin to shed from the biofilm to colonize another surface. The oxygen tension gradient present in the biofilm allows for increased metabolic activity in superficial layers of the matrix while the underlying layers persist in a quiescent phase. Chemical signaling, known as quorum sensing, allows for cell-cell communication within the biofilm. Antibiotics may temporarily reverse symptoms caused by shedding of planktonic bacteria; however, unless the colonized surface is also removed, the infection will recur.

To demonstrate a new paradigm in the pathogenesis of CRS, our laboratory set out to quantitatively describe the anatomical microstructure of adenoids removed from children with CRS vs children with obstructive sleep apnea (OSA). Because microscopy is the only technique whereby bacterial biofilms can be studied at the single-cell level, scanning electron microscopy (SEM) was used to detect and help quantify biofilm architecture. Despite the use of standard measures in attempting to eradicate CRS from our pediatric population, we encounter many treatment failures. Therefore, we contend that biofilm formation might play an important role in the pathogenesis of this disease.

**METHODS**

**SAMPLE COLLECTION**

Institutional review board approval for the study was obtained from Wayne State University, Detroit, Mich. All samples were obtained from adenoidectomies performed by us for CRS and OSA at Children’s Hospital of Michigan (Detroit), St Joseph Mercy Hospital (Pontiac, Mich), and Lahser Ambulatory Surgery Center (Southfield, Mich). In some patients with OSA, the adenoids and tonsils were removed. The ages of the patients ranged from 3 months to 10 years. Sixteen samples were collected from 4 girls and 12 boys. Chronic rhinosinusitis was defined and documented in the medical record as an infectious process of the paranasal sinuses lasting longer than 3 months. All patients diagnosed as having CRS had failed a prolonged course of oral antibiotics lasting at least 4 weeks.

**SCANNING ELECTRON MICROSCOPY**

**Preparation and Fixation**

All samples were prepared for SEM using the following techniques. Tissue was initially fixed for 1 hour in 2.5% glutaraldehyde in 0.1M Sorensen phosphate buffer (pH 7.4). Two rinses of 10 minutes each were then carried out using 0.1M Sorensen phosphate buffer. Next, the samples were treated with 1% osmium tetroxide for 1 hour. After this, the tissue was dehydrated with successively greater concentrations of ethanol as follows: 30% for 15 minutes, 50% for 15 minutes, 70% for 15 minutes, 90% for 15 minutes, and 100% for 15 minutes (this last dehydration step was repeated). Finally, the tissue was washed with hexamethyldisilane (Electron Microscopy Sciences, Hatfield, Pa) 4 times for 15 minutes. A few drops of hexamethyldisilane 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.

**Imaging**

Imaging was done at our SEM laboratory using a microscope (JSM-6400; JEOL Ltd, Tokyo, Japan) (R.B. 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.

**IMAGE ANALYSIS**

The SEM images were analyzed using Carnoy version 2.0 software (http://www.kuleuven.ac.be/bio/sys/carnoy). The software was used to obtain measurements of biofilm coverage of the entire adenoidal surface.

**RESULTS**

**Figure 1** shows and low-power and high-power SEM images of adenoid tissue removed from a patient with documented CRS. In contrast, **Figure 2** shows low-power and high-power SEM micrographs of an adenoid surface devoid of biofilm architecture. This adenoid sample was removed from a patient with documented OSA. Carnoy software quantified the biofilm surface area of each sample imaged using SEM. The **Table** gives these surface areas and the patient demographics and diagnoses.

Adenoids removed from patients with CRS had a mean of 94.9% of their mucosal surface covered with mature biofilms, compared with a mean of 1.9% coverage on the adenoids removed from patients with OSA. This difference was statistically significant at P < .001. Statistics were obtained using nonparametric Mann-Whitney comparisons between the 2 experimental groups.
Pediatric CRS is a disease that is resistant to standard antimicrobial therapy. However, adenoidectomy has been shown to have some efficacy in ameliorating the symptoms of CRS. Possible theories for its success include relieving a source of nasopharyngeal obstruction and removing a nidus for chronic bacterial infection. In studies of otitis media, the benefit of adenoidectomy seems to be unrelated to the size of the adenoid. Hence, the more likely explanation for the effectiveness of adenoidectomy in treating CRS is the elimination of a nasopharyngeal source for reinfection of paranasal sinus mucosa.

Ramadan et al first provided direct evidence of biofilm formation in paranasal sinus mucosa of patients with CRS. Tissue samples were removed from the ethmoid bulla of 5 adult patients undergoing functional endoscopic sinus surgery and were imaged using SEM. All 5 patients showed evidence of biofilms resembling those of *Staphylococcus* species. No control subjects were included in this study.

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The demonstration of resistant biofilm infections in the nasopharynx and paranasal sinuses of children with CRS allows us to explain some well-documented clinical observations previously not understood. In classic teaching, it was believed that the pathogenic bacteria seen in CRS needed to adhere to the nasopharynx, enter the sinus ostia, and then replicate to establish infection. We propose another sequence of events given the recent evidence of biofilms in the nasopharynx and paranasal sinuses. We suspect that the first step in this process is entry of a known sinusitis pathogen into the nasopharynx. Colonization and establishment of the biofilm microenvironment follow. Once established, the biofilm is able to shed planktonic organisms. Therefore, a cycle of re-entry and reinfection of nasopharyngeal pathogens into the sinuses creates the clinical entity known as CRS. Figure 3 shows this paradigm.

Using the biofilm model, we may gain new insight into the pathogenesis and potential beneficial treatments for pediatric CRS. The first step in this process would be inflammation of the nasopharynx. This could be caused by nasal allergy, gastroesophageal reflux disease, or viral up-
The clinical symptoms of such a biofilm infection often bring the patient to medical attention. In the case of chronic infections, such as CRS, the normal sinus anatomy and physiology is expanding our data set in both of the experimental groups that error in processing is expected to be negligible.

Newer techniques, such as confocal laser scanning microscopy with or without fluorescent in situ hybridization, have the advantage of visualizing 3-dimensional structures of fully hydrated specimens noninvasively, in situ and in vivo, in a time-resolved manner. These methods allow for further elucidation of the structure-function relationships in biofilms. Our laboratory is applying these methods to our investigation of middle ear biofilms, and we plan to expand these techniques to our investigation of pediatric CRS. Despite many methods used, we were unable to find any studies in the literature comparing the sensitivity and specificity of the spectroscopic techniques used to detect human host biofilms.

In addition to the aforementioned projects, our laboratory is expanding our data set in both of the experimental groups described herein. Computed tomographic scans are being obtained in patients diagnosed as having CRS. Such information would strengthen our clinical diagnosis of CRS over other infectious or inflammatory disorders such as adenoiditis. We are also cultivating and performing polymerase chain reaction on sinus aspirates and adenoid tissue. Based on our hypothesis,
we would expect to find the same set of pathogens in both anatomical locations.

To our knowledge, our study is the first to identify biofilm architecture on the surface of adenoids in direct association pediatric CRS. This is a crucial first step in supporting the notion of the role of adenoidectomy in mechanical debridement of potential pathogens that re-infect the paranasal sinuses. Although our sample is small, the differences found on SEM examination are notable.

CONCLUSIONS

The difficulty in treating pediatric CRS has long been established. Repeated courses of oral and parenteral antibiotic therapy provide short-term improvement in alleviating the symptoms of CRS. Many physicians and patients have been discouraged to see the same infectious processes recur several times a year. The presence of biofilms in the pediatric nasopharynx may explain why so many apparently successful interventions ultimately fail. We propose a new paradigm in the pathogenesis of CRS that may explain the benefit seen with mechanical debridement of adenoid tissue in the pediatric nasopharynx.

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Author Contributions: Drs Coticchia, Zuliani, Carron, Haupert, and Berk, Ms Coleman, and Mr Gurrola had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Coticchia, Zuliani, and Berk. Acquisition of data: Coticchia and Haupert. Analysis and interpretation of data: Coleman, Gurrola, Zuliani, and Carron. Drafting of the manuscript: Zuliani. Critical revision of the manuscript for important intellectual content: Coticchia, Coleman, Gurrola, Haupert, and Berk. Obtained funding: Coticchia. Administrative, technical, and material support: Gurrola and Berk. Study supervision: Coticchia, Carron, and Berk.

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REFERENCES

Notice of Duplicate Publication: “Biofilm Surface Area in the Pediatric Nasopharynx: Chronic Rhinosinusitis vs Obstructive Sleep Apnea” (Arch Otolaryngol Head Neck Surg. 2007;133[2]:110-114)

It was brought to the attention of the editor of the Archives of Otolaryngology–Head & Neck Surgery that there were numerous similarities between the article by Coticchia et al1 that was published in the February 2007 issue of the Archives of Otolaryngology–Head & Neck Surgery and an article published in the September 2006 issue of the International Journal of Pediatric Otorhinolaryngology,2 authored by the same group. After an extensive review of both articles by the editors of both journals as well as by an independent reviewer, we have come to the conclusion that these 2 manuscripts represent a duplication of publication. Because of this, the Archives of Otolaryngology–Head & Neck Surgery will not consider any submission from Drs Coticchia, Haupert, and Berk, Ms Coleman, or Mr Gurrola for a period of 1 year from the publication of this notification. The response from the senior author can be found herein.


In reply

As one of the authors of the article referenced in the Notice of Duplicate Publication, I would like to take this opportunity to provide an explanation about the reported duplicate submission discussed herein.

Any reported duplicate submission that occurred was an oversight and by no means intentional. During the revision process of the original manuscript submission to the International Journal of Pediatric Otorhinolaryngology, some of the content was added and/or deleted. These additions and/or deletions altered the content of the original manuscript sent to the International Journal of Pediatric Otorhinolaryngology.

I apologize for any problems that these errors may have created. I have subsequently made significant changes regarding how my coauthors and I revise and edit our manuscripts.

Thank you for your consideration.

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