

Case Report

Bacterial Biofilm Persistence in Human Jawbone Following Tooth Extraction: Implications of Surgical Debridement and Resident Population-Shift for Oral Implants

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Abstract: Intra-radicular infection theory assumes that when an infected tooth is extracted, the main source of infection is removed and any residual contaminating bacteria are killed by the immune response, then normal healing ensues. This study investigated whether bacteria and bacterial biofilms persisted in deep bone space following tooth extraction. The study was conducted on two adult male patients seeking dental implant therapy in a private practice setting. The cases presented as (1) A single-tooth with a chronic lesion in the anterior maxilla and (2) As a mandibular full-arch where the few remaining teeth presented endodontic/periodontic lesions, while odsteolytic/osteosclerotic lesions persisted in the edentulous, apparently healed bone. Bone fragments were obtained during full-flap, sterile surgical debridement/osteotomy, for Scanning Electron Microscopy (SEM) and pyrosequencing analysis. SEM images visually confirmed the presence of bacteria and bacterial biofilms in deep apparently healed bone space in health and disease. The extraction of diseased teeth did not result in the spontaneous resolution of pathologically altered bone surfaces. Severely sclerotic bone in both cases required multiple surgical debridements to attain a vascularised health margin beyond sclerotic encapsulation with reconnection to stem-cell-rich periosteum. The clinical effect of debridement was microbial population shift, with histologic regeneration of internal osseous architecture and bone quality, according to habitat fitness. Longitudinal same-site intracommunity extinctions and additions were corroborated by SEM and microbiome analysis, together with clinical and radiographic evidence. The circumvention of resident pathogenic bacterial biofilms before implant deployment becomes a method-protocol for biofilm-based osseointegration, improving outcomes and reducing re-operations.

Keywords: Bacterial Biofilm, Human Jawbone, Dental Implant, Scanning Electron Microscopy, Surgical Debridement, Population-Shift

Introduction

In a previous culture-based microbiological investigation, live bacterial culture was detected in 21% of apparently healed jawbone osteotomies (Nelson and Thomas, 2010). The bacteria present in these apparently healed ridges were not confirmed as biofilm, but their apparent chronicity and persistence

were highly correlated with biofilm phenotype. Lately, an update of the biofilm conceptual model is expanded to include not only surface-associated aggregations but non-surface-associated biofilms which included bacteria forming aggregate microcolonies in a fluid medium without substrate adhesion (Sauer *et al.*, 2022). Furthermore, a contemporary review of biofilm infections has described how bacterial phenotype may

define stages of biofilm infection. This includes a stage 3 biofilm infection presenting a mix of fast growth, slow growth, mutant, and persister cell bacterial phenotypes, while stage 4 biofilm infection is a dormant pathologic persister cell phenotype characterized by relapse infection (Ciofu *et al.*, 2022).

Phenotypic variants often termed “persister cells” may constitute 0.1-10% of a biofilm which offers a “reseeded” population capable of potentiating refractory infection after treatment (Percival *et al.*, 2011). Latent relapse will be initiated by a cell-to-cell communication quorum sensing of stagnating cells in rough surface recesses obscured from cell surface microfluid clearance of autoinducer cells, which produces a detachment signal at a threshold level that controls the biofilm/planktonic plasticity phenotype switch (Mukherjee and Brassler, 2019). A competitive duress for bacterial cells in the race for the implant surface with stem cells (Gristina, 1987) may promote gene code-switching to reduce the energy level of the bacterial cell in the interests of energy conservation and survival (Fisher *et al.*, 2017).

The basis of the intra-radicular infection theory which dominates current teaching and thinking is that the lesion of chronic apical periodontitis is a sterile inflammatory lesion (Ricucci and Siqueira, 2010; Siqueira Jr *et al.*, 2014). The regeneration of the implant-bone bed assumes that the periapical tissues have been defended from microbial invasion and that spontaneous healing follows the removal of diseased teeth. The jawbone may persist in a state of preserved sterility adjacent to a sterile apical granuloma induced by biofilm infection from the root canal (Grossman, 1959).

In a previous enhanced, culture-based study, it was established that live bacterial assemblages could be detected in samples taken from short and long-term apparently healed jawbones, following the extraction of infected teeth. The discovery of live cultured bacteria in apparently healed edentulous jawbone ridges revealed their presence, not only beyond the confines of the infected root canal but following 3 or more months of deep space post-extraction healing (Nelson and Thomas, 2010).

Surgical debridement beyond and through sclerotically confined pathology appeared to alter the bacterial assemblage and correlate with healing but did not eradicate bacterial presence (Nelson and Thomas, 2010). It is central to the definition of jawbone pathology as to whether bacteria are not only present but are present as a chronic bacterial biofilm phenotype.

In orthopedics, SEM was utilized to detect biofilm-mediated growth in the presence of prosthesis-related infections. Culture microbiology returned from swabs may have yielded only one species from what SEM revealed as a polymicrobial population. SEM was also used in orthopedics to observe infecting bacteria during surgical debridement of osteomyelitic bone (Gristina *et al.*, 1985; Lew and Waldvogel, 2004).

Many disease conditions that were previously thought of as a sterile inflammatory process, including chronic osteomyelitis and Chronic Otitis Media with Effusion (COME) (Ehrlich *et al.*, 2002) are now known to be indolent biofilm-mediated infections (Lew and Waldvogel, 2004; Ehrlich *et al.*, 2010). When persistent osteomyelitis was observed by SEM and TEM in an orthopedic context, it was found to be characterized by microcolonies adherent to the bone in a protective glycocalyx. These colonies are linked to form biofilms on the surfaces of necrotic bone. This mode of growth, together with refractory and resistant behavior to host defenses and antibiotics, resembled biofilm infections of orthopedic prostheses and many other chronic bacterial diseases such as cystic fibrosis-related pneumonia (Marrie and Costerton, 1985).

Orthopedic understanding defined chronic infections of bone as quintessential biofilm infections characterized by persistence, resistance, and recurrence (Mader *et al.*, 1999). One of the principal presenting characteristics is to cause confining, dense, sclerotic bone which may need to be thinned, debrided, or penetrated with small drill holes to increase vascular and antibiotic perfusion (Walenkamp, 1997). The severe sclerotic change will reflect the duration of the infection and may adversely affect the prognosis making the infection more resistant to treatment and more significantly related to refractory outcome (Simpson *et al.*, 2001). Penetration through the sclerotic jawbone was adapted from orthopedic “intramedullary reaming” where surgical drills became a method to decrease the intraosseous pressure and revascularize the bone (Gualdrini *et al.*, 2000; Bar-On *et al.*, 2010). The orthopedic definition of chronic osteomyelitis subsequently progressed to the category of biofilm infection which was refractory to non-surgical treatment (Cierny, 2011).

Brynolf (1967) used radiographic and histopathologic evidence from cadavers to redefine the chronic periapical lesion of teeth as a dual osteolytic/osteosclerotic lesion, not a single osteolytic granuloma where sclerosis was given no pathologic relevance. Sclerosis provided bacterial confinement within the extra-radicular periapical bone space (Fig. 1). The bacteria were not confined to the root canal but as a sclerotically encapsulated biofilm nidus (Dahlen, 2002). Multipotent Mesenchymal Stem Cell (MSC) potential was shown in the Alveolar Bone Proper (ABP), the thin cortical plate that forms the socket wall (Fawzy El-Sayed *et al.*, 2013). This provided the histologic basis for the radiographic image of the apical detachment of the ABP from the root surface in endodontic infection and the immunoreactive initiation of MSC osteoblastic synthesis of the bone matrix within the ABP, resulting in osteosclerotic densification. A confining nidus secondary to the primary immunoreactive granuloma which created disconnection and seclusion from the adjacent healthy bone, the capillary micro-vasculature, and the stem-cell-rich periosteum. The periosteum is a specialized tissue that could regenerate all layers of bone (Zhu *et al.*, 2023).

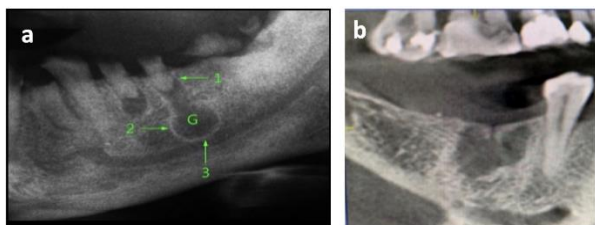


Fig. 1: (a) Chronic periapical lesion showing a dual osteolytic/osteosclerotic lesion as per Brynolf's observations (Brynolf, 1967). The arrows represent (1) Detachment of Alveolar Bone Proper (ABP) from the chronically infected root. (2) Secondary confining sclerotic encapsulation of primary lytic lesion following immunoreactive initiation of mesenchymal stem cell osteoblastic synthesis of the bone matrix within the ABP (Fawzy El-Sayed *et al.*, 2013). (3) Inferior border of lesion, (location G) lytic granuloma; (b) Lower right first molar which had an infective failure of an endodontically treated tooth. This is the CT imaging 12 months post-extraction; (b) Shows a significant three-dimensional persistence of the tooth-borne dual osteolytic/osteosclerotic chronic periapical pathosis (Brynolf, 1967). An asymptomatic sclerotically encapsulated and secluded chronic pathologic biofilm nidus within the edentulous deep bone space. Contemporary classification is a Stage 3 biofilm phenotype infection (Ciofu *et al.*, 2022). This mandates potentially repetitious surgical debridement to a vascularised health margin to progressively regenerate histologic osseous internal architecture (Cierny, 2011). This avoids grafting into a pathologic deep bone space where the colonization of a biomaterial surface may be dominated by dormancy persisting cell biofilm. A stage 4 biofilm phenotype persists cell relapse infection and implant failure may ultimately follow what appeared to be stable osseointegration (Ciofu *et al.*, 2022)

SEM use in dental literature recorded no microbes on the apical root surface of teeth with either pulp vitality or pulp necrosis, with no radiographically visible periapical lesions. However, all samples taken from external root surfaces with radiographically visible periapical lesions recorded the microbial presence and apical biofilm (Leonardo *et al.*, 2002; 2007). Extra-radicular biofilm inhibits the healing of apical and periapical tissues after endodontic treatment. Endodontic surgery may cure refractory chronic apical periodontitis by definitive surgical debridement of extra-radicular biofilms (Su *et al.*, 2010). This study may assist in the progression of defining the dental implant bone bed as a site of preserved sterility, or a site that must recover a disturbed ecosystem, to enable predictable passive osteoblastic anchorage on inert, commercially pure titanium implants.

Materials and Methods

Patient Clinical Cases

Two non-consecutive patients presented in private practice for dental implant treatment. The cases presented as (1) A single tooth, chronic lesion in the anterior maxilla, and (2) As a full arch of chronically infected mandibular teeth with chronic tooth-borne lesions and persistent lesions in edentulous sites. Human ethics was approved by the University of Sydney Human Research Ethics Committee (reference number 07-2007/9962).

Tooth Extraction and Surgical Debridement

Where extraction was required, teeth were removed by conventional forceps extraction or the use of a minimal intervention surgical strategy. All extraction sockets were then subjected to site-specific surgical mechanical debridement, curettage, and saline irrigation.

Sample Collection

Bone samples were acquired with the use of a sterile No. 15 scalpel blade or surgical Rongeurs from the margins of non-healing lesions and from bone retained within the flutes of sterile round surgical burs. All bone samples were obtained during sterile, open-flap surgical procedures, debridement, or placement of primary and revision implants.

Samples for scanning electron microscopy were fixed in 3% glutaraldehyde (Sigma-Aldrich, St Louis, Missouri, USA) in water for 24-48 h and then stored in 0.1 M phosphate buffer at 4°C. Samples for molecular analysis were immediately placed in transport media containing 30% glycerol and stored at -20°C until transported to the laboratory on dry ice.

Scanning Electron Microscopy

The samples were fixed in 3% glutaraldehyde for 24 h, dehydrated through increasing concentrations of ethanol, and immersed in hexamethyldisilane (Sigma-Aldrich) 50% for 10 min and 100% for 3×10 min, before being aspirated dry and evaporated dry overnight. They were mounted on metal stubs and sputter coated with 20 nm gold film. The samples were imaged in a JEOL 6480 LV scanning electron microscope (Japan electron optics laboratory, Japan) with a voltage of 10 kV and a viewing distance of 20 mm.

Microbiome Analysis

Genomic DNA was extracted using a combination of proteinase K (Sigma-Aldrich) and lysozyme (Sigma-Aldrich) digestion followed by phenol/chloroform extraction and ethanol precipitation as previously described (Jacombs *et al.*, 2012). Extracted DNA was resuspended in 100 µL TE (10 mM Tris-HCl pH 8.0 and

1 mm EDTA) buffer. DNA quality and concentration of each sample were checked by Nanodrop 2000 (Agilent Technologies Inc. Santa Clara, CA, USA). Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) was commercially performed by Molecular Research Lab (MR-DNA) using the Titanium platform (Roche) as previously described (Dowd *et al.*, 2008). The V1-V3 regions of the 16S rRNA gene were sequenced and used to identify and quantify the different bacterial species in dental samples. Pyrosequencing data were analyzed by QIIME software (Werner lab, NY, USA) (Lawley and Tannock 2017). After removing low-quality and ambiguous sequences, all the remaining reads were de-multiplexed and chimeric reads were removed by chimera slayer using default parameters. Operational taxonomic units were assigned against a curated green genes database (McDonald *et al.*, 2012).

Results

Patient 1 Case Report

Clinical History and SEM

Patient 1 presented for dental implant therapy with a terminal diseased dentition in both arches. Only the mandible was treated with a full hybrid prosthesis, the maxilla received a conventional full upper denture (Fig. 2).

Following extractions and a healing period of three months, 5 inter-foraminal implants were placed in the mandible using a two-stage protocol. The right-side anterior mandible presented as a block of osteosclerotic bone which obstructed osteotomy preparation at 2000 rpm ad modum Brånemark. Sufficient penetration was obtained to secure bone samples for SEM and microbiome analysis. Implants were placed to the right and left of the sclerotic block. A “wound gape” presented at suture removal on day 10, extending for 1.5 cm corresponding to the mesiodistal arch length of bone exhibiting severe sclerotic change (Fig. 3).

At second-stage surgery, the osteosclerotic block had become radiographically demarcated to the point of sequestration. The presence of what appeared to be a refractory pathologic biofilm and the presence of persisting severe sclerotic change and sequestration provided a provisional diagnosis of chronic osteomyelitis (Figs. 4-5). SEM images of bone samples taken at implant installation from the sclerotic block (Fig. 5) provided visual confirmation of bacteria and bacterial biofilm. Figure 4 indicates the sclerotic demarcation of the sequestering lesion, which was a segment of dead, sclerotic bone tissue caused by osteomyelitis (Cierny, 2011).

Osteosclerotic bone persisted in a three-month apparently healed mandibular edentulous ridge at implant installation. SEM images revealed cocci bacteria covered in maturing Extracellular Polymeric Substance (EPS). A post-surgical wound gape persisted above this reactive sclerotic site until surgical debridements and closure of a blood-filled space.



Fig. 2: Terminal, diseased dentition on a presentation for mandibular implant therapy. Note significant bony degeneration. Location A: Radiolucent osteolytic lesions, location B: Radiopaque osteosclerotic lesions

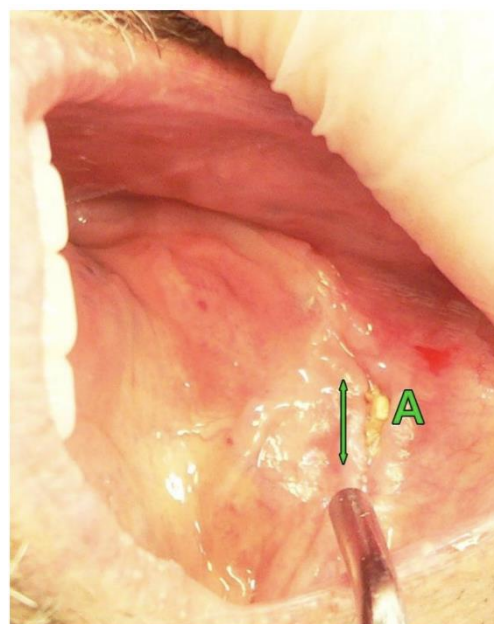


Fig. 3: Devitalised bone below soft tissue "wound gape" with texture and color similar to ivory. Location A is a devitalized ivory bone. The arrow corresponds to the length of the wound gape

Clinical Outcomes after Surgical Debridement

It followed, therefore, that a mandated surgical debridement should be pursued as curative therapy. A block resection was undertaken to punctate point bleeding beyond the sclerosis on all exposed surfaces, without vertical progression to the inferior border of the mandible. Two excisions of reduced dimensions had proved unsuccessful in promoting wound closure prior to this. It was expected that the apical section that remained of the sclerotic lesion would have received sufficient red cell and antibiotic perfusion to heal satisfactorily without structurally endangering the integrity of the mid-line of the mandible by inclusion in the resection (Fig. 6).

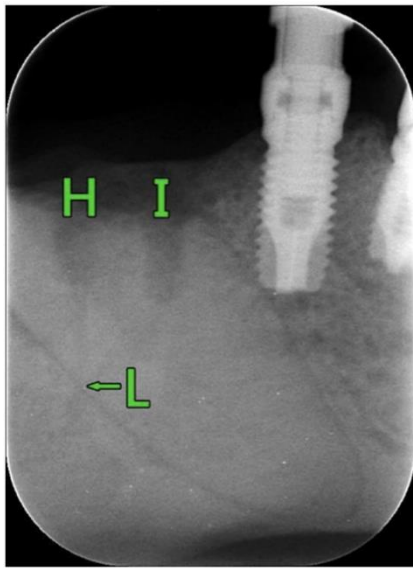


Fig. 4: Bone samples were taken for SEM analysis (locations H and I) at implant installation. (Location L) indicates sclerotic demarcation of the sequestrating lesion. Radiograph was taken 4-months after the placement of five implants

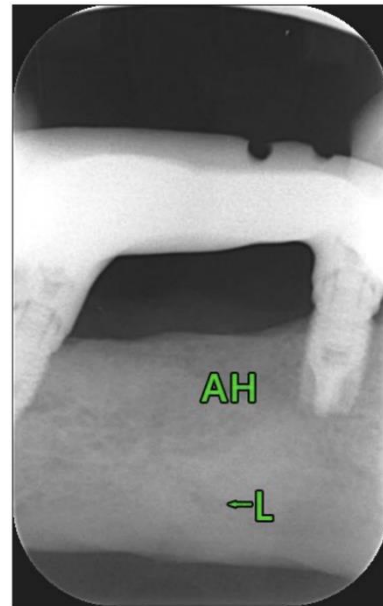


Fig. 7: Review at 12 months following surgical drilling debridements of block section of osteosclerotic lesion regenerated without graft or membranes. Location L: Vague demarcation of a retained apical section of the former lesion with apparent healing (location AH) evident in the debrided area. Multiple debridements with ultimate perfusion of diffuse sclerosis and bone regeneration without graft or membranes. Surgical decompression, capillary perfusion, periosteal reconnection, blood fill, immediate closure

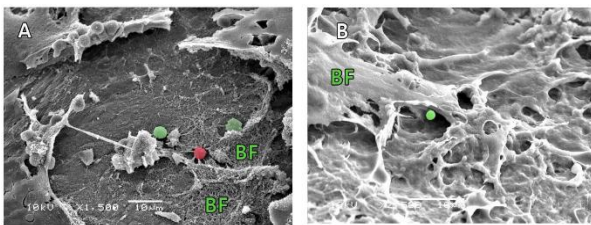


Fig. 5: SEM images of samples taken from location H (Fig. 5A) and I (Fig. 5B) in Fig. 4. BF: Biofilm. Scattered cocci (green highlight). Cocci (red highlight) with biofilm EPS

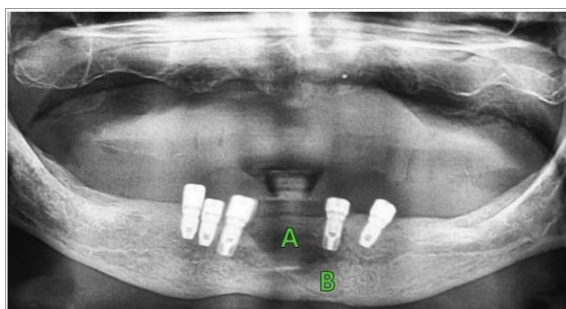
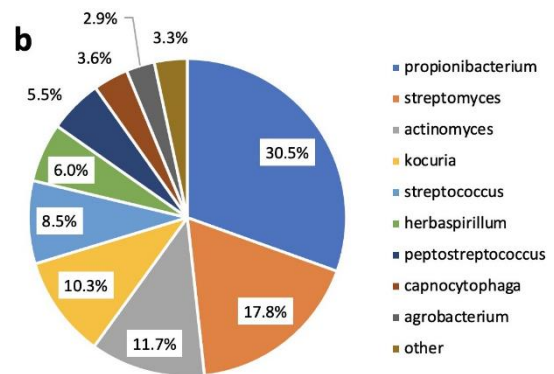
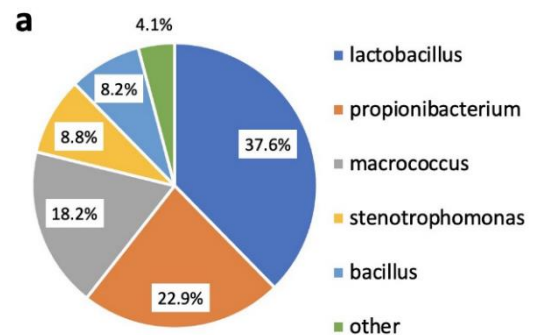


Fig. 6: Surgical defect in anterior mandible. Location A: Blood-filled and closed following Regenerative Surgical forage Drilling Debridements (RSD) of the sclerotic lesion. Location B: Apical section of the lesion was not debrided out of concern for the anatomical size of the infra-bony defect and possible pathologic fracture. Severe sclerotic bone as a pathologic biofilm biomarker (Walenkamp, 1997), treated by multiple small drill surgical forage reaming debridements, capillary perfusion, blood fill, and immediate closure



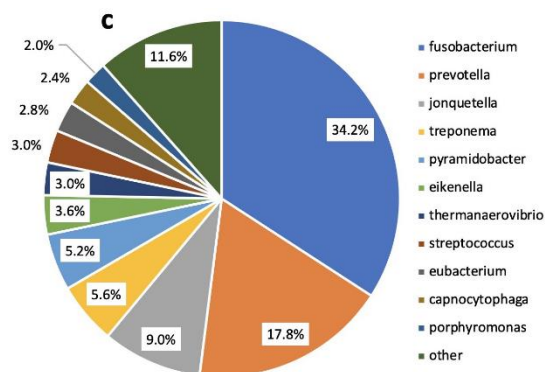


Fig. 8: (a) 454 Pyrosequencing curations confirming resident polymicrobial biofilm from right side mandibular implant osteotomy beyond sequestrum demarcation; (b) 454 Pyrosequencing curations confirming resident polymicrobial biofilm from left side mandibular implant osteotomy beyond sequestrum demarcation; (c) 454 Pyrosequencing curations confirming resident polymicrobial biofilm within sclerotic block sequestrum

A review of the site 12 months following surgical debridement drilling (Fig. 7), with blood-fill and closure and antibiotic administration before and after surgery, revealed satisfactory bone regeneration with no apparent consequences for adjacent implants. No bone graft or exclusion membrane was used (Cierny, 2011). Retained apical section of the sclerotic lesion was more vaguely demarcated and may confirm further regeneration in a subsequent review.

Pyrosequencing of bone samples from sclerotic pathologic block sequestrum (Fig. 8) corroborated SEM visual confirmation of polymicrobial biofilm in severely pathologic sclerotic sequestrum (Fig. 5a-b).

Patient 2 Case Report

Clinical History and SEM

The tooth extraction was performed on patient 2 according to the sterile bone paradigm, where tooth removal theoretically removed the intra-radicular source of bacteria that had established and sustained a sterile inflammatory lesion. However, no spontaneous healing occurred following the extraction and a stipulated healing time of 3 months. Primary implant placement failed with purulent infection 10 days after installation and soft tissue closure. Lesion persisted 9 months post-extraction, despite repetitious surgical debridement (x3). The lytic lesion is characterized by fiercely adhesive histopathologic granulation/fibroconnective tissue, embedded in deep bone space. An osteosclerotic lesion can be very difficult to penetrate and perfuse by drilling. Healing is progressive, as pathologic dominance is reduced to less pathogenic and eventually non-pathologic biofilm communities. Grossman said that an infected root canal could co-exist with a sterile

periapical granuloma (Grossman, 1978). Why then, did this lesion not spontaneously heal with preserved sterility? It is important to use multiple diagnostic tools to rule out the presence of any microorganisms before declaring their sterile status (Santoro *et al.*, 2008).

Patient 2 presented for dental implant treatment with an asymptomatic fractured upper left lateral incisor with a large extra-radicular radiolucent lesion surrounded by diffuse sclerotic spongiosa, continuous to the nasal plate (Fig. 9).

A vertical surgical drilling debridement comparable to orthopedic intramedullary reaming (Gualdrini *et al.*, 2000; Bar-On *et al.*, 2010) was performed in the extraction socket and beyond using a round surgical guide drill and 2 mm twist drill but was obstructed from securing bicortical debridement at 2,000 rpm ad modum Brånemark by a diffuse and severe sclerotic pattern.

The implant was installed 13 weeks post-extraction/debridement with a presumed, non-regenerative, sterile lesion persisting in the osteotomy. A vertically mobile implant presented 10 days after the installation as activated osteomyelitis with a discharging purulent swelling and was removed as an early infective failure (Fig. 10).

Following healing, bi-cortical debridement was achieved 3 months after implant failure (6 months post-extraction) with penetration to the nasal cortex. Curettage was also undertaken of significant, adhesive granulation/fibroconnective tissue in dead space. Following healing, bi-cortical surgical debridement and curettage were repeated, 6 months after implant failure (9 months post-extraction). Bone samples were taken from the borders of the persistent lesion for SEM (Fig. 11).



Fig. 9: Patient presents with a fractured crown and large extra-radicular pathosis consistent with a short-exit apical foramen (location AF) on the distal surface. The spongiosa visible is without trabeculation and presents as a diffuse, reactive sclerosis

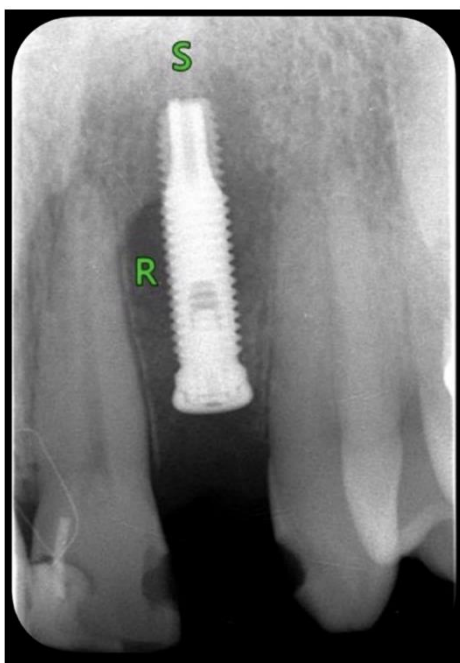


Fig. 10: The implant suffered an early infective failure 10 days after installation in an osteotomy characterized by persistent radiolucency (location R) and sclerosis (location S). The implant was removed, the site surgically debrided once more, and closed

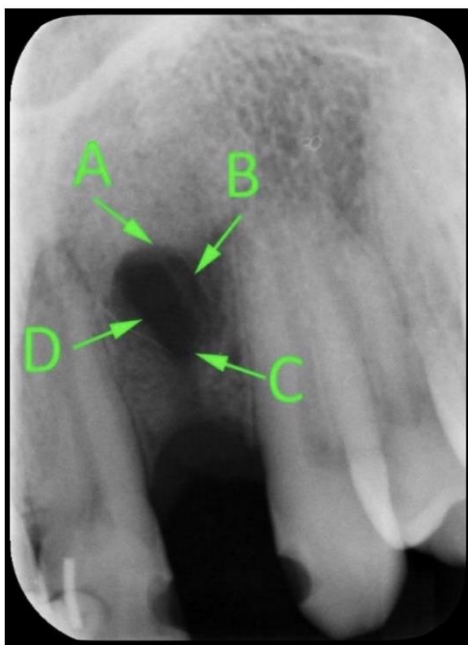


Fig. 11: Persistent radiolucent/radiopaque lesion 6 months after implant failure and 9 months post extraction. Bone samples were taken with sterile sharp blade instruments from the borders of the lesion for microbiome analysis (locations A, B, C, and D) and SEM analysis (locations A, B, and D)

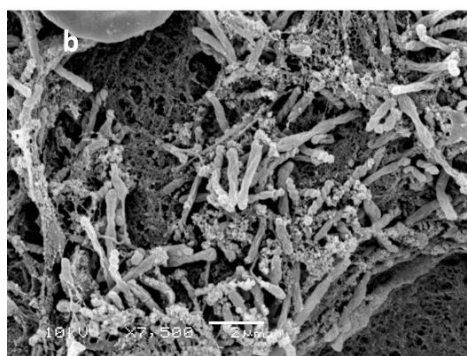
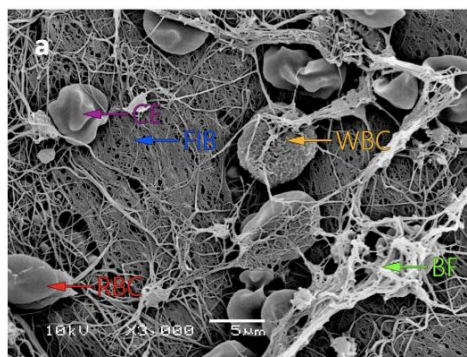


Fig. 12: (a) SEM of a bone sample taken from (location A) side of the lesion in Fig. 11. 6 months after implant failure and 9 months post extraction. The definitive image shows White Blood Cells (WBC) in Biofilm (BF) with red blood cells (RBC), Fibrin (FIB), and Cretinated Erythrocytes (CE). This sample demonstrates extensive fibrous deposits with lace biofilm plus small areas of patchy biofilm clusters covering the bone surface; (b) SEM image of persistent pathologic sample (41F) taken from “location B” side of lesion invading deep within the bone with areas of bony destruction $\times 7500$. Deeply invading pathogenic rods persisting 9 months post extraction and multiple (3 \times) debridements in diffuse sclerotic bone (D1 Nelson and Viljoen bone quality index) (Viljoen, 2019); (c) SEM image of same site healed sample (57E) taken at 12 months post extraction from D side in Fig. 11 following further debridement and further 3 months post-surgical debridement healing. Rods are no longer present, now dominated by cocci

Visual Confirmation of Persistent Bacterial Biofilm

SEM reveals an apparent population shift, corroborated by pyrosequencing in the healing trajectory. The clinical and radiographic presentation of the lesion was of necrotic bone confined by severe sclerotic change and a radiolucent dead space containing adhesive fibroconnective/granulation tissue. SEM demonstrated visual confirmation of bacteria and bacterial biofilm (Fig. 12).

As a progressive SEM sample in the same patient/same site, the healing bone lesion demonstrated a much thicker established biofilm and the identifiable bacteria are no longer pleomorphic rods but are coccoid. This suggests that there has been an evolution in the biofilm and its microbial inhabitants and that the biofilm might now be polymicrobial. There was no visual evidence of the original rod-shaped bacteria seen in the previous pathological persistence profile at 9 months post-extraction. Longitudinal SEM suggests an apparent population shift in the resident biofilm requiring four surgical debridements in search of a regenerative health margin over 18 months. The healing bone lesion had recovered a large radiolucent ischaemic necrosis into a vascularised vertical cleft in the labial plate.

Microbiome Population Shift after Multiple Surgical Debridements

Microbiome analysis revealed that change in the resident bacterial assemblages may correlate with a microbial community health/disease shift, corroborated by ‘corresponding same site SEM images. Persistent continuity of chronic pathology revealed by pyrosequencing at 6 months and 9 months post-extraction (Sample 41F) (Fig. 13a-b) corroborated SEM visually confirming deeply invading pathogenic rod-shaped bacteria (Fig. 12b). Health recovery population-shift with a turnover of 7 phyla between 9 and 12 months, following multiple debridements, at 12 months post-extraction (Sample 57E) at revision (Fig. 13a-b).

Repetitious surgical debridement drove a healing trajectory through habitat change and resident microbial population shift, according to habitat fitness. This allowed the placement of a revision implant into an ecologically healthy bone bed with a bony return to normal internal architecture. At a 9-year review, a normal bony architecture was fully returned (Fig. 14).

The invading pathogens appear to have completely replaced the resident community with a disease-orientated community. SEM visually confirmed biofilm phenotype pathogenicity and persistence as an ecological shift in a commensal (resident) population (Costerton, 2007).

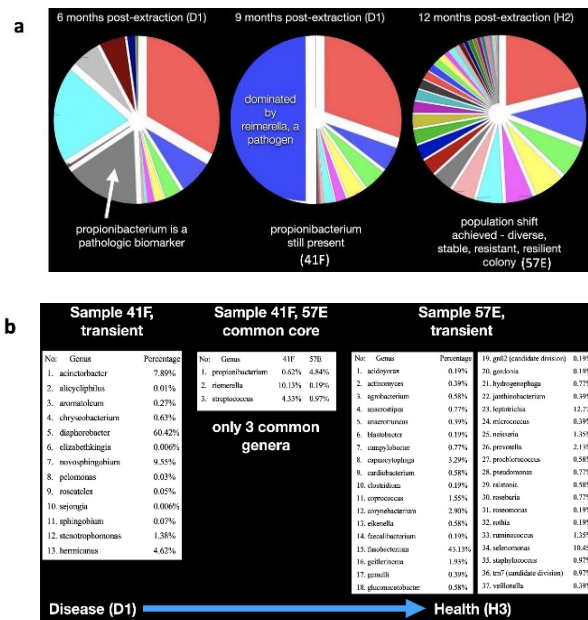


Fig. 13: (a) Microbial population shift achieved before implant revision. Diversity and stability are almost synonymous. Turnover of 5 Phyla between the pathologic sample at six months post-extraction, and the pathologic sample at nine months post-extraction (41F). There was a 7 Phyla turnover between diseased bone at nine months post-extraction, and healthy bone at twelve months post-extraction (57E); (b) Example of population-shift, post-RSD. The shift of 7 phyla between 9 months (41F) post-extraction persistent pathology and 12 months (57E) healing recovery and population shift following further debridement and stipulated healing time. Resident bacterial diversity shifted from 13 genera to 37 genera with only 3 common genera after a major healing event of chronic resistant pathology. Ecologic diversity is recovered along with stability and bone health

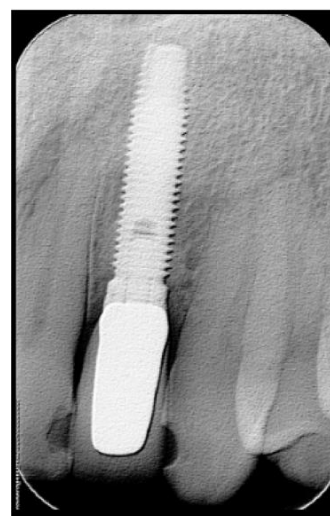


Fig. 14: 9-year case review in 2021. Noted a full return to normal bony architecture

The bacterial biofilm health/disease population shift has the primary aetiological role in disease and persistence which impedes and delays healing. Pathologic communities will produce direct toxin lysis and compression lysis of capillaries where ischaemic bone beds are subject to infection and disease by population shift. Persister cell populations will persist as substrate-bound subclinical biofilm niduses which will be resistant to non-surgical treatment (Cierny, 2011).

Discussion

SEM was a methodology critically pursued in 1985 by Gristina *et al.* to detect adhesive and coherent polymicrobial micro-colonies in failed orthopedic prostheses and bone samples obtained during surgical debridement of long-bone chronic osteomyelitis (Gristina *et al.*, 1985; 1987). This helped promote the definition of chronic osteomyelitis as a bacterial biofilm infection of the bone. SEM provided visual confirmation of bacteria and bacterial biofilm in the jawbone in health and disease. This included pathologically altered sclerotic sequestrum, a pathologically altered chronic periapical lesion, and an apparent same-site population-shift health recovery, corroborated by same-site longitudinal microbiome analysis. We may now suggest that the enhanced live bacterial culture (Tunney *et al.*, 1998) that we had previously reported of 21% in a minimum three-month healed edentulous jawbone1, was a result of the surgical disturbance of live resident biofilms. A biofilm/planktonic phenotype switch followed resulting in the detection of much higher numbers of dispersed planktons, compared to perhaps 2% or repetitive negative culture recorded from passive swabs (Tuttle *et al.*, 2011). Similarly, 22% live bacterial culture was recorded when subjecting biofilm-infected hip prostheses to the disturbance delivered by ultrasonication (Tunney *et al.*, 1998).

SEM provided high-magnification spatial images of bacteria located on bone surfaces and within the biofilm of bone from samples of both patients and allowed good visualization of the bacteria and biofilms (Alhede *et al.*, 2012). This evidence could then be integrated with clinical and radiographic observations in association with microbiome analysis to provide correlated evidence for the biofilm-based infectious disease pathogenesis (Costerton, 2005).

In a disturbance profile of chronic pathology, SEM visually confirmed bacterial biofilm disease persistence in the jawbone 9 months after extraction. Furthermore, SEM provides visual corroborative evidence of the altered bacterial presence in health recovery and population shift. This further demonstrated that bacteria naturally exist in the biofilm mode in health and disease and are not eradicated with the resolution of chronic pathologic lesions. It further

supports the hypothesis that there is a routine commensal (resident) live biofilm.

Surgical drilling debridements to vascularised margins beyond sclerotic encapsulation in both patient cases in this study progressively shifted and replaced live biofilms that were a result of pathogenic bacteria with live biofilms that were a result of less pathogenic or non-pathogenic bacteria. Circumvention of ubiquitous pathologic bacterial biofilms (Weinstein and Darouiche, 2001; Deva *et al.*, 2013) as opposed to adherence to the sterile bone paradigm requiring debridement to remove all bacteria and bacterial biofilm (Schultz *et al.*, 2017).

To the best of our knowledge, SEM has not previously shown evidence of live biofilm population shift in a same-site, longitudinal case in jawbone biofilm infection (Case 2), corroborated by microbiome analysis recording 7 phyla shift between 9 and 12 months post extraction, clinical and radiographic evidence.

Previous studies using SEM in dentistry recorded polymicrobial biofilms in a lower molar site 4 years post-extraction despite extended antibiotic administration (Stoodley *et al.*, 2011). In addition, SEM had shown biofilm on resected root surfaces, extruded gutta-percha root filling material, and in sulfur granules from lesions of apical actinomycosis (Leonardo *et al.*, 2007; Signoretti *et al.*, 2011).

The development of biofilm-preventing implant surfaces or dampening of the immune response during infection or surgical trauma are talked of as future strategies to avoid host tissue damage and refractory pathologic failure (Ciofu *et al.*, 2022). We have existing answers to both critical issues. Jogging the corporate memory of implant history tells us that ad modum Brånemark had a Swedish naval gun manufacturer (Bofors) "turn" or machine smooth and uniform commercially pure titanium screws with a surface roughness of less than 1 micron (Adell *et al.*, 1981; Brånemark and Albrektsson, 1985). This surface inhibited quorum sensing by biomimicking the smooth and uniform surface topography of pathogen-evading, healthy human cells (Percival *et al.*, 2015). The surface was accessible to cell surface microfluid wash and did not allow stagnation of 1-2 micron bacteria in surface recesses (Mukherjee and Bassler, 2019; Graham and Cady, 2014).

When the health ecology of the bone is recovered, there is the potential for beneficial bacterial sRNA to dampen the immune response to surgical trauma/infection to a recoverable short-term pulse disturbance and return to the pre-disturbance homeostatic ecologic state (Moriano-Gutierrez *et al.*, 2020). Botton defined ecologic stability as the ability of a system to return to equilibrium after a temporary disturbance (Botton *et al.*, 2006). Beneficial bacterial small sRNA is rarely involved in gene

code switches and survival default to persist cell dormancy and relapse infection. The debridement-driven trajectory of the recovery of beneficial health bacteria regulates a dampening or restraint of surgically induced immune response to enable stable symbiont colonization of health microbiota on both biologic and non-biologic surfaces during passive osteoblastic anchorage at the osseointegration interface (Moriano-Gutierrez *et al.*, 2020, Nelson, 2015).

Conclusion

Strong evidence is provided supporting the presence of live resident polymicrobial biofilm in apparently healed edentulous human jawbone in health and disease, independent of healing time or surgical intervention. We recovered cellular health homeostasis with evidence of a resident, beneficial, ecologically diverse health microbiota, where sterility was not relevant.

Ecologic and internal histologic architectural health of bone may be recovered longitudinally by Regenerative Surgical Debridement (RSD) to a vascularised health margin beyond sclerotic encapsulation by population shift, thereby recovering the microbial diversity and stability of live beneficial health microbiota which may then support a consortial, symbiont colonization of topographically similar abiotic and biotic surfaces in an enduring, passive osteoblastic anchorage, at an inert osseointegration interface, where cellular homeostasis avoids immunomodulation and foreign body response. This is the microbial ecological extension of what P.I Branemark called “Restitution Ad Integrum” and “*ad modum* Branemark” original osseointegration model. Return to pre-disturbance condition and passive integration of inert commercially pure titanium surfaces without an immunobiological event.

To improve osseointegration outcomes and reduce revision numbers, we recommend that eradication of quintessential chronic biofilm infections of bone must occur before implant deployment. This is a clinically viable, biofilm-based methodology, in a new model of biofilm-based osseointegration.

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Data are available from corresponding authors on request.

Author’s Contributions

Stephen Nelson, Honghua Hu, Graham Thomas, and Karen Vickery: Conception and design, acquisition of data, analysis, and interpretation of data. Drafted the critical review for significant intellectual content. Final approval was given for submission.

Anita Jacombs: Study design, data collection, and analysis. A critical review of drafted and intellectual content. Final approval was given for submission.

Anand Deva: Conception and design analysis and interpretation of data. Final approval was given for submission.

Andre John Viljoen: Analysis and interpretation of data drafted and critically reviewed for significant intellectual content. Final approval was given for submission.

Institutional Review Board Statement

The study was conducted in accordance with the declaration of Helsinki and approved by the University of Sydney human research ethics committee (reference 07-2007/9962).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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