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A scanning electron microscope characterisation of biofilm on failed craniofacial osteosynthesis miniplates

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ABSTRACT

Introduction: Between 3 and 18% of craniofacial osteosynthesis plates are removed due to chronic infection. Removal of the plate is necessary to manage the chronic infective state i.e. miniplate removal results in resolution of the infection. These observations are suggestive of a biofilm-related infection. The aim of this retrospective study was to characterise the presence of biofilm on the removed miniplates from oral and maxillofacial surgery.

Materials and methods: A total of 12 plates and associated screws were recovered from eleven patients suffering from persistent, trauma site infection. The recovered plates plus 1 control plate were imaged using scanning electron microscopy (SEM). One recovered plate was also imaged using confocal microscopy (CM) for comparative purposes.

Results: Of the 12 plates, 3 (25%) demonstrated highly localised polymicrobial biofilms, five (42%) demonstrated coccal biofilms, one possessed a filamentous biofilm and one showed attached yeast. Overall, 75% of the plates and 82% of the patients exhibited evidence of biofilm to varying degrees. All of the infections resolved following removal of the plates and antibiotic treatment.

Conclusion: Microbial biofilms can explain the clinical course of chronic infections associated with miniplates.

1. Introduction

Titanium miniplates are used in the routine practice of oral and maxillofacial surgery for a variety of reasons. The more common uses are following trauma or orthognathic surgery and the majority of these plates do not require removal due to good biocompatibility. However, plate removal rates due to complications are between 3 and 18% (Brown et al., 1989, Mosbash et al., 2003, Bhatt et al., 2005, Rallis et al., 2006, O'Connell et al., 2009, Kuhlefelt et al., 2010, Kyrgidis et al., 2013) and infection is the primary cause for plate removal (Rallis et al., 2006, O'Connell et al., 2009). The causative pathogen is reported to be predominantly *Staphylococcus aureus* (Yamamoto et al., 2013). Patients present with classical symptoms of infection; defined as swelling, pain, wound dehiscence and discharge of pus in the region of the plate (Theodossy et al., 2006).

The characteristics of the infection course are in concordance with those resulting from biofilm infection (Hall-Stoodley and Stoodley, 2009, Parsek and Singh, 2003). The infection is localised, persists despite antibiotic therapy, causes chronic inflammation and is associated with a foreign body (Hall-Stoodley and Stoodley, 2009). A biofilm is defined as an 'aggregate of microbial cells adherent to a living or non-living surface, embedded within a

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matrix of extracellular polymeric substances (EPS) of microbial origin' (Donlan, 2001); the EPS slime contributes to strong adherence of biofilms to surfaces and the resulting biofilm infection is notoriously intractable (Kobayashi, 2001).

Biofilms have been previously identified in knee, hip and elbow arthroplasty infections (Stoodley et al., 2011, Kathju et al., 2009). A study on a failed total ankle arthroplasty revealed that bacterial biofilms were present on the prosthesis and the periprosthetic tissue despite the fact that the clinical specimen culture was negative on preoperative aspiration (Stoodley et al., 2011). The authors were able to confirm their hypothesis that biofilms might be an underlying cause of the infection by using a culture independent approach of direct imaging with confocal microscopy and a PCR-mass spectrometry assay for pathogen DNA. A similar approach, used to detect bacterial biofilms in chronic surgical site infections, found a polymicrobial biofilm, associated with sutures used in hernia repair (Kathju et al., 2009) as well as a clinical culture negative ventricular bacterial surveillance drain (Stoodley et al., 2010). Scanning electron microscopy has also been pioneered as being reliably diagnostic of biofilm infections associated with clinical specimens, by the late Professor J.W. Costerton (Gristina and Costerton, 1985). In additions, biofilms have been found associated with various infections in the neck and head region including otitis media, rhinosinusitis, adenoiditis dental disease (Post et al., 2007). In ossicular reconstruction prostheses, biofilms have been identified as forming on both titanium and plastic prostheses. The biofilm was identified using electron microscopy and quantitative microbiology (Jaryszak et al., 2009).

It is well established that dental biofilms are formed within the oral cavity and are a recognised cause of oral disease e.g. caries, gingivitis, periodontitis (Taubman et al., 2005, Ferraro et al., 2007) and that dental biofilms can infect dental implants (Lang et al., 2002) and that osteosynthesis repair plates and miniplates are frequently infected but, to our knowledge there have been no reports on biofilm as being a possible underlying cause of the infection (PubMed data base search using keys words "biofilm" combined with "maxillofacial", "osteosynthesis", "plate" or "miniplate" alone or in combination returned zero citations).

We designed a non-interventional observational retrospective study where retrieved miniplates and screws were collected over a year (2012) from 11 consecutive patients who had previous zygomatic or mandibular fracture or undergone osteotomy, to test our hypotheses that biofilm on the foreign body might be involved in the disease process. We used scanning electron microscopy (SEM) examination of the retrieved hardware for the detection of possible biofilms. Confocal microscopy was used for one miniplate to support the SEM imaging.

2. Materials and methods

2.1. Patient enrolment and clinical details

The study submission Biofilm Characterisation Number: 3771 was approved by the Ethics Committee of the Faculty of Medicine, University of Southampton, on January 20 2012. In total a consecutive series of 11 patients undergoing the routine removal of miniplates as a result of chronic, antibiotic-resistant infection in the locality of the implant at the departments of Oral & Maxillofacial Surgery at Southampton General Hospital and Queen Alexandra Hospital, Portsmouth during 2012 were consented for the study (Table 1). The patients were assessed in an outpatient clinic setting due to ongoing symptoms and removal of the osteosynthesis plates was indicated to resolve the infection. For seven of the patients where we had reliable records the median and average time taken for symptoms to develop after plate placement was 3 and 18 months respectively. The range was from 7 days to 8 years. Symptoms experienced included swelling, tenderness and wound dehiscence. Within this patient cohort, the primary reason for initial plate insertion was mandibular fracture (50%). In 11 cases chronic infection and in 1 case abscess formation indicated plate removal. There was successful union in 8 out of the 9 plates for which this information was available.

Retrospective analysis of the case notes was performed, data was collected and Microsoft Excel software was used for demographic analysis. For the data collection, the parameters included were; patients hospital number and date of birth, age, gender, smoking status, indication and date of plate insertion, reason and date of plate removal, time period (in months) from date of plate insertion to reported symptoms, antibiotic treatment regime, union at site, clinical signs, antibiotic treatment given during plate removal and length of use, inflammatory markers at the time of plate removal (CRP), white blood cell count at time of removal and any co morbidities (Table 1).

2.2. Sample Preparation

Following removal of the miniplates and screws, they were rinsed gently with 10% formalin to remove loosely attached material and stored in a specimen container containing 10% formalin fixative.

2.3. Imaging the Samples for Biofilm

To image the plates three imaging modalities were used. Firstly the plates were imaged at 1200 dpi in a pool of fixative on the bed

Table 1
Relevant clinical patient data and samples collected.

Patient/sample number	Samples	Gender	Smoker	Indication for insertion	Indication for removal	Antibiotics	Union?
P1/S1	1 plate	–[a]	–	–	–	–	–
P2/S2	1 plate + 3 screws	M	Y	Mandibular fracture	Infection	–	Y
P2/S3	1 plate + 4 screws						N
P3/S4	I plate + 5 screws	F	N	Zygomatic fracture	Infection	Augmentin®	Y
P4/S5	1 plate + 3 screws	M	N (ex smoker)	Mandibular osteotomy	Infection	–	Y
P5/S6	1 plate + 4 screws	F	N	Le Fort osteotomy	Infection	–	Y
P6/S7	1 plate + 4 screws	M	N	Mandibular fracture	Infection	–	Y
P7/S8	1 plate + 1 screw	M	N	Mandibular fracture	Infection	Augmentin®	Y
P8/S9	1 plate + 3 screws	F	Y	Mandibular osteotomy	–	–	–
P9/S10	1 plate + 1 screw	F	–	Mandibular fracture	Infection	–	Y
P10/S11	1 plate	–	–	–	–	–	–
P11/S12	1 plate	M	–	Zygomatic fracture	Infection	Augmentin®	Y
CONTROL	1 plate						

[a] – = not known.

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