Biofilm’s Role in Chronic Cholesteatomatous Otitis Media: A Pilot Study

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Abstract
Cholesteatoma is a destructive lesion involving the temporal bone, which may induce severe complications due to its expansion and erosion of adjacent structures. Bacterial biofilm plays a crucial role in the pathogenesis of many otolaryngologic inflammatory/infectious chronic diseases. In this pilot study, we investigated, by means of cultural examination and with scanning electron microscope, the presence of bacterial biofilm in a series of samples from the epitympanic and mastoid region in patients affected by cholesteatoma and from the promontory region in patients with healthy mucosa who were undergoing to stapes surgery. The preliminary data support the association between biofilm and cholesteatoma (81.3% of the cases) and allow us to hypothesize that keratinized matrix of cholesteatoma may represent the ideal substrate for biofilm colonization and survival; this finding is consistent with the clinical course of aural cholesteatoma, characterized by recurrent exacerbations and recalcitrant course.

Keywords
cholesteatoma, biofilm, chronic otitis media, pseudomonas aeruginosa.

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The first report of bacterial biofilm in cholesteatomas, by Chole and Faddis¹ in 2002, described the massive colonization by bacterial biofilm within human and experimental matrix of infected cholesteatoma. Gram-positive, gram-negative, and fungal pathogens have been isolated from cholesteatoma tissue; in particular, numerous biofilm-forming Pseudomonas aeruginosa colonies have been isolated.²⁻⁴

Patients and Methods
We examined cholesteatoma samples from the epitympanic and mastoid region, obtained from 15 patients (mean age, 46 years) admitted to our otorhinolaryngology unit undergoing to ear surgery (12 canal wall down and 3 canal wall up tympanoplasty; cholesteatoma group) and 10 specimens of healthy middle ear mucosa (promontory region) of 10 patients undergoing to stapes surgery (control group). This study was approved by the Ethics Committee of the Medical Faculty of the Catholic University of the Sacred Heart in Rome; all patients provided informed consent before participation.

All patients showed recurrent otorrhea associated with hearing loss for >6 months, resistant to repeated systemic antibiotic therapy. Computed tomography scan of the temporal bone demonstrated inflammatory tissue in the middle ear, with partial erosion of temporal bone. Each specimen was divided in 2 fragments. One fragment was submitted to histologic examination by means of optical microscope, and
the other fragment was fixed in Karnovsky buffer, treated, and coated with colloidal gold for SEM examination.

**Results**

All samples from the cholesteatoma group and the control group were negative at bacterial culture. At SEM examination, no evidence of bacterial biofilm was found in samples from the control group, whereas the presence of bacterial biofilms was diagnosed in 14 of 15 samples from the cholesteatoma group (81.3%). Bacterial colonies appeared as densely packed microbial cells with rod-shaped and/or spherical profiles and a variety of capsular staining patterns. Close inspection showed that the cells were embedded in a homogeneous amorphous background substance, which was well preserved in the solvent-processed tissues (*Figure 1*). The epithelium observed at SEM examination showed abundant anucleate keratin squames, gradually transformed into ciliated pseudo-stratified columnar epithelium, with no evidence of the simple squamous epithelium usually lining the middle ear. In specimens in which biofilm was detected, we found evident destruction of the ciliated epithelium, minimum residual of intact cilia, and goblet cells partially recognizable and disarrayed (*Figure 2*).

It was also observed that the biofilm was adhered to the superficial layer of the squamous debris. Only in 1 case was bacterial biofilm absent (*Figure 3*) and isolated spherical microorganisms, consistent with planktonic bacteria, were detected. Mucociliary structures appeared, in this unique sample, less damaged, and a higher number of cilia and a homogeneous distribution of goblet cells were assessed.

No statistical analysis was performed because no specimens showed positive bacterial culture. Moreover, in the control group, biofilm was not detected; these data do not allow any statistical comparison.

*Figure 1.* Evidence of bacterial biofilm adhered to the superficial layer of the squamous and densely packed microbial cells with rod-shaped and/or spherical profiles (scanning electron microscopy).

*Figure 2.* Cholesteatoma sample colonized by biofilm, with abundant anucleate keratin squames, with no evidence of the simple squamous epithelium usually lining the middle ear (scanning electron microscopy).

*Figure 3.* Single case with no bacterial biofilm (scanning electron microscopy).
Discussion

Bacterial biofilm plays a crucial role in the pathogenesis of an increasing number of otolaryngologic diseases. The role of bacterial biofilms in chronic otitis media with effusion has been described by many studies. Acquired cholesteatoma frequently becomes chronically infected, especially with \( P. \) aeruginosa. Biofilm colonization of the middle ear seems to be responsible for resistance to topical and systemic antimicrobial agents; chronically infected cholesteatomas are described as highly relapsing, rapidly progressive, and more subject to multiple surgical treatments. Increased bacterial retaining and biofilm formation are histologically found in association to massive entrapment of keratin and keratinocyte proliferation resulting in an expanding matrix with osteoclasts recruitment and bone erosion.

In our study, the high rate of bacterial biofilm (81.3%) confirms the association between bacterial biofilm evidence and cholesteatoma, even though the causal relationship remains unclear. Few authors have analyzed the relationship between disorder/absence of functioning mucociliary clearance and biofilm detection in the sinonasal region, but the behavior of mucociliary clearance in cholesteatomatous tissue colonized by biofilm is controversial. Our data support the association among ciliary damage/loss, goblet cell disarray, and biofilm detection.

Cultural results in all our samples were negative; we can assume that this result was determined by pre- and intraoperative antibiotic prophylaxis, administered to all our enrolled patients, which could invalidate cultural examination. These assumptions are also in agreement with previous reports in which the presence of bacteria was positively assessed by means of \( SEM \) and polymerase chain reaction in children affected by chronic otitis media, showing negative results at bacterial culture. Our purpose is to prove, in a future research, the direct pathogenic role in cholesteatoma of virulent species such as \( P. \) aeruginosa (wild type) by means of immunohistochemistry and polymerase chain reaction and its biofilm-forming capacity as a correlate to the clinically aggressive pattern.

Our current research demonstrates the strong association between biofilm and cholesteatoma (81.3% of the cases). SEM examination, moreover, allowed us to demonstrate that biofilm massively colonizes the keratinized matrix.

In our opinion, the keratinized matrix of cholesteatoma and the destruction of the ciliated epithelium of the respiratory tract may represent an ideal substrate for biofilm colonization and survival. The role of biofilm in maintaining active chronic inflammation in the interface between matrix and bone, its role in the active bone resorption and enlargement of cholesteatoma, and the characteristics of mucosa at sites away from the matrix need to be clarified in the future.

Author Contributions

Jacopo Galli, designed study, revised article; Lea Calò, designed study, revised article; Monica Giuliani, collected data, wrote article; Bruno Sergi, analyzed data, wrote article; Daniela Lucidi, collected data, wrote article; Duino Meucci, collected data, wrote article; Ezio Bassotti, collected data, wrote article; Maurizio Sanguinetti, designed study, revised article; Gaetano Paludetti, designed study, revised article

Disclosures

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