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The Role of Biofilm Infections in Pediatric ENT Patients

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Introduction
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All knowledge in medical fields is based on basic sciences: anatomy, microbiology, physiology, pathology. We try to use this knowledge in our daily surgical practice. In all health-disease processes, especially when there are infections, theory used to be based on planktonic bacteria, bacterial forms that "fluctuate" freely in bodily liquids (blood, cerebrospinal fluid). Unfortunately, we are seeing more and more cases become chronic and require, many times, invasive procedures, frequently with poor results.

New advances have clarified many of these concerns. Thanks to studies such as those of Dr. Post, advances in cell- and molecular biology, laboratory techniques, tissue cultures, and even confocal microscopy and genetic markers, we have made not only progress but integration among these areas that has enabled us to gather more information and to know and recognize biofilms. Remember that a biofilm is not the same thing as a disease. There are beneficial biofilms. They are not formed exclusively by bacteria. There can be gram-positive bacteria, gram-negative bacteria, anaerobic bacteria, and even fungi. Biofilms have been studied now for a number of years by researchers in marine biology, environmental engineering, food engineering, pharmacology, and dentistry. Fortunately, in the last few years many studies have also been conducted in medical areas, including pediatric ENT.

For those who have not had contact with this subject of biofilms, it is important to remember that biofilms are nothing more than one of the ways bacteria exist. The biofilm is a complex and structured microbial community characterized by cells attached to a live or inert interface and to one another, and that communicate. They are surrounded by a extracellular polymeric matrix that creates a barrier, and they exhibit a different phenotype from that of free forms (the planktonic forms I mentioned) especially in their rate of growth and genetic transcription. So they form "cities" with a polymeric "wall," and they have channels that enable access to oxygen and nutrients, and other channels to release residues.

Biofilms follow a formative process—here divided to aid understanding. The bacteria must have a propensity to form this kind of structure. They send out a

signal calling other organisms (*quorum sensum*), and there is a process of growth, maturation, and disaggregation, a dynamic process occurring simultaneously. This consideration is naturally only didactic. The organization creates complexes of physiological cooperation that provide a more stable base for growth, enabling survival in hostile environments, requiring less oxygen and nutrients and, at the same time, increasing resistance to antimicrobials and the immune system of the host. Having these properties, they end up having specific characteristics, especially in cultures with negative results. Sensitivity to detect these organisms has improved with PCR, with genetics. Organisms in biofilms have a poor response to antimicrobials, and there is potential for "metastasis." The shape and composition of these biofilms depend on the surface and the environment (site of adhesion)—whether it is necrotic tissue, a ventilation tube, a laminar blood-flow or a turbulent blood-flow at the site.

The Center for Disease Control and Prevention (CDC) estimates that 65% of all bacterial infections in humans originate from biofilms. We have some examples of infections: the most classical one would be bacterial plaque (dental caries), bacterial endocarditis, sepsis, pneumonias, chronic infections, those related to prostheses, pacemakers, cochlear implants, catheters, valves, ventilation tubes, and the like. Zuliani and co-workers (Zuliani G, Carron A, Gurrola J, Coleman C, Haupert M, Berk R, Coticchia J. Identification of adenoid biofilms in chronic rhinosinusitis. Int J Pediatr Otorhinolaryngol (IJPORL), 70 (9): 1613-1617, 2006) used a bi-laser confocal microscope to evaluate the adenoids in 16 patients with repeated rhinosinusitis and nasal obstruction. They found that 94.9% of cases with recurrent rhinosinusitis had biofilms in the adenoids. In cases of obstruction without recurrent rhinosinusitis, the incidence of biofilms was only 1.9%. Thus, adenoidectomy is useful when we approach the treatment of infections caused by biofilms, but we are restricted to palatine tonsils and adenoids. When dealing with the middle ear (otitis) and rhinosinusitis, a more aggressive approach cannot be used, we cannot remove all tissues. How should we approach these infections?

We have some expectations. In a study by Scott Manning from Seattle, we have to acknowledge that biofilms exist and are present.

What can we do to act against these biofilms?

First we inhibit the formation of bacteral adhesions: if the bacteria do not attach to tissue, the biofilm will not be formed. Manning refers to xylitol, chelating agents, biomaterials such as we have in ionized ventilation tubes (indicated in recurrent otitis media with effusion), fluoroplastics, that decrease bacterial adhesion. According to Richard Rosenfeld, we can also inhibit signaling by using competition, changing the environment in ventilation tubes. The tubes not only drain effusions from the middle ear but also change the environment, increase oxygen-tension, ventilate, improve pressure in this region. It is, however, a little more difficult to interfere in structuring of biofilms.

In our daily practice we already do a lot, even before "knowing" about these biofilms, when we scrub our hands before surgery, clean surgical materials and sterilize them. All readers, I am sure, brush their teeth, floss, and there are many mouthwash solutions containing xylitol, with enzymes that help avoid the adhesion

of *Streptococcus mutans* to enamel, remove bacterial plaque, and prevent caries. And xylitol, which is also a sugar, changes not only the adhesion of *S.mutans* but also that of *H.influenzae* and *S.pneumoniae*. In a micrograph of *S.pneumoniae* after exposure to xylitol, it practically disassembles a portion of the cell wall of the bacterium, even changing its phenotype to a less virulent one.

There are expectations that xylitol can be used to break the biofilm barrier. This conclusion comes from a study carried out in Finland. Another study still "in press," by Dr. Martin Desrosiers from Canada, developed equipment to literally "wash" the region of the paranasal cavities using a surfactant containing a calcium sequestrant that can disrupt the biofilm barrier in cases of chronic rhinosinusitis. The author used a dental jet, a hydrodynamic way to remove biofilm from the paranasal cavities. Mechanic removal is extremely important, but you can't use detergent and a brush on all parts of the human body, unfortunately. It is known that surfactant used alone can help in removing these viable bacteria, these biofilms, but the hydrodynamic factor is most important. Thus, there is an expectation that in the future we will have a treatment where this "washing" can be used in association with nasal endoscopic surgeries.

Updating is important, breaking old paradigms. I have nothing against antimicrobials, and what concerns me is not their use, but abuse, banalization. Medicine based on evidence is very important. It is important to have researchers such as those on Dr Post's team, to show us things, to clear our doubts

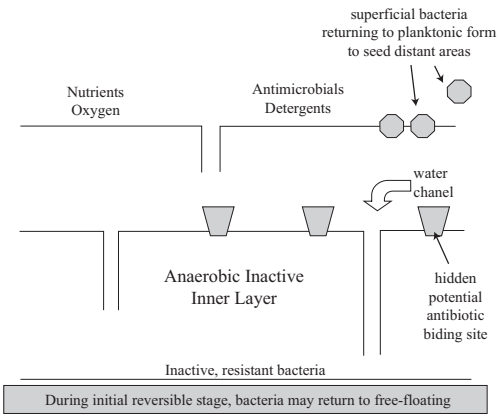
Introduction
Christopher Post

Bacterial biofilm development is increasingly recognized as a major factor in the development of pediatric ENT infections. Biofilm morphology and physiology resist antimicrobial therapy, often leading to recurrent, resistant, or chronic infections. The unique characteristics of biofilms help to explain their persistence and antibiotic resistance. Understanding the influential role of biofilms in the development and perpetuation of common pediatric ENT infections is paramount to developing strategies for both the assessment and treatment of these patients.

Bacteria can exist in two forms: free-floating planktonic bacteria and biofilms. Planktonic bacteria are independent, individual organisms, while biofilms are sophisticated communities of pathogens living within an extracellular glycocalyx matrix that creates a protective living environment for bacteria. Due to their complex nature, biofilms often contain several pathogens. A complex process converts bacteria from individual planktonic cells into a biofilm population (**Figure 1**). First, planktonic bacteria reversibly attach to an acceptable, moist surface where they divide and begin to form a colony. Later, bacterial attachment becomes irreversible as a glycocalyx matrix of water and macromolecules (including exopolysaccharides, proteins, and nucleic acids) forms a stable and protective dynamic structure for the embedded bacteria. As more bacteria aggregate into a microcolony, the production of signaling molecules increases until a threshold level is reached, activating a quorum sensing mechanism

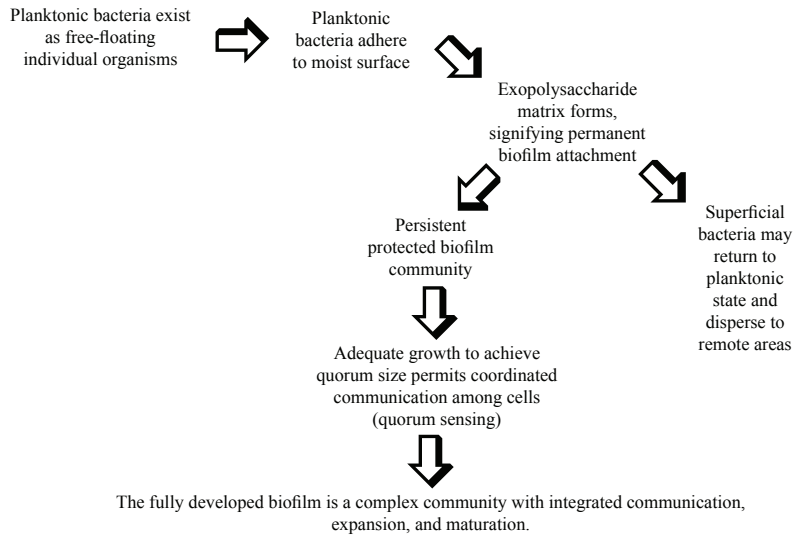
that biofilm bacteria use to coordinate their activities. Quorum sensing directs glycocalyx production, bacteria division rate, and gene regulation. The fully developed biofilm is a complex community with integrated communication, expansion, and maturation. The biofilm grows and may also seed distant areas as superficial bacteria can shed to disperse to new locations.

Figure 1. Complex development and maturation of biofilms



Variations within the biofilm structure promote pathogenic survival, with unique living environments offered within diverse layers within a single biofilm community (**Figure 2**). These varied regions make biofilms more resistant to antimicrobial therapy.

Figure 2. Biofilm layers



The most vulnerable region is the metabolically active, outer layer that is exposed to higher concentrations of oxygen, nutrients, and, potentially, antimicrobial therapy. This active, outer layer encloses less active, more anaerobic interior layers. Key antigens and ligands can become hidden within biofilm, effectively masking target sites where antibiotics might bind. Furthermore, antibiotics like beta-lactams target metabolically active cells; consequently, beta-lactam treatment may effectively eradicate bacteria from the active, outer layers of the biofilm, without influencing persistent inner bacteria. Communication and nutrient diffusion can occur between layers through water channels. Genetic material can also be exchanged within the biofilm, increasing genetic diversity, permitting adaptation to new pathological niches, and improving survival.

Biofilms in pediatric ENT disease

Warm, moist surfaces in the ears, nose, and throat provide ideal substrate for bacterial attachment and biofilm growth. The persistent and adaptable characteristics of biofilms makes their understanding essential when considering pediatric infections, particularly resistant and chronic infections. Biofilms are particularly important for understanding chronic rhinosinusitis (CRS) and otitis media (OM) with effusion.

Bacteria can form biofilms on both biotic surfaces and abiotic surfaces. Abiotic surfaces lack inherent antimicrobial mechanisms and are consequently less resistant to bacterial attachment and subsequent biofilm formation. For this reason, biofilm growth can be seen on both living tissues as well as devices, such as tympanostomy tubes and other devices used in pediatric ENT patients.

Chronic rhinosinusitis

Biofilms associated with CRS are typically polymicrobial, most commonly including *S. aureus*, *P. aeruginosa*, coagulase-negative Staphylococci, *S. pneumoniae, M. catarrhalis*, and *H. influenzae* [1]. Fungi are also frequently included. In a prospective, observational study of 18 CRS patients undergoing endoscopic surgery, biofilms were identified on 78% of tissue samples, predominantly *H. influenzae*, *S. pneumoniae,* and *S. aureus* [2]. In a controlled study, mucosal specimens were evaluated from surgical endoscopic samples from 12 patients with CRS and 6 control patients with obstructive sleep apnea [3]. Bacterial biofilms were identified in 83% of the samples collected from patients with CRS and none of the controls. These data underscore the preponderance of biofilms in patients with CRS.

Otitis Media with Effusion

OM with effusion has traditionally been considered to represent a sterile inflammatory process. More recent research, however, has demonstrated the presence of pathogenic biofilms in the middle ear cleft of pediatric patients with culture-negative effusions [4]. Analysis of middle ear mucosa biopsies from 26 children (mean age 2.5 years, range 0.5-14 years) undergoing tympanostomy tube placement for the treatment of OM cultured one of three major pathogens (*H. influenzae*, *S. pneumoniae*, *M. catarrhalis*) in only 19% of effusions using traditional culture methods [4]. Polymerase chain reaction is a culture-free method for detecting pathogens by amplifying specific genetic sequences. Using

polymerase chain reaction testing, all effusions were found to be positive for at least one of the three tested bacteria, with 17% positive for all three bacteria and 13% positive for two bacteria. These important studies emphasize the inadequacy of identifying active pathogens in surfaces prone to the development of biofilms using traditional culture methods.

Device infections

ENT devices frequently become contaminated with biofilms, with biofilm growth identified on middle ear ventilation tubes, speech valve prostheses, tracheotomy tubes, cochlear implants, and bone-anchored hearing aid implants, resulting in device failure or recurrent infections [5,6]. Device-related biofilms in children were demonstrated in a study evaluating consecutively enrolled staphylococcal strains isolated from peripheral intravenous devices, venous blood, device insertion sites, and nasal mucosa of patients admitted to pediatric ward with peripheral intravenous devices for >48 hours [7]. A total of 100 invasive, 50 colonizing, and 50 commensal isolates were studied, with biofilms identified in 74% (74/100), 68% (34/50), and 32% (16/50), respectively.

Biofilms as pathogenic reservoirs for ENT infections

Biofilms establish reservoirs of pathogenic bacteria by colonizing moist surfaces, such as the adenoids and nasopharynx. The adenoids may serve as another important bacterial pool for recurrent OM. In a recently published study, adenoid mucosal samples were evaluated from 6 children with recurrent acute OM that was resistant to antibiotic therapy [8]. Biofilm ultrastructure was demonstrated by scanning electron microscopy and co-localized with middle ear pathogens utilizing fluorescent *in situ* hybridization (FISH) and confocal laser scanning microscopy. Biofilms were identified in all specimens, with >86% surface area biofilm coverage. FISH staining for middle ear pathogens was positive for at least one of the pathogens in all samples, with polymicrobial colonization in 83%. *S. pneumoniae*, *H. influenzae*, and *S. aureus* were identified in 67% and *M. catarrhalis* in 50% of adenoids.

The nasopharynx may also provide an important reserve of resistant bacterial biofilm, promoting recurrent or chronic OM. Among isolates of nontypeable *H. influenzae* from children with acute OM, 84% were biofilm-forming strains[9]. Furthermore, identical strains were isolated from both middle ear fluids and the nasopharynx. Biofilms were more substantial among cases that had failed to respond clinically to amoxicillin.

Targeting biofilms when treating pediatric infections

Fully comprehending the resistant and resilient nature of biofilms suggests a critical role for minimizing the development of biofilms, when possible. Guidelines restricting antibiotic use of broad-spectrum antibiotics are supported by data showing that limiting exposure and treatment duration in newborns decreased late-onset infection, without an increase in infectious relapse [10]. While this study did not evaluate biofilms growth, excessive antibiotic exposure may predispose to the development of biofilms with stronger survival characteristics.

In patients with established infections, testing results of antibiotic sensitivity against cultures of planktonic bacteria often fail to provide useful information

about the efficacy of antibiotics against more resistant biofilms. For example, bacterial isolates from sputum samples from 110 cystic fibrosis patients with acute exacerbations were grown planktonically and as biofilms 11. Antibiotics selected for treating acute exacerbations were effective against planktonically-grown bacteria for 60% of patients. Biofilm-grown bacteria, however, responded to antibiotics for only 22% of patients. As expected, patients treated with antibiotics to which biofilm-grown bacteria were susceptible were significantly more likely to have a decrease in sputum bacteria and length of hospitalization.

Chronic rhinosinusitis treatment

CRS may be more effectively managed by selecting antibiotics and delivery route to which biofilms are more susceptible. CRS is more effectively treated with macrolides than other classes of antibiotics [12], with macrolides able to effectively inhibit quorum-sensing [13]. Direct delivery of antimicrobials via nasal washes may effectively reduce CRS biofilm. In *in vitro* studies, hydrodynamic delivery of a soap-like surfactant and a calcium-ion sequestering agent effectively reduced bacterial colony counts from biofilm isolates obtained from patients with refractory CRS [14]. Topical antibiotics offer an avenue for increasing the dose beyond what can safely be achieved with systemically-administered antibiotics, with mupirocin nasal lavage shown to reduce biofilms and CRS in both animal and human models [15-17].

CRS may also be eradicated with surgical removal of infected adenoids [18,19]. A small retrospective study recently described good resolution of CRS in 23 pediatric patients treated with a stepwise protocol including concurrent adenoidectomy and bilateral maxillary sinus irrigation followed by long-term double oral antibiotic therapy [20].

Otitis media treatment

In vitro, fluoroquinolones have been shown to be more effective against OM from nontypeable *H. influenzae* than β-lactams, cephalosporin, and macrolides [21]; however, *in vivo* data are lacking. Subinhibitory concentrations of the macrolide clarithromycin inhibit biofilm formation by interfering with the twitching mobility of *P. aeruginosa* [22]. Antimicrobial treatment, however, may adversely affect susceptibility of treated bacteria. In one study evaluating the treatment of OM, the initial resistant organism in the nasopharynx replaced a susceptible organism in the middle ear within a few days of initiating antibiotic therapy for OM for 47% of treated patients [23].

Preventive therapy through vaccination programs has also been proposed to reduce the occurrence of OM. While some experts have suggested that pediatric vaccinations to minimize nasopharngeal colonization by *S. pneumoniae, H. influenzae*, and *M. catarrhalis* might reduce risks for contracting OM, a recent study following healthy children ages 6-36 months for one year indicated that changes in colonization by one bacterial species would likely result in modifications in other species [24]. For example, these data suggested that elimination of nasopharyngeal *S. pneumoniae* and *H. influenzae* may increase colonization risk from pathogenic *S. aureus*.

Device-related infections

Tympanostomy tubes are routinely used for treating chronic OM effusion. Post and colleagues theorized that the effectiveness of tympanostomy tubes results from increased oxygen tension within middle ear space with reventilation, thus, restoring mucosal defenses [25]. The tympanostomy tube itself, however, can become a target of bacteria attachment and biofilm formation. Phosphorylcholine-coated fluoroplastic tympanostomy tubes are resistant to *S. aureus* and *P. aeruginosa* biofilm formation, while uncoated fluoroplastic tubes develop *P. aeruginosa* biofilm and silver oxide impregnated tubes develop biofilm from both *S. aureus* and *P. aeruginosa* [26]. Albumin coating of the tympanostomy tube may also reduce biofilm growth by preventing foreign material adherence through inhibition of fibronectin binding [27].

Definitive treatment for established biofilm-related device infection is device removal. Prevention of bacterial attachment to abiotic surfaces has been tested with endotracheal tubing, including selective digestive decontamination, utilizing antibacterial endotracheal tubes, and synchronized mucus aspiration of the distal endotracheal tube [28]. Aggressive device cleaning with scraping and photodynamic therapy might also be used to eliminate biofilm that has already formed [28,29]. Recently, the combination of power ultrasound and ozonation were demonstrated to be effective for the removal of biofilm from stainless steel [30] Efficacy in pediatric ENT infections has yet to be demonstrated.

Summary

Pediatric ENT infections often become resistant and persistence due to characteristics of biofilm that enhance bacteria growth and survival. Moist, mucosal ENT surfaces provide ideal conditions for biofilm growth. Colonization of the nasopharynx and nearby structures by pathogenic bacteria is common and may provide a reservoir for the development of recalcitrant infections, such as CRS and OM. While antimicrobial therapies, such as detergents and antibiotics, are typically effective against planktonic bacteria, biofilm defenses provide effective barriers against bacterial eradication from these treatments. Selection of antibiotics may need to consider those agents with activity against inactive anaerobic cells. For this reason, fluoroquinolones may be more effective against biofilm bacteria than β-lactams. Antibiotic therapy alone, however, may be insufficient to eradicate complex biofilms, with additional steps necessary to reduce biofilms and minimize bacterial development in biofilm reservoirs, like the adenoids and nasopharynx.

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