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Main Article

Recurrent upper airway infections and bacterial biofilms

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Abstract

Background: Bacterial biofilms identified in various medical devices used in otorhinolaryngology, including tympanostomy tubes, voice prostheses, and cochlear implants, can directly colonise mucosal tissues. The upper airways seem to be at high risk for this type of colonisation. Chronic and/or recurrent upper airway infections may be related to the complex structural and biochemical (quorum sensing) organisation of the biofilm which interferes with the activity of antibiotics (including those with proven *in vitro* efficacy), thus promoting the establishment of a chronic infection eradicable only by surgical treatment. Biofilm formation plays a role in upper respiratory infections: it not only explains the resistance of these infections to antibiotic therapy but it also represents an important element that contributes to the maintenance of a chronic inflammatory reaction.

Objectives: To document the presence of biofilms in surgical tissue specimens from patients with recurrent infection diseases, and identify their possible role in the chronicity of these infectious processes.

Method: We examined 32 surgical specimens from the upper respiratory tract (tonsils, adenoids, mucosa from the ethmoid and maxillary sinuses) of 28 patients (20 adults, eight children) with upper airway infections that had persisted despite repeated treatment with anti-inflammatory agents and antibiotics with demonstrated *in vitro* efficacy. Tissues were cultured using conventional methods and subjected to scanning electron microscopy for detection of biofilm formation.

Results: Over 80 per cent (26/32; 81.3 per cent) of the tissue specimens were culture-positive. Bacterial biofilms (associated in most cases with coccoid bacteria) were observed in 65.6 per cent of the tissue samples.

Key words: Biofilms; Tonsil; Adenoids; Paranasal Sinuses; Otolaryngology

Introduction

In most natural ecosystems, the majority of bacteria exist within highly structured communities enclosed in a self-produced polymeric matrix that is irreversibly associated with the surface of an inert material or living tissue.[1] These microbial communities, which are known as biofilms, were first described in the seventeenth century by Van Leeuwenhoek, whose simple light microscope allowed him to observe the micro-organisms within the dental plaque.[2] However, the clinical significance of biofilm formation was not fully appreciated until the late twentieth century.[2]

The formation of a biofilm requires co-ordinated chemical signalling between cells. Under conditions of low bacterial density, the costs of biofilm production to an individual bacterium outweigh the benefits, and planktonic (free-living) growth predominates. The 'decision' to produce a biofilm thus depends on the presence of a sufficiently large bacterial burden, and the process by which this presence is ascertained involves the secretion of small signalling molecules known as quorum sensing.[3]

The sessile bacterial populations within biofilms are profoundly different from their planktonic counterparts. The hydrated matrix of polysaccharides and proteins mediates bacterial adhesion to a variety of surfaces, including living tissues, but it also provides protection from unfavorable external conditions.[2] Furthermore, the oxygen and nutrient requirements of biofilm-associated microbes are significantly reduced, and they also present distinct gene transcription profiles. One of the results of these adaptations is increased resistance to antibiotics.[4]

Bacteria such as *Pseudomonas* species, *Staphylococcus* species, and *Haemophilus influenzae*, are well known for their capacity to adhere to inert materials as well as living tissues. The Centers for Disease Control and Prevention have recently estimated that at least 65 per cent of all human bacterial infectious processes involve biofilm formation,[5] and this phenomenon has been linked to the chronicity of numerous infections.

The purpose of our study was to identify bacterial biofilms in surgical tissue samples obtained from patients with chronic and/or recurrent upper airway infections and to identify their possible relation to the chronicity of these infectious processes; furthermore, the same bacteria identified in the cultural

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examinations have been studied to show their ability to grow as biofilm.

Materials and methods

We examined 32 samples of upper respiratory tract tissues obtained from 20 adults (mean age 45 years) and eight children (mean age six years) consecutively admitted to the ENT ward of the 'A. Gemelli' Medical Center of the Catholic University of the Sacred Heart.

This study was approved by the Ethics Committee of the Medical Faculty of the Catholic University of the Sacred Heart in Rome, and patients gave their informed consent before participation.

All 28 were suffering from recurrent and/or chronic upper airway infections documented by clinical findings, blood chemistry data, and the results of computed tomography (CT) scans. The infections had proved to be refractory to repeated cycles of antibiotic regimens with demonstrated *in vitro* potency, and surgical treatment was thus being planned. The open procedures performed included adenotonsillectomy (four patients), adenoidectomy (four patients), and tonsillectomy (four patients). Functional endoscopic sinus surgery was performed in the remaining 16 cases (anteroposterior ethmoidectomy in 10 patients, medium maxillary antrostomy in six). Four of the six who underwent antrostomy had a previous diagnosis of allergic rhinitis (based on clinical findings and positive allergy tests).

Thirty-two tissue samples were collected: adenoid tissues ($n = 8$), tonsillar tissue ($n = 8$), and mucosa from the ethmoid ($n = 10$) and maxillary ($n = 6$) sinuses. Each specimen was washed in a sterile saline solution and divided into two fragments. One fragment was processed for bacterial cultures, which were analysed with the API 20 STREP, API ID 32 STAPH and API NH systems (all from Bio Merieux; Marcy-l'Etoile, France). The other (which varied in size from 3.0 × 3.0 mm to 5.0 × 5.0 mm) was subjected to scanning electron microscopy for detection of biofilms. The latter fragments were fixed in Karnosvsky buffer (2.5 per cent glutaraldehyde, 1.5 per cent paraformaldehyde, 0.1 M cacodylate, and 0.05 M sucrose) for two hours at 4 °C, washed in sodium cacodylate for five minutes, and immersed in 1 per cent osmium tetroxide for one hour. After a second five-minute wash in sodium cacodylate, the tissues were dehydrated in a graded ethanol series (50 per cent, 79 per cent, 95 per cent, 100 per cent), dried with liquid CO_2 (Dried Balzers CPD 030), lightly coated with colloidal gold, and examined with a Philips 515 scanning electron microscope.

Results

The results are summarised in Table I. Twenty-six of the 32 tissue samples (81.2 per cent) were culture-positive. We identified biofilms in 21 of the 32 (65.5 per cent) specimens. Bacterial colonies appeared as densely packed microbial cells with rod-shaped and/or spherical profiles and a variety of capsular staining patterns. Close inspection revealed that the cells were embedded in a homogeneous amorphous background substance, which was well preserved in solvent-processed tissues (Figure 1). Bacterial biofilms were not observed in tissues obtained from the four patients with a history of allergic rhinitis and in five of the six specimens collected from patients with maxillary sinusitis alone.

Cultures of tonsillar tissues carried out using conventional methods yielded *Staphylococcus aureus* (three specimens) or alpha-haemolytic *Streptococcus* (five specimens). Adenoid tissues grew *Haemophilus influenzae* (six specimens), *Streptococcus pyogenes* (one specimen), and alpha-haemolytic *Streptococcus* (one specimen). Specimens of mucosa from the ethmoid sinus grew *Moraxella catarrhalis* (six of 10 samples), *Haemophilus influenzae* (three samples) or alpha-haemolytic *Streptococcus* (one sample). *Streptococcus pyogenes* was recovered from two of the six maxillary sinus tissue samples and four others yielded *Haemophilus influenzae* (Table I).

At scanning electron microscopy observation, in three of the eight samples of maxillary sinus mucosa, ciliary epithelium was intact and well represented, in spite of the positive cultural examination; tissue samples obtained from the other sinonasal mucosa revealed squamous cell metaplasia, with epithelial degeneration associated to cilia loss.

Discussion

Microbial biofilms seem to play important roles in a large number of human infections, including those associated with contamination of central venous lines and endotracheal and tracheostomy tubes. Bacterial emboli originating from these biofilms can enter the bloodstream and cause fever or prolonged septic conditions.[5,6] Even if they do not provoke sepsis, biofilms associated with medical devices can cause functional problems that may require complete replacement of the device. Biofilms have been identified in various medical devices used in otorhinolaryngology,[7] including tympanostomy tubes, voice prostheses, endotracheal tubes of patients admitted to the intensive care unit[8–10] and cochlear implants[11] removed because of recurrent infections.[12] Biofilms can directly colonise mucosal tissues, producing chronic and/or recurrent infections that are resistant to all types of antibiotic treatment. The upper airways seem to be at high risk for this type of colonisation. They have been documented in the nasal and sinus mucosa of subjects with chronic hyperplastic sinusitis[13–17] and in the tonsillar crypts.[4] Furthermore, Wang *et al.*[18] have demonstrated that variant strains of otopathogenic *P. aeruginosa* are capable of forming biofilms on cholesteatomas, which explains persistent infection that can be eradicated only by surgery. Biofilm bacteria are more resistant to immunity clearance mechanisms and to antibiotics compared to the planktonic bacteria.[19,20]

In our study, *Haemophilus influenzae* was the most commonly isolated bacterium (61.5 per cent of the total positive cultural examinations) and was prevalent in adenoid tissue and in maxillary sinus mucosa; *M. catarrhalis* was recovered from six of the 10 samples of ethmoid mucosa tissue, and

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TABLE I

BIOFILM FORMATION AND BACTERIAL CULTURE RESULTS IN 32 SPECIMENS OF CHRONICALLY INFECTED UPPER RESPIRATORY TRACT TISSUES

Tissue samples	*n* (%) of samples with biofilms	*n* (%) of samples with positive cultures	Micro-organisms isolated (*n*)
Adenoid ($n = 8$)	8 (100%)	7 (86.5%)	*H. influenzae* (6), *S. pyogenes* (1) Alpha-haemolytic *Streptococcus* (1)
Tonsil ($n = 8$)	5 (62.5%)	5 (62.5%)	*S. aureus* (3) Alpha-haemolytic *Streptococcus* (5)
Ethmoid sinus mucosa ($n = 10$)	7 (70%)	8 (80%)	*M. catarrhalis* (6) *H. influenzae* (6) Alpha-haemolytic *Streptococcus* (1)
Maxillary sinus mucosa ($n = 6$)	1 (16.7%)	6 (100%)	*S. pyogenes* (2) *H. influenzae* (4)
Total ($n = 32$)	21 (65.6%)	26 (81.25%)	

alpha-haemolytic *Streptococcus* in five of the eight tonsillar specimens. Moreover, bacterial biofilms were found in well over half of the tissue specimens collected from patients undergoing surgery for the eradication of chronic upper respiratory tract infections.

These data led us to suppose that bacteria from biofilms, within the tissues of chronically infected adenoids, tonsils and ethmoid sinus mucosa, can resist being eradicated by antibiotics and host defences. Data obtained from patients suffering from maxillary sinusitis alone are more difficult to interpret. In such cases (six), the sinusitis was documented by marked opacity on CT, and the sinus mucosa obtained during endoscopic maxillary antrostomy consistently yielded positive cultures. However, biofilm formation was demonstrated in only one of the maxillary sinus mucosa specimens obtained during surgery. It is possible that these findings are a reflection of the shorter period of infection diagnosed early due to the routine use of CT. Moreover, the maxillary sinus mucosa in three of the six samples without biofilm evidence, was characterised by well represented cilia in the respiratory epithelium (at the scanning electron microscopy examination) (Figure 2), compared to the absence of cilia in the other samples of nasosinusal mucosa with evidence of biofilm (Figure 3). In the literature Chun *et al.* found that differentiated human airway epithelia can inactivate quorum sensing molecules of *P.aeruginosa*, the main opportunistic pathogenic bacterium that causes a variety of infections, especially in the

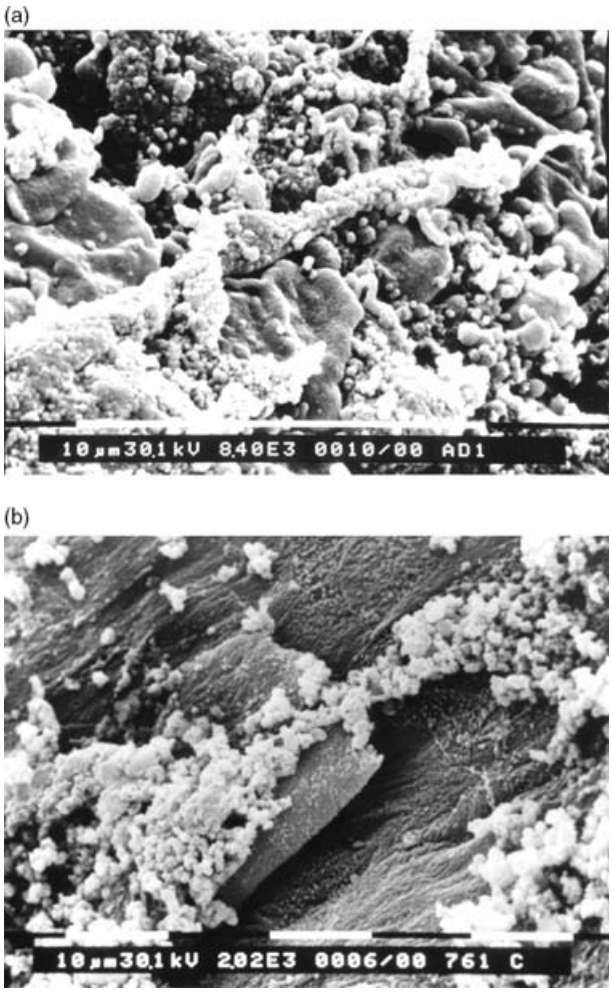

FIG. 1

Scanning electron microscopy observation of bacterial biofilm in adenoid (a) and tonsil (b) tissue samples.

FIG. 2

Scanning electron microscopy observation of maxillary sinus mucosa with positive cultural examination and without evidence of biofilm, characterised by well represented cilia in the respiratory epithelium.

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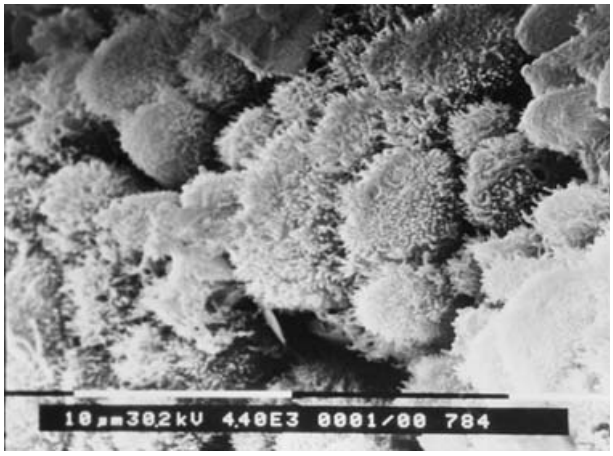

FIG. 3

Scanning electron microscopy observation of ethmoid mucosa tissue sample, with evidence of bacterial biofilm and epithelial degeneration associated with cilia loss.

pulmonary airways of patients with cystic fibrosis.[21] We could suggest that normal upper airways epithelium can be an obstacle to the growth of biofilm, but also these data require further confirmation in larger case studies.

- **Microbial biofilms play an important role in a large number of human infections, including those associated with contamination of central venous lines and endotracheal and tracheostomy tubes**
- **Biofilms can directly colonise mucosal tissues, producing chronic and recurrent infections that are resistant to all types of antibiotic treatment**
- **In this study bacterial biofilms were found in well over half of the tissue specimens collected from patients undergoing surgery for the eradication of chronic upper respiratory tract infections**

In conclusion, our initial experience, which is consistent with data in the literature, indicates that biofilm formation plays a role in upper respiratory infections: it not only explains the resistance of these infections to antibiotic therapy but it also represents an important element that contributes to the maintenance of a chronic inflammatory reaction.

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