

Main Article

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Address for correspondence:

Dr Stine A Schrøder, Department of Otorhinolaryngology, Nordsjaellands University Hospital, Hillerød, Denmark
Fax: +45 4829 3811
E-mail: stine.eva.attrup.schroeder@regionh.dk

Morphological evidence of biofilm in chronic obstructive sialadenitis

S A Schrøder^{1,2}, S Eickhardt³, T Bjarnsholt^{3,4}, T Nørgaard⁵ and P Homøe⁶

¹Department of Otorhinolaryngology, Nordsjaellands University Hospital, Hillerød, Departments of ²Otorhinolaryngology Head and Neck Surgery, and ⁵Pathology, Rigshospitalet – Copenhagen University Hospital, ³Costerton Biofilm Center, Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, ⁴Department of Clinical Microbiology, Copenhagen University Hospital, and ⁶Department of Otorhinolaryngology and Maxillofacial Surgery, Zealand University Hospital, Køge, Denmark

Abstract

Objective. Bacterial infection is a common finding in acute sialadenitis and may play a role in the chronicity of the condition. This study investigated if bacterial biofilm is present in submandibular chronic obstructive sialadenitis.

Methods. A descriptive case–control study was conducted that compared 10 histological sections of submandibular glands with chronic obstructive sialadenitis, to 10 histological sections of the healthy part of submandibular glands with pleomorphic adenoma. Fluorescence in situ hybridisation and confocal laser scanning microscopy visualised evidence of bacterial biofilm.

Results. In the chronic obstructive sialadenitis group, 5 out of 10 histological sections showed morphological evidence of bacterial biofilm. In the control group, there was no sign of bacterial biofilm formation.

Conclusion. Morphological evidence of bacterial biofilm was found in the submandibular gland sections from patients with chronic sialadenitis and suggests a role in the chronicity of submandibular chronic obstructive sialadenitis.

Introduction

Sialadenitis is an acute or chronic inflammatory and infectious condition of the salivary glands causing swelling and pain of the affected gland. Sialadenitis constitutes one of the most frequent clinical conditions of the salivary glands.¹ Classification of sialadenitis is based on aetiology and pathophysiology, and includes bacterial, viral, immune or granulomatous causes.²

Obstructive salivary gland diseases such as sialolithiasis are a common course of chronic sialadenitis of the submandibular gland.^{2,3} Obstruction of the duct reduces the flow of saliva. For chronic obstructive sialadenitis, two factors seem to be important: reduced salivary flow, and retrograde contamination of the salivary gland and duct with bacteria from the oral cavity.³

Bacteria either occur as free-swimming ‘planktonic’ or aggregated ‘sessile’; the latter is also known as biofilm.⁴ Biofilms are increasingly important in chronic infections,⁵ including those of the ENT region.⁶ Micro-organisms are also proposed to contribute to the formation of sialoliths.⁷ In addition, bacterial biofilms have recently been suggested as a pathogenic factor in sialolithiasis.⁸ However, it is not known whether biofilms are involved or present in chronic obstructive sialadenitis of the submandibular gland.

This descriptive case–control study aimed to test the hypothesis that biofilm is morphologically present in histological submandibular gland sections from patients with chronic obstructive sialadenitis.

Materials and methods

Study population

The study comprised patients who, between 2012 and 2013, suffered from submandibular chronic sialadenitis, had an additional diagnosis of sialolithiasis, and who underwent submandibular excision at one of two hospitals. Patients were selected longitudinally.

The inclusion criteria were: submandibular chronic sialadenitis diagnosis for at least one year, with an additional diagnosis of sialolithiasis showing typical symptoms such as pain and recurrent swelling. Furthermore, endoscopic treatment and/or sialdochotomy in the included patients had failed or had been considered not feasible. The exclusion criteria were: acute infection, age of less than 18 years and antibiotic treatment during the previous 3 months.

The surgical procedure for the gland excision was a classic submandibular adenectomy. The gland was fixated in formalin immediately after the excision. The location of the

TABLE I OVERVIEW OF PATIENT DEMOGRAPHICS, SYMPTOM DURATION AND TREATMENT

Gender	Age (years)	Symptom duration (years)	Endoscopic or surgical intervention	Other treatment	Biofilm confirmed
Female	34	1.5	Sialendoscopy × 1, combined approach × 1	Antibiotic × 4	No
Female	65	1	No previous endoscopic treatment	None	No
Female	26	4	Sialendoscopy × 1	Antibiotic × 2	No
Male	21	1	Sialendoscopy × 1	Antibiotic × 6	No
Female	39	1	No previous endoscopic treatment	None	No
Male	55	2	No previous endoscopic treatment	None	Yes
Male	65	1	No previous endoscopic treatment	Antibiotic × 2	Yes
Female	43	1	Sialendoscopy × 1, sialodochotomy × 2	Antibiotic × 3	Yes
Male	60	3	No previous endoscopic treatment	None	Yes
Male	38	15	Sialendoscopy × 1, sialodochotomy × 2	Antibiotic × 3	Yes

ex vivo histological section of the gland was randomly chosen, and was taken either from the gland poles or the central parts of the gland.

Within the same study period, from the same two hospitals, *ex vivo* sections of submandibular glands (adenectomised because of pleomorphic adenoma) were included as a control group, from patients matched by gender and age. These sections were taken solely from the healthy part of the glands (sections did not include the pleomorphic adenoma).

The local ethical committee of the Capital Region of Denmark approved the study (H-4-2011-022).

Biofilm analysis

Biofilm was visualised by conventional light microscopy, as well as fluorescent bacterial probes (peptide nucleic acid fluorescence *in situ* hybridisation 'PNA-FISH') and subsequent confocal laser scanning microscopy. Samples were stained with a peptide nucleic acid fluorescence *in situ* hybridisation probe UniBac targeting bacteria in general (red) (AdvanDx, Woburn, Massachusetts, USA). To detect DNA, DAPI (4',6-diamidino-2-phenylindol) was used as a counter stain. The manufacturer's instructions were followed for the staining procedure. Bacterial biofilm was defined as aggregated bacteria, with each bacteria being 1–2 µm in size, morphologically either cocci- or rod-shaped, and fluorescent only in the spectrum of the fluorophore used.

All samples were examined blinded and independently by a physician (SAS) and a specialist (SED) in the peptide nucleic acid fluorescence *in situ* hybridisation method. Comprehensive microscopic analyses of the sections were made to ensure that the bacteria identified during confocal laser scanning microscopy investigation were within the tissue and not due to contamination from outside the tissue.

Statistics

Fisher's exact test was used to compare the groups, and the level of significance was set to $p < 0.05$.

Results

Population

The sialadenitis group comprised 10 patients suffering from chronic obstructive sialadenitis of the submandibular gland;

5 were male and 5 were female. The patients' median age was 41 years (range, 21–65 years). The control group comprised 10 patients with pleomorphic adenoma; 5 were male and 5 were female. Median age was 40 years (range, 22–65 years). The patients' demographics and treatment are summarised in Table I.

Biofilm

Five of the 10 submandibular glands (50 per cent; 95 per cent confidence interval = 24–76 per cent) from patients with chronic obstructive sialadenitis showed morphological evidence of bacterial biofilm, both in terms of biofilm formation with aggregation of bacteria, and immune response (Figures 1 and 2). There was no discrepancy of the results between the two examiners.

Based on size and shape, the bacteria in the biofilms morphologically resembled cocci. In all five glands, the biofilms were found within the tissue of the gland and surrounded by inflammatory cells, primarily polymorphonuclear leukocytes (Figures 1 and 2).

All 10 histological sections from the control group (comprising non-sialadenitis submandibular glands) were without morphological signs of bacterial biofilm. In the sialadenitis group, the number of biofilm positive sections was significantly higher than in the control group ($p = 0.03$). In four sections (three from sialadenitis patients and one from a control patient), bacteria were found lining the tissue; this was interpreted as contamination because of the location and morphological appearance.

No differences were found between the cases in which biofilm was confirmed and not confirmed in the sialadenitis group with respect to age ($p = 0.1$), gender ($p = 0.2$), duration of symptoms ($p = 0.4$) and endoscopic intervention ($p = 1$) (Table I).

Discussion

Bacterial biofilm is reported as a factor in many chronic infections.⁹ In this descriptive study with controls, we demonstrated morphological evidence of bacterial biofilm in half of the histological submandibular sections investigated from the sialadenitis group, but in none from the non-sialadenitis group (Table I).

Morphologically, the bacteria in the biofilms resembled cocci, which is in accordance with non-haemolytic

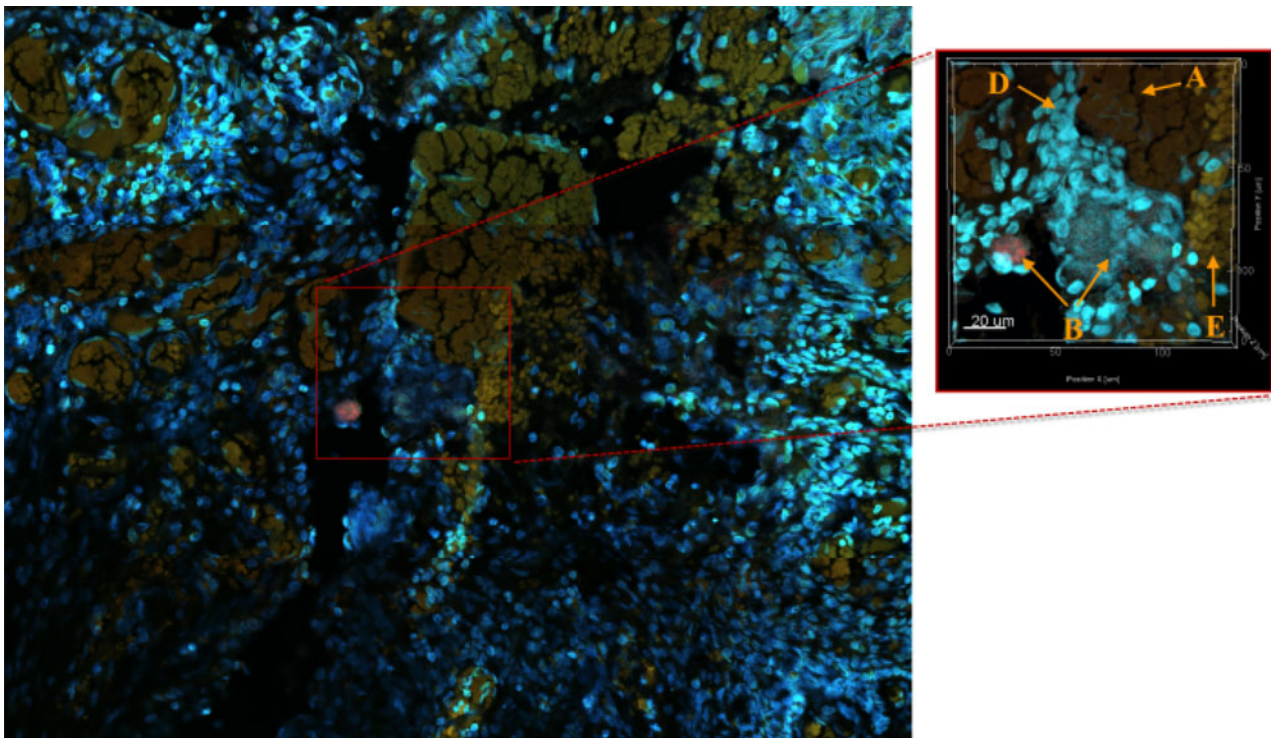


Fig. 1 Images show a section of the submandibular gland (sialadenitis group) stained with peptide nucleic acid fluorescence in situ hybridisation (universal bacterial probe (Texas red) and DAPI as a counter stain (blue); magnification $\times 63$). 'D' = DAPI-stained nucleus of immunological cells; 'A' = glandular acini (golden yellow area); 'B' = fluorescent bacterial biofilms (violet and more intense red colour); 'E' = erythrocytes

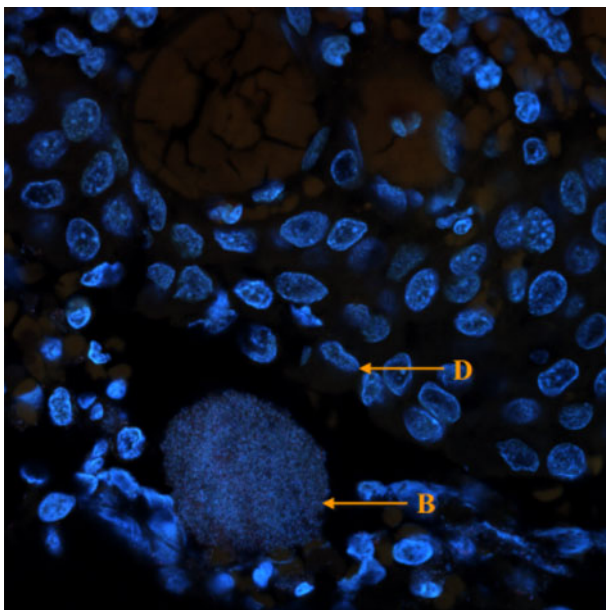


Fig. 2 Magnification of a section of the submandibular gland from a different patient (sialadenitis group) showing bacterial biofilm ('B') surrounded by DAPI-stained immunological cells ('D'). The section is stained with peptide nucleic acid fluorescence in situ hybridisation (universal bacterial probe (Texas red) and DAPI as a counter stain (blue); magnification $\times 63$).

streptococcus being one of the most abundant bacteria of the oral cavity¹⁰ and *Staphylococcus aureus* being frequently linked to acute bacterial sialadenitis.³

Sialadenitis is a common clinical condition of the salivary glands.¹ Bacterial sialadenitis is typically considered a consequence of ascending bacterial infection and reduced salivary flow, and is known to be linked to the obstructive disease sialolithiasis.³ Thus, sialolithiasis may be considered a consequence

of sialadenitis or vice versa.^{8,11} Biofilm has recently been identified inside a sialolith,⁸ supporting the notion that bacteria in the gland duct are causal to the sialolith rather than secondary to duct obstruction.

Treatment of chronic obstructive sialadenitis is often conservative, with massage, saliva stimulation and antibiotics given for acute exacerbation. However, if a sialolith is present, treatment may include stone removal, either endoscopically or by open approach surgery. Chronic, intractable sialadenitis is managed by surgical excision of the entire gland.^{3,12} Recurrent infection is not a rare event, and biofilm in the glands may contribute to this. In the present study, 5 of the 10 sialadenitis patients had previously undergone sialendoscopy and/or sialodochotomy (Table I), and it could be speculated that previous surgical or endoscopic intervention introduced bacteria in the duct system and promoted biofilm formation. However, there was no difference in the number of biofilm cases between patients who had previously undergone endoscopic or surgical treatment and those who had not. Prior antibiotic treatment also did not seem to influence the presence of biofilm (Table I).

The strength of the study is the direct morphological evidence of bacterial biofilm formation in the submandibular gland excised because of chronic sialadenitis with sialolithiasis. The biofilms identified are unlikely to be a result of contamination as they were present within the tissue. It should be stressed, however, that the presence of bacterial biofilm does not constitute proof of causality for chronic sialadenitis.

Regarding the study limitations, the sample size was somewhat small, only one sample was available from each patient and the negative findings in the control group may be a consequence of the limited material available. Furthermore, only a eubacterial probe was used, and no culturing or other molecular methods were utilised to confirm bacteria in the samples.

The observation of biofilm in the submandibular gland suggests that early and aggressive antibiotic treatment, together with stone removal, may prevent and/or postpone recurrent chronic obstructive sialadenitis. Inasmuch as the biofilm per se is challenging to treat with the normal regimen of antibiotics, the presence of biofilm in sialolithiasis may have prognostic implications.

In conclusion, we present morphological evidence of bacterial biofilm formation in histological sections of submandibular glands from patients suffering from chronic obstructive sialadenitis. The results indicate that biofilm may play a role in the pathogenesis, recurrence and/or chronicity of chronic obstructive sialadenitis of the submandibular gland.

- Bacteria and reduced salivary flow are associated with acute sialadenitis
- Bacterial biofilm was found in sections of the submandibular gland with chronic obstructive sialadenitis
- It is speculated that biofilm may play a role in the chronicity of sialadenitis

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Competing interests. None declared

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