Biofilms have been recently linked to human disease in several different ways. This talk attempts to educate the listener regarding biofilms in chronic rhinosinusitis.

Biofilms are known to exist in two forms. Planktonic is the traditional free-floating form which biologists have studied for hundreds of years, and the basis for which culture and sensitivity has traditionally been used. Treatment modalities such as antibiotics are aimed at the assumption that the Planktonic individual form of bacteria is the primary form. More recently in the medical literature, however, Biofilm forms of bacteria have come to be appreciated.

Biofilms are a much more complex organization of multiple different types of bacteria in a 3-D structure. They have been identified in many different processes such as water treatment, flow cells, and can be seen even in hot springs. Most notably, you probably have some biofilm currently on your teeth, otherwise known as plaque. Biofilms have a predictable growth and maturation cycle, starting with the initial attachment of free-floating planktonic bacteria onto a host surface. This initial phase of growth can be reversible. However, if conditions are favorable, a permanent chemical attachment will start to be formed around the bacteria, and the bacteria will make a slime coating of glycocalyx for protection while they began to build in a vertical phase of growth. As they develop vertically, so the different kinds of the bacteria involved in the Biofilm can sub-specialized depending on their level within the film and the nutrient and oxygen available to them in that area.

Once a Biofilm matures, seeding and dispersal of free-floating planktonic bacteria that are adept at creating biofilms can then be released into the environment. Research has shown that multiple different kinds of bacteria exist together as well as yeast and sometimes mold within the Biofilm environment. The aerobic bacteria tend to aggregate nearest the surface where oxygen supplies are more plentiful, and anaerobic bacteria can aggregate towards the center of biofilms where there is less oxygen penetration into the matrix. Similarly nutrients diffuse through the Biofilm matrix in gradients of higher to lower concentrations. This can create micro-environments within which certain types of
bacteria flourish. These micro-environments also subselect within the bacterial colonies involved. At areas of minimal penetration of oxygen or other nutrients, the bacteria within the interface can react with a stress response and express different genetics as well as exchange genetic material. Within the base of the Biofilm, where the penetration of oxygen and nutrients is very low, bacteria can become senescent where they are no longer dividing or have any measurable metabolic activity. Bacterial Biofilm has been shown to be a barrier to wound healing, because of the matrix surrounding the bacteria, oxygen cannot penetrate into the tissues and can create even more tissue necrosis underlying the Biofilm.

DETECTING BIOFILM CLINICALLY

Now that we know that biofilms are far more common than planktonic bacteria, how do we detect them clinically? Currently only laboratory based research can detect biofilms reliably, and they use a few different methods to do so. The oldest is scanning electron microscope imaging. This technology uses a high vacuum or Gold plating in a high vacuum to observe structures at a micron level. Because of requirement for fixation with a drying agent, the moist glycocalyx of the Biofilm becomes altered and while this technology is still used, new developments have started to take its place. One benefit of scanning electron microscopy, however, is that it has widespread availability to many research facilities.

A new modality for detecting biofilms is technology called confocal laser scanning microscopy. This imaging modality provides the capacity for direct, noninvasive, serial optical sectioning of intact, thick living specimens with a minimum of sample preparation. The images are enhanced with fluorescent dyes, particularly with staining of live and dead bacteria, or with staining of tissue cells versus bacteria. Unfortunately, confocal laser scanning microscopy is not a cheap and readily available set of equipment. Recently, Biofilm researchers have compared hematoxylin and eosin stained tissues enhanced with Gram staining to prove Biofilm existence using commonly available pathology methodologies to detect biofilms. These methodologies have not yet been widely embraced within the research community, but may prove to be quite helpful as clinical diagnosis of biofilms may become more widespread.

These researchers have noted some characteristic changes to the nasal epithelium that are associated with biofilms. They see fragmented or destroyed epithelium as opposed to the ciliated columnar epithelium normally seen in a healthy mucosa. They have also noted some squamous cell metaplasia, neutrophil dominance and some plasmacytic infiltration of tissues in associated with Biofilm in the nasal passages. They have also noted that tobacco smoke can induce Biofilm formation in the nasal mucosa, from bacteria normally inhibited by cigarette smoke in a healthy subject.

DIAGONOSIS OF CHRONIC RHINOSINUSITIS

Diagnosis of chronic rhinosinusitis has been well-established. Chronic rhinosinusitis is defined as 12 weeks or longer of 2 or more of some of the following signs and symptoms: mucopurulent drainage, nasal obstruction or congestion, facial pain, pressure, or fullness, or decreased sense of smell. Inflammation must also be documented by one or more the following findings of: purulent mucus or
edema in the middle meatus, polyps in the nasal cavity or middle meatus, and radiographic imaging showing inflammation of the paranasal sinuses. Recurrent acute rhinosinusitis is defined as four or more episodes per year of sinusitis without signs or symptoms between episodes.

**DIAGNOSING A BIOFILM**

So how does someone diagnose a Biofilm? In Biofilm studies, patients with failure of maximum medical therapy and endoscopic sinus surgery have a Biofilm positive rate of 20-100%. These studies use a wide variety of criteria for Biofilm positivity, but most agree that about 60% of these patients are positive for biofilms. One study found a Biofilm positivity correlated with the SNOT-20 items of the need to blow nose, cough and post nasal drip. However, this study did not find a strong correlation with endoscopic or CT findings as far as Biofilm positivity. One study found a strong correlation between the grade of osteitis by CT and pathologic evaluation of chronic rhinosinusitis. During endoscopic sinus surgery they found pathological and CT evidence of osteitis for 84.8% of the bones underlying mucosa with Biofilm in the ethmoid sinuses. This study confirmed approximately 46.4% of chronic rhinosinusitis patients were from a subgroup with both Biofilm and osteitis.

A wide variety of biomarkers are being investigated for correlation with Biofilm positivity. One study found an increased number of dendritic cells that express CD 209 to correlate with Biofilm positivity. When dendritic cells are activated they release proinflammatory cytokines such as IL-1 and tumor necrosis factor alpha, as well as growth factors. This response may induce epithelial apoptosis and metaplasia seen in sinus tissues underlying biofilms. TNF-alpha receptor positivity was also found to be upregulated in epithelial cells associated with biofilms in chronic rhinosinusitis patients. Similarly, eosinophils and plasma cells are noted to have significantly greater counts in biofilm positive tissue compared to controls. There are significantly higher counts of these cells in patients with polyps as well.

**TREATMENT OF BIOFILM**

Unfortunately, biofilms are difficult to treat. Antibiotics don't work very well at penetrating through the resistant layers and structures of Biofilm. The protective glycocalyx within which the Biofilm operates, as well as the multiple species of bacteria, disruption of the cell cycle, and shared resistance among bacteria are all barriers to antibiotic effectiveness. Nasal steroids, a mainstay of treatment of chronic rhinosinusitis, are also found to have no effect on the presence of biofilms. However, the pattern of inflammatory cells in the subepithelial layer are altered by steroids and they have also been shown to down regulate release of tumor necrosis factor alpha. Physical disruption of biofilms may help to reduce the biomass and improve the mucociliary function of the epithelium, as well as improve the penetrance of topical treatments. However endoscopic sinus surgery has also been implicated in dispersion of biofilms. A variety of irrigation solutions have been proposed to help with chronic rhinosinusitis such as baby shampoo other surfactants. These are proposed to work through mucolytic effects. Sinus rinses with antibiotic solutions are purported to have improved penetration as opposed to oral preparations.
However surfactant potential for nasal irritation and possible ciliotoxicity limits their use. One preparation shows some promise in recent literature. Manuka honey has an active component of methylglyoxal that has been shown in a sheep model to reduce Biofilm concentrations without harming native tissue. Honey has also been noted for anti-pseudomonal activity in other studies. Another promising area of research is photodynamic therapy. A 670 nm red light used to illuminate the maxillary sinus model pretreated with methylene blue and EDTA have demonstrated significant reduction in Biofilms. This treatment has not yet been approved for use in humans however. Several studies of ultrasound treatment of biofilms have shown some promise. In a small clinical trial, low-frequency ultrasound was applied over the maxillary sinuses of CRS patients in 6 sessions and pre-and post-treatment SNOT-20 scores were shown to be improved by 34%.

CONCLUSION

In conclusion, biofilms are ubiquitous and pervasive, a stable multi-microbial colony that is difficult to diagnose. They have been implicated in chronic rhinosinusitis cases and can be very difficult to treat. However several promising improvements in diagnosis and treatment are being developed and warrant attention as they come to market.

DISCUSSION: BRUCE LEIPZIG, MD

Let me start out by saying in my personal opinion that these are the most important Grand Rounds that we do this year. Dr. Natili, that was an excellent presentation. You vocalized well to your audience. We can both hear you and understand what you were saying. Your slides were not overly busy. In fact they were quite pleasant and easy to read, and I think we can all learn from a good presentation.

This is so important -- especially for the residents: this is going to show you some new science that is coming into play that all of us old folks don’t even know about. It’s just coming to the fore for the first time. There are a number of novel therapies for chronic sinusitis because we fail at it so often and we see it so often. People come in with sinus disease, we treat them empirically and some feel good. Most of them aren’t real sinusitis -- they are viral in origin, but our tendency is to make them worse, and we don’t know why. We don’t know why when we give them antibiotics, and they come back worse at a later date and or we do surgery more quickly than we should because it’s well known that the CT scan lags in its clearance of sinus disease from several weeks. So we go ahead and operate, and we create these little areas for biofilms to form. But we’d never heard about this in the past so we didn’t know we were creating this problem.

Your presentation showed us that we really don’t have the answers yet. We know we can find them if we take tissue cultures but we also know that even if we culture the tissue we’re going to find multiple organisms and it’s hard to say which one we should be treating and we’ve had very poor
success utilizing antibiotics. When we irrigate these, we know that by chance irrigations can help, but many people give their patients tap water, and all that does is to add antibiotics to the mixture. All that does is to form new biofilms or new super-bacteria and cause further infection.

The reason this is becoming important is because all of us are going to have to follow the literature. We can’t be satisfied with what we learned today. For us as practitioners we also have the even worse problem of not of the patient who comes in with a sinus problem but of those who continue to get maximal medical therapy as you mentioned but gets worse. You develop draining ears and we now know that you develop biofilms in the ear, one of the many novel sources of failure. We re-operate on them-- we do more antibiotics on them, we send them to Infectious Disease. Our antibiotics are often found to be appropriate by culture, but as you’ve shown us we can’t get at the resistance that we’ve often created by ourselves. The question then comes up -- what to do and as surgeons. We always want to do something. So I’ve seen that I can’t tell you how many hundreds, if not thousands of times over the years. Every one of you will see it too because -- maybe sixty percent -- who knows what the real number is -- of these recurrent or persisting sinus problems, have maybe biofilm, or maybe something more unique that we don’t know yet. Most of the treatments that we give these patients simply make them worse.

Dr. Natili has shown us that there are some irrigations that are useful. Gentian violet that has worked so well in the external ear probably works because it disrupts the biofilm and allows us to get at the bacterium. Ultrasound may be the answer for the future, but clearly the answer isn’t surgery except in a very limited number of cases where there is obstruction. As a historical reference to Dr. Quinn, when I was in my training we did Calwell-Lucs and antral windows. An antral window was a hole underneath the inferior turbinate because we thought the reason the sinus wasn’t open and draining was because the ostium was too high. We knew about ciliary movement but we didn’t seem to believe it. So we did these windows. They were safe as could be, they were easy, and all they did was to create more problems. We stripped all the mucosa that we could, got into incredible bleeding problems, and again we treated infections that we weren’t curing. Then in a way we finally went backwards and learned to use the ostiomeatal complex as a surgical as well as a biological proper way of treating obstructive rhinosinusitis but it doesn’t get at the basis of infection, and that’s what we’re starting to learn now.

All of the newer non-novel treatments are nonsurgical, and they’re based on inflammation. So we have all these anti-inflammatories that we give to patients. I think cultures are important but they’re not the be-all or end-all because you don’t always get the best antibiotic that is needed for treated. We know about fungi, allergies, and we know we have to use distilled water and not tap water when we have our patients irrigate and when we try to do all these things. We try to keep up with what’s being learned in the laboratories which we don’t have in our offices. We’re going to
come a long way with the treatment of chronic rhinosinusitis. I congratulate you. This is a great topic and a very excellent presentation. Thank you.

REFERENCES:

Angelia Smith, MD, Farrel Joel Buchinsky, MD, and J. Christopher Post, MD, PhD, MSS, “Eradicating Chronic Ear, Nose, and Throat Infections: A Systematically Conducted Literature Review of Advances in Biofilm Treatment” Otolaryngology -- Head and Neck Surgery 2011 144: 338


Han Li, et al “Relationship between bacterial biofilm and clinical features of patients with chronic rhinosinusitis” Eur Arch Otorhinolaryngol (2012) 269:155–163

Dong Dong, MM, Zhao Yulin, MD et al “Correlation Between Bacterial Biofilms and Osteitis in Patients With Chronic Rhinosinusitis” Laryngoscope, 00:000–000, 2013

Tama’s Karosi, Pe’ter Csomor, Zolta’n Hegyi, Istva’n Sziklai “The presence of CD209 expressing dendritic cells correlates with biofilm positivity in chronic rhinosinusitis with nasal polyposis” Eur Arch Otorhinolaryngol (2013) 270:2455–2463


Pe’ter Csomor, Istva’n Sziklai, Tama’s Karosi “Effects of intranasal steroid treatment on the presence of biofilms in non-allergic patients with chronic rhinosinusitis with nasal polyposis” Eur Arch Otorhinolaryngol, 25 Aug 2013


Tamas Karosi, MD, PhD; Istvan Sziklai, MD, DSc; Peter Csomor, MSc, “Low-Frequency Ultrasound for Biofilm Disruption in Chronic Rhinosinusitis With Nasal Polyposis: In Vitro Pilot Study” Laryngoscope, 123:17–23, 2013