



# Biofilms in Otolaryngology

## Otolarengolojide Biofilmler

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### ABSTRACT

Biofilms have been shown to play an important role in otolaryngologic disorders. Recent advances have demonstrated that there are different bacterial species within the biofilms in different disease states. Since the recognition that chronic otolaryngologic infections are associated with biofilms, new treatment strategies with new agents have been trying to be developed for antibiotic-resistant bacterial biofilms. It has been well known that inadequate doses of antibiotic treatment can trigger biofilm formation and can lead to chronic infections. In fact, to diagnose, treat and prevent biofilm-related illnesses, different strategies are required. Determining the bacterial genetics of biofilm formation and the interaction between host and bacteria will provide substantial targets for biofilm drug therapies. The main goal of this review is to evaluate and discuss in detail some of the recent findings in biofilms-related otolaryngologic states. (*JAREM 2013; 3: 1-4*)

**Key Words:** Biofilm, otolaryngology

### ÖZET

Biofilmlerin otolarengolojik hastalıklarda önemli bir rolü olduğu gösterilmiştir. Yakın zamanda yapılan çalışmalarda farklı hastalık durumlarında biofilmlerin içinde farklı mikroorganizma türlerinin bulunduğu saptanmıştır. Kronik otolarengolojik enfeksiyonların biofilmler ile ilişkili olduğu anlaşıldıktan sonra, özellikle antibiyotiklere dirençli olduğu bilinen bakteriyel biofilmler için yeni ajanları içeren tedavi stratejilerinin geliştirilmesine çalışılmaktadır. Uygun olmayan dozlarda antibiyotik tedavisi biofilm oluşumunu ve kronik enfeksiyonların gelişimini tetikleyebilir. Biofilmler ile ilişkili hastalıkların tanısı, tedavisi ve önlenmesi için farklı stratejilere gereksinim vardır. Biofilm oluşumunun bakteriyel genetik alt yapısının saptanması ve konak ile bakteri arasındaki etkileşimin anlaşılması, biofilme bağlı enfeksiyonların tedavisi için doğru hedeflerin belirlenmesini sağlayacaktır. Bu derlemenin esas amacı biofilm ile ilişkili otolarengolojik hastalıklardaki yeni gelişmelerin detaylı bir şekilde tartışılarak değerlendirilmesidir. (*JAREM 2013; 3: 1-4*)

**Anahtar Sözcükler:** Biofilm, otolarengoloji

### INTRODUCTION

Microbial biofilms exhibit a major lifestyle instead of a planktonic life and are commonly formed on inert or living surfaces (1). A biofilm is an aggregate of microbial cells that is irreversibly attached to the surface and enclosed in a matrix which is primarily composed of self-produced extracellular polymeric substances (EPSs).

Van Leeuwenhoek, with his simple microscopes, first observed microorganisms on tooth surfaces and can be mentioned with the initial discovery of microbial biofilms. However, a detailed examination of biofilms would not be possible until the discovery of electron microscopy, which allowed high-resolution photomicroscopy at much higher magnifications than did the light microscope. At the beginning of 1973, Characklis (2) studied microbial slimes in industrial water systems and showed that they were not only very obstinate but also highly resistant to disinfectants such as chlorine. Based on observations of dental plaque and settled communities in mountain streams, Costerton et al. (3) in 1978 came up with a theory of biofilms that explained the mechanisms through microorganisms adhere to living and nonliving materials.

It can be predicted that more than 65% of all human bacterial infections such as dental caries, periodontitis, bacterial prostatitis, endocarditis and cystic fibrosis may participate in biofilm formation (4, 5).

### More Resistant Bacterial Community: To Be or Not To Be

Biofilms are initiated when planktonic bacteria attached to a biologic or inert surfaces. The attached bacteria proliferate and progress from a state of monolayer to a microcolony and then to a bigger colony, in which interbacterial communication (quorum sensing) arises. The bacteria respond collectively to express factors that are specific to the biofilm phenotype, which evoke the secretion of an exopolysaccharide matrix. This biofilm phenotype is characterized morphologically by the formation of microbial towers, which are consisted of embedded, live bacteria layers with intervening water channels. Under the appropriate environmental conditions, free-floating bacteria are released from the biofilms, and the cycle is continued at neighbouring surfaces. Approximately 80% of the world's microbial biomass exists in the biofilm state, and the National Institutes of Health estimates that more than 75% of microbial infections that occur in the human body are encouraged by the formation and persistence of biofilms (6, 7). Biofilms are the favored state of all bacterial lifestyles in nature. Bacterial populations within a biofilm, have a reduced growth rate as opposed to their planktonic lifestyle due to a nutrient limited environment (8, 9) Furthermore, they exchange genetic material at an increasing rate so that achieving favorable traits obligatory to their persistence. Bacteria in a biofilm also have substantially increased resistance to effectors of innate and acquired immunity, the action of antibiotics and osmotic, acid, and oxidative stresses as compared with planktonic cells due to its reduced growth rate

and strong physical barrier comprised by the EPS (10). Bacteria in biofilms can be up to 1000 times more resistant to antibiotic treatment than its free-floating counterparts (11).

In addition, biofilms are environments where bacteria can exchange their DNA by transfer of genetic information via plasmids to enhance variability and to achieve adaptive mutations, such as antibiotic resistance. All of these properties provide persistence of bacteria for an extensive duration of time despite antibiotic treatment, resulting in chronic disease with intermittent acute infections. Biofilms also provide a source for recurrent infections by releasing planktonic bacteria, resulting in implantation and population of new anatomic locations (12). Thereby, survival of bacteria in host is guaranteed by biofilms. Foreman et al suggested that different biofilms are associated with different disease patterns-both disease severity and surgical responsiveness (13).

#### Detection of Biofilm

Even biofilms can be detected by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (14), these techniques have limited clinical utility due to inherent problems associated with tissue preparation and sampling (15, 16). Currently fluorescent in situ hybridization (FISH)/ confocal laser scanning microscopy (CLSM) is accepted as a gold standart for detection of biofilms, due to nondestructive trait and using species-specific oligonucleotide probes (17, 18). Foreman et al. (19) recently revealed BacLight/CSLM, possessing an equivalent sensitivity of FISH/CSLM, by analyzing sinus mucosal tissues from 20 CRS patients. Recently Berk et al. (20) improved an in vivo labeling strategy to allow the extracellular matrix of developing biofilms to be visualized with conventional and superresolution light microscopy. By means of this matrix-labeling strategy it was possible to observe *V. cholerae* biofilms in real time, developing with single-protein and single-polymer precision. However, these methods are not easily carried out because of being expensive and requiring highly sophisticated equipment and well-trained staff.

#### Biofilms in Otolaryngologic Disease States

As seen in the vast majority of the infectious diseases it is not surprising to see the biofilms in otolaryngologic infections. These infections are more difficult to treat because of the highly resistant nature of the bacterial biofilms against antibiotics and host response (21). In the field of otolaryngology, biofilms have been reported in otitis media with effusion, cholesteatoma and tonsillitis, rhinosinusitis and adenoids removed from children with chronic rhinosinusitis (CRS), and they have also been isolated on some prosthetic devices, such as tracheotomy and tympanostomy tubes and cochlear implants (8, 22-25).

#### Chronic Rhinosinusitis

The higher incidence of biofilms in CRS patients suggests a role in the pathogenesis of CRS, but no correlation with severity of CRS was found (21). Bacterial biofilms that have formed on mucosal surfaces are referred to as mucosal biofilms. Bacteria of mucosal biofilms must overcome the normal airway mucociliary clearance that usually protects the upper airways and sinonasal tract. Studies have reported that the presence of biofilms is associated with worse postoperative outcomes after endoscopic sinus surgery. Therefore treatments of biofilm-associated CRS are generally aimed at impairing the biofilm life cycle (26, 27).

Increased antibiotic resistance is a trait common to biofilm bacteria. Bacteria in biofilms show 10- to 1000-fold less sensitivity to antibiotics than bacteria growing in culture. A recent study by May et al. (28) demonstrated that subinhibitory concentrations of antibiotics triggered biofilm formation in *E. coli* and the induction of antibiotic efflux pumps. This study recommends that inadequate doses of antibiotic treatment can trigger biofilm formation and lead to chronic infections.

One of the leading causes of medically refractory rhinosinusitis is the opportunistic gram-negative bacteria, *P. aeruginosa* with biofilm-forming capacity. Biofilm formation in chronic sinusitis has been also demonstrated by many other bacterial species including *S. aureus*, *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* (4).

Not only bacteria but also a variety of fungal species have been demonstrated to form biofilms in vivo and in vitro (29). Among the pathogenic fungi, *C. albicans* is the most frequently associated with biofilm formation, especially with infection of indwelling medical devices. Chandra et al. (30) showed that *C. albicans* isolates in the form of biofilm exhibited increased resistance to amphotericin B, nystatin, chlorhexidine, and fluconazole when compared with planktonic cultures. Fungal elements have been demonstrated within sinus mucosal biofilms in patients with CRS (31). Foreman et al. (32) also identified mixed bacterial-fungal biofilms from intraoperative specimens in patients with CRS. In their study, 82% patients had mixed bacterial-fungal biofilms with either *S. aureus* or *H. influenzae*, whereas the remaining patients had only fungal biofilms.

#### Biofilms in adenoids and tonsils

Adenoidectomy is considered to be beneficial in children with CRS and chronic otitis media. Recent studies identifying biofilms in adenoids may support this clinical assumption. Approximately 95% of the mucosal surface of adenoids removed from children with CRS, covered with biofilms (33).

Confocal images of adenoidal tissue from patients with otitis media exhibited the presence of biofilms from multiple species including *S.aureus*, *H.influenzae*, *M. catarrhalis* and *S. pneumoniae*.

Chole et Faddis (34) confirmed the presence of biofilms in tonsils by using CLSM with double fluorescent staining, in which 70.8% tonsil specimens removed from patients with chronic or recurrent tonsillitis, contained biofilms (35).

Toretta et al. (36) demonstrated the presence of tonsillar biofilm producing bacteria in children with recurrent exacerbations of chronic tonsillar infections and suggested that tonsillar size is an important indicator of the presence of tonsillar biofilm producing bacteria and raise the question as to whether tonsillar biofilm is a causative factor or just a consequence of recurrent exacerbations of chronic hyperplastic tonsillitis.

#### Otitis Media

Using CLSM examination, FISH and immunostaining it was demonstrated that bacterial biofilms were present on the middle ear mucosa of children with both otitis media with effusion (OME) and recurrent otitis media on the 92% middle ear mucosa specimens, supporting the concept that biofilms play a role in chronic ear infections (8).

Hoe et al. (37) demonstrated that biofilms in adenoid tissue may act as a reservoir for reinfection of the tubotympanum in otitis-prone children. Passaging of planktonic middle ear pathogens existing in resistant adenoid biofilms during a viral upper respiratory tract infection may be an important factor in the development of recurrent otitis media.

### Biofilms in Other Fields of Otolaryngology

Like all biomaterials endotracheal and tracheostomy tubes, cochlear implants and frontal recess stents are subject to formation of bacterial biofilm on their surfaces. A number of studies investigated the evidence of biofilms in these biomaterials (38-42).

Tympanostomy tube (TT) biofilm formation may lead to refractory otorrhea and occlusion. Malaty et al investigated whether TT biofilm formation may be promoted by mucus or blood exposure and they demonstrated that blood exposure during surgery enhanced *P. aeruginosa* biofilm formation on fluoroplastic tympanostomy tubes and recommended minimizing bleeding during surgery to reduce the risk of biofilm formation (43).

### Treatment of Biofilms

Several different treatment modalities have been studied against biofilms; surgery, topical antimicrobials such as mupirocin irrigation for *S. aureus* biofilm, honey for *S. aureus* and *P. aeruginosa* biofilms, surfactants such as baby shampoo and finally citric acid/zwitterionic surfactant and agents that disrupt quorum sensing such as macrolide therapy (44-50). Surgery stimulates host's defense mechanism via increasing oxygen tension and mechanically disrupting biofilms. Topical antibiotics may be more advantageous than systemic ones because systemic ones requires higher doses to achieve the desired tissue level on the target tissue that may cause side effects. Alandejani et al. (46) reported that honey was effective against *S. aureus* and *P. aeruginosa* biofilms in vitro. Krespi et al. (45) revealed a nonpharmacologic treatment method for methicillin-resistant *S. aureus* (MRSA) biofilm disruption and killing using 2 different lasers. Further highlighting is needed despite the promising potential releasing advances in the treatment of biofilm-associated diseases.

Future challenge is whether it will be possible to prevent the formation of biofilm such as preventing bacterial adhesion or to disrupt the structure of biofilms and finally to provide penetration of agents easily to the biofilm layers and kill the bacteria inside the EPS.

### CONCLUSION

In many otolaryngologic diseases biofilms were demonstrated but what triggers to initiate this biofilm formation and how to remove biofilm producing bacteria from mucosal surfaces and neighbouring anatomic locations are still in doubt, needs investigation.

### Conflict of Interest / Çıkar Çatışması

No conflict of interest was declared by the authors.  
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**Hakem değerlendirmesi:** Diş bağımsız.

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