

LITERATURE REVIEW**Biofilms and nasal wound healing in postsurgical patients with chronic rhinosinusitis - A review of literature****Veronica Trombitas¹, Alina Anda Nagy¹, Diana Vlad¹, Ilea Aranka², Silviu Albu¹**¹IInd Department of Otolaryngology, University of Medicine and Pharmacy Cluj-Napoca, Cluj-Napoca, Romania²Department of Oral Rehabilitation, Oral Health and Dental Office Management, University of Medicine and Pharmacy Cluj-Napoca, Romania**ABSTRACT**

Chronic rhinosinusitis (CRS), fairly common disease in the field of otorhinolaryngology, is the focal point of thorough study of the pathophysiology and treatment strategies for a favourable evolution of patients with this problem. Although there have been important advances in the treatment of CRS, signs and symptoms still remain long after functional endoscopic sinus surgery (FESS), indicating the persistence of the biofilm infection on the sinus mucosa. This review aims to highlight the relationship between bacterial biofilm and CRS, and also the available treatment strategies of the latter.

KEYWORDS: chronic rhinosinusitis, biofilm, chronic disease, wound

INTRODUCTION

Chronic rhinosinusitis (CRS) is a very common condition and, although there are an abundant number of studies on this topic, there still are uncertainties when it comes to understanding its pathophysiology and the correct therapeutic attitudes. In fact, Benninger et al. suggested, in a recent article, that CRS is a medical disorder of mucosal inflammation which affects the nose and sinuses, lasting at least three months, with numerous, disparate and frequently overlapping potential causes^{1,2}. The role of infection is very controversial in rhinology, but with a recognized importance in recalcitrant chronic rhinosinusitis^{3,4}. Recent studies argue the presence of the biofilm, especially in the case of pathogenesis of refractory CRS. Topical nasal steroids, saline lavage and antibiotics are the usual treatment in the management of CRS. Functional endoscopic sinus surgery (FESS) represents the easiest surgical treatment method with many advantages over more conventional techniques, like Caldwell-Luc and others⁵. The purpose of this method is to reestablish the normal function of the sinus, normal ventilation, and ciliary clearance⁶. The outcomes after

FESS depend on CRS pathogenesis and on the therapeutic route after surgical treatment. This is why it is essential to understand biofilm interactions, the microbial organism behaviour and the ability of microorganism components to detach and disperse^{7,8}.

BIOFILM: STRUCTURE AND FUNCTION

During the 18th century, Robert Koch was the author of an essential work that formed the source of modern microbiology, and that explained the relationship between bacterial microorganisms and diseases. For the first time, these sessile communities, bacterial aggregates that live on the human body, were described and named in 1978, while later direct examination demonstrated that the biofilm had a complex and individual structure, different from their planktonic counterparts⁹⁻¹².

Biofilm represents an organized community of bacteria, which live on both the mucosal surface and foreign body, and are resistant to difficult conditions of life, controlled by different genetic pathways depending on growth conditions and exposure to membrane-

targeting antibiotics^{10,11}. Their structural composition is characterized by a three-dimensional complex with a cell component ($\pm 15\%$ of their volume), matrix material ($\pm 85\%$ of their volume) represented by polysaccharides, nucleic acids, proteins and extracellular DNA and a rich network of ramifying water channels^{12,13}. The structure of biofilms is sophisticated, the distribution of the cells in the matrix being remarkable as well. It could be concluded that the matrix components may dictate these cells' precise location. The structure and the function of biofilms are coordinated through regulatory signals, like hormones or pheromones, identified for the first time in 1998¹⁴.

Stoodley et al. have demonstrated that these bacterial aggregates have the possibility of moving, by creeping properties, under the influence of regulatory signals, and enhance the biofilm's survival and virulence⁹⁻¹⁷.

A variety of bacteria, which are called fixed nasal floras, normally not pathogenic floras, can live in a normal nasal cavity. It can be formed of common aerobic bacteria like *Staphylococcus*, *Enterobacter*, *Corynebacterium diphtheroids*, *Pseudomonas aeruginosa*. Anaerobic bacteria could also be found, like *Peptostreptococcus*, *Prevotella melaninogenica* and *Propionibacterium*. *Corynebacterium diphtheroids* and *B catarrhalis* are found, as normal bacterial flora, in the respiratory tract, with a non pathogenic character. Frequently, *Staphylococcus aureus* and *Streptococcus pneumoniae* are considered pathogens, and form nasal biofilms. Sanderson et al. show in a recent study that the *Haemophilus influenzae* is the most predominant species, in combination with biofilms of *S. aureus* and *S. pneumoniae*, which are also present, but to a lesser degree. The balance between normal flora and pathogens creates a local resistance against the invasion of foreign pathogens and stimulates the body's immune defense¹⁸⁻²⁰.

For a better understanding of biofilm action, we need to know the formation and the maturation of these sessile communities. In the presence of a supportive nutrient, that promotes the replication of bacteria, the trigger event is the adhesion of planktonic bacteria to a mucosal surface. This event is determined and supported by reversible physical forces. If the stability of the bacteria on this surface is not immediately stopped, or the antimicrobial defence of this surface is not strong enough, a phenotypic change that permits powerful connections, by means of adhesins, will take place. After that, the adhesion of extra bacteria to the surface is facilitated, with the formation of bacterial colonies. The attachment of bacteria itself initiates the synthesis of an extracellular matrix with protective properties. The continual adhesion of other bacteria and the cellular division ensure the continued growth and maturation of the biofilm, with a powerful archi-

itecture of microcolonies and channels²¹⁻²³. Research on biofilm maturation emphasizes an important phenomenon about the acyl-homoserine lactone signals, which are generated by individual bacterial cells, having the potential to gather and determine the appearance of explicit collections of genes⁸.

From an evolutionary approach, bacteria from biofilms have a number of strong points to stay alive and prokaryotic continuation. Life in the form of bacterial aggregates is safer, and so is the opportunity to act and to collaborate together, and more than that, to use in common hereditary material with other cells. Also, biofilms are actually not so vulnerable to the influence of the environment, for example ultra-violet emission, dryness, fluctuation of pH and osmolarity²⁴. In this context, it was in the 1970s that Characklis studied biofilms in industrialized water facilities and highlighted their obstinacy and strength in front of disinfectants, for example chlorine²⁵.

BIOFILM AND CHRONIC DISEASE

The human body nestles from 10 to 100 trillion microbes which play a fundamental role in our well-being, so defining a healthy microbial state is a critical step for discovering how variations in the microbiome may contribute to or cause a wide range of diseases²³. Following the latest developments in the field, scientists began to understand and explain the influence of bacterial biofilms in case of persistent diseases. By contrast with acute infections, caused by bacteria presented exclusively in planktonic type, biofilms are extremely able of causing persistent feedbacks from the immune system, creating a persistent infection that might be better considered "tenacious survival" more willingly than hostile virulence². Biofilms prefer inert surfaces, necrotic tissue or medical devices, grow slowly with silent symptoms and can live in one or more locations. The host's reaction is prompt, stimulating the production of antibodies, which cause complex damage to surrounding tissue, and the cellular and humoral immune reactions can rarely eradicate the biofilm infection⁸. The structure of biofilm is favourable to a decline of the oxygen and nutrient gradient, from periphery to center, which determines the fall of the metabolic gradient in the same way; also, the resistance of antibiotics and the capacity of native and adaptive host defences are compromised. These characteristics explain the implication of the biofilm in persistent and recurrent infectious diseases^{13,24}. As such, biofilms play an important role in the pathogenesis of many human body infections like otitis media, chronic prostatitis, pneumonia, line sepsis, osteomyelitis, periodontal disease and recurrent recalcitrant or persistent CRS or just CRS^{8,26}.

BIOFILMS AND CRS

One of the most common chronic diseases in the field of rhinology, CRS affects 4 to 28% of the European and US populations and has many socio-economic implications. The patient's quality of life is altered because of at least two symptoms: nasal congestion or nasal discharge (anterior/posterior nasal drip) and nasal blockage, to which one can add facial pain, headache and decreased sense of smell²⁷.

CRS is one of the most frequent pathologies met at nasal and paranasal level, an important role being played by inflammatory changes occurring to the ostiomeatal complex, composed by maxillary sinus ostia, anterior ethmoidal cells and their ostia, ethmoid infundibulum, hiatus semilunaris and middle meatus. These changes affect the mucociliary clearance and patent ostia, causing hypoxia, mucosal congestion, ciliary epithelial damage, pH alteration, increased secretions and bacterial proliferation^{28,29}.

A variety of factors were found to be involved in CRS pathogenesis, such as histopathology, tissue reshaping, ciliary dysfunction and epithelial barrier malfunction, inflammatory cell and T cell patterns³⁰⁻³².

The role of infection as a potential pathophysiologic mechanism in CRS has been a source of considerable debate in rhinology³³. Hochstim, Joung et al. demonstrated that bacterial biofilms are strongly associated with persistent mucosal inflammation, for a longer period of time^{34,35}.

The microbiology of CRS is polymicrobial, and the predominant organisms reported include *Staphylococcus aureus*, coagulase negative *Staphylococcus*, *H. influenzae* and various gram-negative organisms, such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Stenotrophomonas maltophilia*^{20,36-39}. Trifillis et al., in a 1998 study, described different degrees of denudation for

mucosal changes in CRS, which can cause favourable conditions for biofilm formation⁴⁰. The surface roughness and hydrophobicity are frequently met conditions in CRS; also, ample food supply and favourable temperature are positive factors for biofilms development⁴¹.

The objective evidence of CRS is provided through anterior rhinoscopy, endoscopy or CT. Anterior rhinoscopy can recognize polyps, purulent leakage or polypoid modifications, mucosal abnormalities, confirmed through nasal endoscopy. Mucosal malformations of the middle meatus are identified through nasal endoscopy and CT (Figure 1). Imagistic investigations, like CT or MRI, offer more information about the ostiomeatal complex or sinuses; MRI is moreover explicit and is not suggested for the diagnosis of regular CRS²⁷.

To highlight the presence of biofilm in a wound, scientific evidence is needed, represented by microscopy visualization. A series of staining and molecular procedures include the staining for extracellular polymeric substances, like calcofluor white/ethidium bromide, Congo red/Ziehl carbol fuchsin, safranin/FITC-ConA, and DAPI/PAS. Peptide nucleic acid-fluorescence in situ hybridization is a molecular technique which can identify bacterial aggregates in host tissue⁴²⁻⁴⁴. Electron microscopy and confocal laser scanning microscopy represent the methods frequently used in previous studies. James et al. found that electron microscopy of biopsies from chronic wounds is significantly populated by dense bacterial aggregates (60% of the specimens) by comparison to fewer bacteria (6%) in the case of biopsies from acute wounds⁴⁵. Those interpretations could clarify the prognosis for recalcitrant CRS or the different evolution in the case of chronic versus acute wound healing^{42,44}. Hematoxylin and eosin staining is a cheap and handy method for detecting biofilms in CRS, that could predict the evolution after FESS (Figure 2).



Figure 1 CT scan of the paranasal sinuses

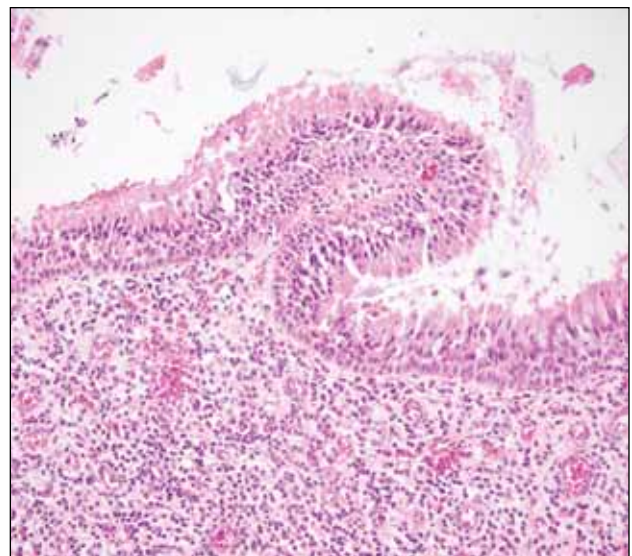


Figure 2 Hematoxylin and eosin staining

MANAGEMENT OF CRS WITH BIOFILM IMPLICATION

Systemic antibiotics

The occurrence of biofilms in CRS may have considerable influence on therapy attitude, as it is known that bacteria that form biofilms are commonly resistant to typical forms of antimicrobial therapy⁴². The mechanisms of antimicrobial resistance are not completely understood, but it is presently known that the biofilm matrix is resistant to usual antimicrobial agents. The main objectives for antimicrobial treatment efficacy are to reestablish sinus ventilation and mucociliary clearance, to kill pathogenic bacteria. Thereby, we concentrate our attention on topical chemical and mechanical treatments. The goal of antibiotics is to treat especially acute exacerbations of CRS. Antibiotics are usually prescribed for 2 – 4 weeks, and supply broad-spectrum coverage beside the pathogens described previously^{22,44}. A number of studies underline the resistance of antibiotic for isolates in biofilms. The most common is methicillin-resistant *S. aureus*, also important is vancomycin-resistant *Enterococcus*, and rarely met multidrug-resistant *Acinetobacter baumannii*.

Macrolide therapy for CRS, in low doses, is considered unique for its anti-inflammatory properties. It has recently been demonstrated that Clarithromycin therapy alters the structure of biofilms^{22,35,45}. Also, moxifloxacin administered at levels approaching minimal inhibitory concentration can determine a significant reduction (99%) in the number of lively bacteria in a controlled in vitro mature *Staphylococcus aureus* biofilm. Ceri et al. demonstrated that *Pseudomonas* biofilms are susceptible to minimal inhibitory concentration of gentamicin and very susceptible to ceftazidime and piperacillin⁴⁶. Recent studies show that mupirocin treatment can significantly reduce all *S. aureus* isolates²². It can be assumed that this treatment for *Staphylococcus aureus* biofilms could have an important role.

Topical treatment

Saline lavage is a good option for topical treatment in CRS, while in CRS with bacterial biofilm it could also be maintained as a good treatment option, because the polymers that compose the extracellular polymeric substance have a good solubility in water. So, it is expected that the biofilm will disperse into the saline solution when we apply it to the nasal mucosa. But many biofilms are resistant to saline lavage, the best example is that of *Pseudomonas aeruginosa* biofilms – due to the calcium-ion bridging that cross-links mannuronic/guluronic acids, the biofilm dispersion is prevented, this making saline irrigations inefficacious. However, theoretical advantages of saline lavage are

the reestablishment of the mucociliary flow, the mucosa hydration, and the mucosa cleaning from toxic and irritative substances^{22,47,48}.

Topical antibiotics therapy is an alternative to systemic antibiotherapy, having the goal to deliver the antibiotics at the sinus mucosa surface. In this case, concentration may be adjusted.

In vivo rabbit models of maxillary sinusitis, Ceri et al. attempted to eradicate *Pseudomonas* mucosal biofilms with high concentrations of topical tobramycin, but the results underline a persistence of live bacteria at the sinus mucosa surface^{49,50}. A recent study shows that topical irrigations with mupirocin can significantly reduce all *S. aureus* isolates. It can be assumed that this treatment for *Staphylococcus aureus* biofilms could have an important role²². Also, in a recent study, Ezzat et al. reported a significant improvement in patients after treatment with topical ofloxacin⁴⁹.

Topical corticosteroids are strongly recommended as well, but evidence is moderate. A real recommendation of this treatment is justified in the case of CRS with polyps, which is associated with a great risk of biofilm formation, and for which the reduction of inflammation has been shown. Optimal action of corticosteroids is evident during the immediate post-surgical period, when the opened sinuses are associated with an optimal penetration inside the sinus cavities, and the application is better to the sinus mucosa. A diversity of corticosteroid delivery methods to the paranasal sinuses exists, such as steroid drops, and, with a high utilization, atomizers and steroid irrigations. With a highly efficient area of action (especially the middle meatus), a combination of budesonide and saline is commonly used in the treatment of refractory disease^{49,50}.

Surgical treatment

For patients with refractory symptoms, which persist after appropriate medication has been administered, surgery represents the key in the management of CRS with bacterial biofilm. FESS has as goals to remove polyps, pus or debris from the sinus, to reestablish good sinus ventilation, smooth drainage, and to promote its mucosal, morphologic and functional improvement and recovery^{12,50,51} (Figure 3).

It is important to have a proactive attitude during the immediate post-surgical period, because only rigorous post-surgery attention and hygiene, including crust debridement, removal of secretions, saline lavage and prolonged use of topical corticosteroid sprays can lead to a proper healing of the wound⁵¹.

However, a serious challenge for clinicians remains the fact that biofilms persist after treatment, and may cause the unfavourable outcomes of CRS surgery.



Figure 3 Surgical treatment - FESS

CONCLUSIONS

Biofilm has an important role in persistent and recurrent symptoms after FESS, in the case of CRS that is unresponsive to intense medical management. Bacteria that compose biofilms are frequently met on the surface of wounds and are considered to have an important role in the incapacity of these wounds to heal. 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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