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Biofilms in Ear, Nose, and Throat Infections: How Important are They?

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Background: **Biofilms present a new challenging concept in sustaining chronic, common antibiotic-resistant ear, nose, and throat (ENT) infections. They are communities of sessile bacteria embedded in a matrix of extracellular polymeric substances of their own synthesis that adhere to a foreign body or a mucosal surface with impaired host defense. The aim of this paper is to review the literature on ENT diseases that can be attributed to biofilm formation and to discuss options for future treatment.** ***Materials and Methods:*** **Literature review from Medline and database sources. Electronic links and related books were also included.** ***Study Selection:*** **Controlled clinical trials, animal models, ex vivo models, laboratory studies, retrospective studies, and systematic reviews.** ***Data Synthesis:*** **Biofilm formation is a dynamic five-step process guided by interbacterial communicating systems. Bacteria in biofilms express different genes and have markedly different phenotypes from their planktonic counterparts. Detachment of cells, production of endotoxin, increased resistance to the host immune system, and provision of a niche for the generation of resistant organisms are biofilm processes that could initiate the infection process. Effective prevention and management strategies include interruption of quorum sensing, inhibition of related genes, disruption of the protective extrapolymer matrix, macrolides (clarithromycin and erythromycin), and mechanical debridement of the biofilm-bearing tissues. With regard to medical indwelling devices, surface treatment of fluoroplastic grommets and redesign of cochlear implants could minimize initial microbial colonization.** ***Conclusion:*** **As the role of biofilms in human infection becomes better defined, ENT surgeons should be prepared to deal with their unique and tenacious nature.** ***Key Words:*** **Biofilms, bacteria, infections, otitis media with effusion, chronic rhinosinusitis.**

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INTRODUCTION

It has been observed that ear, nose, and throat (ENT) infections encountered in clinical practice are becoming more resistant to common treatment.[1–3] Moreover, the chronic nature of some of them (i.e., chronic rhinosinusitis [CRS], chronic otitis media [COM], otitis media with effusion [OME]) makes the situation more difficult with regard to diagnosis and management. The latter often fails, and long-term antibiotic administration is often inadequate to eradicate disease that gradually affects patients' quality of life. Even though one can argue that these situations could merely represent an increase in antibiotic resistance, because of the overuse of antibiotics in current clinical practice, the challenging concept of biofilms can be considered as an etiologic factor, among others.

Bacterial biofilms are three-dimensional aggregates of bacteria that have been shown recently to play a major role in many chronic infections.[4] Biofilm formation is an ancient and integral component of the prokaryotic life cycle and is a key factor for survival in diverse environments.[5] In the human host, biofilms exist as a community of sessile bacteria embedded in a matrix of extracellular polymeric substances (EPS) they have produced, which adhere to a foreign body or a mucosal surface with impaired host defense[6,7] or ample roughness.[8]

It is becoming increasingly clear that the biofilm mode of growth may play an important role in many otorhinolaryngologic infections and result in their persistence and difficult eradication, mainly because of two distinct biofilm characteristics: 1) biofilms are highly resistant to immune killing and clearance and to treatment with antimicrobial agents,[9] and 2) biofilms might be capable of shedding individual bacteria to the surrounding tissues and into the circulatory system, thus causing bouts of infection, which may recur despite intensive antimicrobial treatment.

The aim of the present paper is to review the current knowledge on ENT diseases that can be either attributed to, or perpetuated by, biofilm formation. Implications for future treatment are also addressed.

MATERIALS AND METHODS

An extensive search of the literature was performed in Medline and other available database sources using the keywords

"biofilms," "infection," "otolaryngology," "ENT," "ear," "nose," "tonsils," "treatment," "antibiotics," and "resistance." Information from electronic links and related books were also included in the analysis of data.

RESULTS

Five controlled clinical trials, 7 animal models, 7 ex vivo models, 38 laboratory studies, 3 retrospective studies, and 23 systematic reviews met the defined criteria and were included in study selection.

DISCUSSION

Formation of Biofilms

Biofilm formation represents a protected mode of growth that allows microbes to survive in hostile environments and also disperse to colonize new areas. With the use of scanning electron microscopy and, more recently, the confocal laser scanning microscope, it became clear that biofilms are not unstructured, homogeneous deposits of cells and accumulated slime but complex communities of surface-associated cells enclosed in a polymer matrix containing open water channels.[10]

The formation of these microbial accretions is a dynamic five-step process. The first substances associated with the surface of the area of colonization may actually not be bacteria but trace organics. These organics are thought to form a layer, which neutralizes excessive surface charge and surface free energy, which may prevent the initial bacterial approach, as it has been acknowledged that microorganisms attach more rapidly to hydrophobic, nonpolar surfaces.[11–13] Furthermore, these organic molecules often serve as nutrients for the attached bacteria. The rate of bacterial settling and association with the area of colonization also depends on the velocity characteristics of the surrounding liquid medium because individual cells in a liquid environment behave as particles.[14] The attachment of bacteria onto a surface initiates a cascade of changes. In fact, it has been shown that a whole different set of genes is triggered by cell attachment, which are responsible for the biofilm phenotype. A series of RNA-polymerase associated sigma factors that derepress a large number of genes have been implicated in this process[15,16] In *P. aeruginosa* biofilms grown for 6 days, only 40% of the expressed proteins were identical to the planktonic form.[17] Moreover, algD, algU, rpoS, and genes controlling polyphosphokinase synthesis were found to be up-regulated.[18] However, detailed studies of differential gene expression in *P. aeruginosa* biofilms using sophisticated DNA micro-array technology showed that, as a percentage, genes that are differentially expressed in planktonic and biofilm cells are relatively few (1%).[19] The phenotypic change is guided by an interbacterial communicating system called "quorum sensing."[20] Quorum sensing employs the use of small, diffusible molecules, members of the class of N-acylated homoserine lactones, which are released by biofilm bacteria into their local environment, where they can interact with neighboring cells.[21] Quorum sensing is crucial in determining the density of the bacterial population, and it increases locally as more bacteria attach. Regulation of this type coordinates bacterial behavior at the population level.[21] At this stage, attachment is reversible because it is based on electrostatic attraction rather than chemical bonds. However, some of the cells form structures for firmer anchoring, thus advancing in the second step of biofilm formation, the irreversible adhesion. This step requires the mediation of bacterial surface proteins, the cardinal of which is similar to *S. aureus* autolysin and is denominated AtlE.[6]

The aggregation of bacteria and the production of the EPS represent the third step of biofilm formation. In staphylococci, the EPS matrix is a polymer of β-1, 6-linked N-acetylglucosamine, whose synthesis is mediated by the ica operon.[6] The chemistry of EPS, in general, is quite complex and includes polysaccharides, nucleic acids, and proteins.[22,23] EPS polysaccharides differ between Gram-positive and Gram-negative bacteria. In the latter, bacteria polysaccharides are neutral or polyanionic. By contrast, Gram-positive bacteria have primarily cationic polysaccharides.[22] The composition and structure of polysaccharides determine the primary EPS conformation.[22]

Step 4 of the process is the maturation of the biofilm structure. The latter includes cell growth (and potential reproduction) within a given microenvironment as determined by exopolysaccharide substances, neighboring cells, and proximity to a water channel.[24] The open water channels represent a primitive circulatory system for the preservation of homeostasis within the biofilm. In the mature biofilm, more volume is being occupied by the EPS matrix (70%–95%) than by bacterial cells (5%–25%).[25] At this stage, secondary colonizers (other bacteria or fungi) can become associated with the biofilm surface.[26]

Finally, bacteria can be detached from the biofilm (step 5) either by external forces or as a part of a wave-like migrating physical movement[27] or even as a self-induced process to disseminate to the environment. Even though biofilm dispersion is an almost untouched area of research, it has been reported that the RNA-binding protein CsrA acts as an activator of biofilm dispersal in *E. coli* by way of regulation of intracellular glycogen biosynthesis and catabolism.[28]

Specific Diseases

Ear infections. Although acute infections have been associated with the planktonic form of bacteria, chronic ear infections or persistent effusions in the middle ear may very well be perceived as biofilm related diseases. Indeed, traditional culturing methods have been proven inadequate to detect many viable bacteria present in OME,[29] which is an extremely frequent situation in the pediatric population, and this has resulted in OME being questioned as a microbial inflammatory process. However, there is mounting evidence indicating the potential relationship between biofilms and OME, and this may in turn change current scientific perceptions with regard to etiology and conservative management.

Thus, mucosal biofilms formed in an experimental model of otitis media (OM) suggested that biofilm formation might be an important factor in the pathogenesis of chronic OME.[30] In addition, bacterial DNA has been found in a significant percentage of middle ear effusions sterile by culture using polymerase chain reaction (PCR)-based assay systems.[31] Although this finding is not actually a

proof of an active bacterial infectious process, the large number of bacterial genomic equivalents present in the operated ears is suggestive of an active process.[31,32] Furthermore, the presence of endotoxin (detected by the Limulus amebocyte lysate assay) has been compared with the presence of viable *H. influenza* and *M. catarrhalis* (detected by PCR) in 106 middle ear effusions from pediatric patients with COM. The results suggested that viable Gram-negative bacteria detectable by PCR, but often undetectable by culture, may be the source of endotoxin in middle ear effusions.[33] Biofilm produced endotoxins actually induce less potent host innate responses,[34] as was documented experimentally in nontypeable *H. influenza*, thus contributing to the chronicity of middle ear disease.

Nevertheless, some reservations have been expressed on whether middle ear effusions have the ability to inhibit nuclease activity, thus resulting in the detection of "fossilized" DNA remnants by PCR assays, which, in turn, can be interpreted as indications of noncultivable bacteria.[35] However, an reverse-transcription PCR-based assay system detected the presence of bacterial mRNA in a significant percentage (31%) of culturally sterile middle ear effusions, thus establishing the presence of viable, metabolically active, intact organisms in some culture-negative OME.[36] Furthermore, findings indicate that purified DNA and DNA from intact but nonviable bacteria do not persist in the middle-ear cleft in the presence of an effusion, even after high-copy inoculation. In contrast, antibiotic-treated bacteria persist in some viable state for weeks, as is evidenced by the differential ability of the PCR-based assay systems to detect the live bacteria but not detect the heat-killed organisms.[37] In any case, direct detection of biofilms on middle ear mucosa biopsy specimens, from children with OME and recurrent OM, supports the hypothesis that these chronic middle ear disorders may be biofilm related.[38]

In addition, experimentally induced chronic suppurative otitis media (CSOM) in a nonhuman primate model infected by *P. aeruginosa* in only one ear resulted in the detection of *P. aeruginosa* biofilms by scanning electron microscopy on the middle ear mucosa of the infected ear only.[39] However, it should be taken into account that in both the infected and the control ears, biofilm formation caused by cocci was also seen; this finding warrants further investigation to determine the exact role of both rod and cocci biofilms in the pathogenesis of CSOM.

Exacerbations of COM in patients with cholesteatomas may also be associated with biofilms. This notion has been initially supported by the fact that common organisms cultured from experimentally induced cholesteatomas are biofilm formers.[40] In addition, the keratin matrix of a cholesteatoma appears an ideal environment for the support of biofilm formation, and strains of otopathogenic *P. aeruginosa* isolated from cholesteatoma show firm adherence to keratinocytes.[41] Chole and Faddis[40] evaluated the histomorphologic characteristics of 24 human and 22 experimental cholesteatomas for evidence of biofilm formation. Examination using light and transmission electron microscopy revealed Gram-positive and Gram-negative bacteria within acellular deposits among the keratin accumulations in 21 of 22 gerbil and 16 of 24 human cholesteatomas. Regions of accumulated bacteria possessed the ultrastructural appearance of typical amorphous polysaccharide biofilm matrix. The authors, thus, concluded that there appeared to be strong anatomic evidence for the presence of bacterial biofilms in experimental and human cholesteatomas. As a conclusion, biofilm formation may explain the clinical characteristics of infected cholesteatomas, that is, persistence and recurrence of infection, with surgical eradication being the only effective treatment.

Refractory superinfections of either tympanostomy tubes or more sophisticated medical indwelling devices in the middle ear, such as cochlear implants or artificial ossicles, have been also attributed to biofilm formation. Indeed, various reports suggest that biofilms can form on tympanostomy tubes placed in children's ears[29] and might play a major etiologic role in post-tympanostomy otorrhea.[29,42] Biofilms may also account for the extrusion of cochlear implants or the recalcitrant infection of implanted ears, which necessitate device removal, with loss of function.[43] Scanning electron microscopy performed on cochlear implants removed from two patients because of recalcitrant infection, on two implants removed secondary to device failure, and on two devices that had never been implanted (which served, therefore, as controls) showed microorganisms and amorphous extracellular debris on the surface of the infected cochlear implants and the implants removed because of device failure. Biofilm formation was deemed definite in one infected device and possible in the other explanted devices. The never-implanted controls demonstrated microbial contamination without exopolymeric matrix, inconsistent with biofilms.[44]

Infections of the nose and throat. Although cases of paranasal sinusitis with severe suppuration are reportedly becoming less frequent, the incidence of chronic sinusitis is increasing.[45] The presence of bacterial biofilms may explain the recalcitrant nature of some forms of chronic sinusitis.[46]

In a study by Ramadan et al.,[47] specimens from five CRS patients who were undergoing functional endoscopic sinus surgery (FESS) were taken bilaterally from the ethmoid and maxillary sinuses. Electron microscopy revealed bacterial biofilms in all specimens. In addition, bacterial biofilms were identified in animals with sinuses experimentally infected with *P. aeruginosa* using scanning electron microscopy.[48] In an even larger series of patients, biofilms were demonstrated to be present in patients undergoing surgery for CRS, whereas none of the patients without CRS had any evidence of biofilm formation.[49] However, further investigation on the precise role that biofilms play in CRS is warranted because, in another study conducted by Sanderson et al.,[50] biofilms were present not only in 14 of 18 samples from CRS patients who underwent sinus surgery but also in 2 of 5 healthy control samples. Interestingly, a correlation between in vitro biofilm producing capacity of *P. aeruginosa* and *S. aureus* and unfavorable evolution after FESS was established,[51] suggesting a role for biofilm production in chronic sinusitis. Furthermore, there is evidence for the possible presence of bacterial biofilms on frontal sinus stents in patients with chronic sinusitis who underwent FESS. These stents may actually serve as biofilm reservoirs.[52]

It is also interesting that adenoid tissue removed from children with CRS had almost its entire mucosal surface covered with biofilms, whereas adenoids from children with obstructive sleep apnea had only scant coverage.[53] Therefore, biofilms in the nasopharynx of children with CRS may actually act as a chronic reservoir for bacterial pathogens, and this might explain the observed clinical benefit associated with adenoidectomy in this subset of pediatric patients (in terms of the mechanical debridement of nasopharyngeal biofilms).

The presence of bacterial biofilms within the tissue and crypts of inflamed tonsils may also explain the chronicity and recurrent characteristics of some forms of tonsillitis. Thus, biofilms from Gram-positive and Gram-negative bacteria were seen in a study conducted by Chole and Faddis[54] in 11 of 15 infected tonsils and in 3 of 4 tonsils removed because of hypertrophy using light and transmission electron microscopy. The authors, therefore, concluded that there appears to be strong anatomic evidence for the presence of bacterial biofilms in chronically diseased tonsils. However, the clinical significance of these findings remains to be determined because of the lack of controls and extensive research in the area.

Treatment

The therapeutic strategies that have served medicine so well in regard to the partial eradication of acute epidemic bacterial diseases have not yielded favorable outcomes when applied to biofilm diseases.[55] Part of this can be attributed to the fact that biofilm cells are at least 500 times more resistant to antibacterial agents,[15] potentially because of the presumed reduced rates of cellular growth and respiration of biofilm bacteria and the protection conferred by biofilm matrix polymers.[56,57] The expression of specific protective factors, such as multidrug efflux pumps and stress-response regulons, further enhance biofilm resistance against antibacterials[21,58–64] as well as plasmidial gene transfer, which is facilitated in the biofilm environment.[14] Moreover, the heterogeneity in metabolic and reproductive activity within a biofilm correlates with a nonuniform susceptibility of enclosed bacteria.[65] In biofilms, resistance appears to depend on multicellular strategies.[66] Because the numerous antimicrobials used in everyday practice act at the molecular, cellular, or organismal level, very few can actually act at the community level. This is so because it was difficult to conceive microbial communities as causative agents and to develop antimicrobials effective against them.[67] However, the recent advances in our understanding of the genetic and molecular basis of the bacterial community behavior point at therapeutic targets that may provide a means for the control of biofilm infections.[68]

Thus, the detection of two different intracellular signaling systems, lasR-lasI and rhlR-rhlI, which are involved in the development of *P. aeruginosa* biofilms, indicates signal manipulation as a possible target to control biofilm growth.[20,55] Interruption of quorum sensing and inhibition of the transcription of biofilm-controlling genes or genes involved in cell attachment might also prove to be a successful strategy in inhibiting biofilm infections by interfering with various stages of biofilm maturation. Markers associated with the detachment of individual bacteria from the biofilm could be found and potentially used to determine the status of a biofilm infection and direct the administration of the appropriate therapy.[21] In addition, the disruption of the protective extrapolymer matrix, through mechanical or chemical means, can make the biofilm more susceptible to antimicrobials and to the immune system mechanisms (i.e., phagocytosis, antibody-mediated defense, etc.)[15,68–72] In a more holistic sense, a probiotic approach, as, for instance, the colonization of susceptible mucosal surfaces with nonpathogenic bacteria that inhibit the growth of pathogens, might prove an effective antibiofilm strategy[24].

With regard to antibiotics, those with activity against nongrowing cells (i.e., fluoroquinolones) appear to be more active against biofilm bacteria compared with those which are only effective against growing bacteria (i.e., β-lactams).[73–76] Furthermore, some macrolides (i.e., clarithromycin and erythromycin) inhibit biofilm formation, possibly because of properties other than bactericidal activity. Thus, as proven in patients suffering from chronic pulmonary inflammatory syndromes, macrolides show certain immunomodulatory effects[77] mediated at least in part by effects on the activation of gene transcription mediated by nuclear factor-kappabeta activation.[78] Moreover, in vitro concentrations of macrolides below the minimum inhibitory concentration enhance the phagocyte properties of polymorphonuclear leukocytes against *P. aeruginosa* biofilms,[79] and subclinical doses of macrolides may also affect signaling within and between bacterial communities.[78] Because even drastic concentrations of macrolide antibiotics can be achieved in tissues, nasal discharge, and sputum with actual clinical doses, a potential favorable effect against biofilm infections, at least those caused by *P. aeruginosa*, can be achieved.[80]

Interestingly, some studies have shown that biofilm bacteria may be more susceptible to conventional antibiotics in direct current electric fields[81] or when treated with ultrasonic radiation.[82] Mechanical debridement of the biofilm-bearing tissues may be the ultimate solution in persistent situations.

With regard to medical indwelling devices, prevention of biofilm formation and the related refractory otorrhea in tympanostomy tubes has been attempted with the surface treatment of fluoroplastic grommets. Thus, ionized, coated fluoroplastic grommets have been considered as highly effective tubes in preventing biofilm contamination.[83] Ion-bombarded silicone might also be helpful in preventing chronic tube contamination, compared with other silicone ventilation tubes.[84,85] Moreover, albumin coating of the tubes has been shown to inhibit the binding of fibronectin on their surfaces, thus preventing the adherence of foreign material.[86]

As mentioned earlier in this paper, cochlear implant material can provide a surface for bacterial biofilm formation. Impressions in the surface of the implant appear more susceptible to biofilm establishment and growth,[43] thus necessitating further designing interventions toward preventing initial device colonization and minimizing microbial cell attachment to the device.

CONCLUSION

Modern otorhinolaryngology is facing the spread of biofilm-related infections. Although biofilms, as a concept, are relatively novel to many ENT surgeons, a basic understanding of their mode of growth and the recognition that strategies developed to treat planktonic bacteria are ineffective against bacteria in a biofilm is essential in developing rational strategies for prevention and treatment.

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