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Bacterial Biofilm Bioremediation of the Human Jawbone before Implant Installation - the Paradigm Shift is Resident Microbial Population Shift

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Abstract

Our previously published, peer reviewed research examines the fact that the human jawbone, like multiple other body spaces, is not sterile, but rather, it supports permanent, native, living, microbial communities, which may range in community structure from homeostatic (healthy, stable, and ecologically diverse), to a graded spectrum of pathologic communities, often depending on the history of the previously supported tooth. The health or otherwise of human jawbone can be recognised radiographically, with healthy bone showing a moderate to medium intact cortical plate without sclerotic thickening, which confines spongy bone with trabecular connectivity varying in open or more closed mesh anatomy with variation in thickness or trabecular density. The presence of lytic (radiolucent) or sclerotic (radiopaque) areas within the trabecular space are both biomarkers of diseased bone, often seen post-extraction, which support pathologic biofilm communities. We have previously identified 5 bone qualities which directly relate to the health/disease of the resident biofilm communities. The primary requirement for enduring osseointegration is that the installation of the dental implant must occur into a healthy, disease-free bone-bed, which supports non-pathogenic biofilm communities. Accepting that only ecologically healthy bone is suitable for implant installation, is it possible to return diseased bone to ecological and architectural health and normalcy, suitable for enduring osseointegration? In this paper we will present a surgical technique, named Regenerative Surgical Debridement (RSD), as a method of returning diseased bone to health, making it suitable for implant installation. Using statistical NMDS ordination of the pyrosequencing data taken from both the pre- and post-RSD bone beds, we present case studies showing complete and partial population shift of diseased microbial communities that ordinate close/closer to our health-control communities, with a radiographic return to normal bony architecture and anatomy. We suggest that the presence of native, beneficial resident bacteria does not elicit an immunobiological response as they are a tolerated population. In the presence of symbiotic colonisation of these beneficial health microbiota and a pathogen-evading, smooth (Sa<1 micron), uniform, commercially pure titanium surface (that bio-mimics the surface topography and microarchitecture of the heathy human cell), we can progress to passive osteoblastic implant anchorage, where neither the microbial presence nor the biomaterial surface, elicit chronic inflammation, (outside of an initial, brief, surgically-induced inflammation), which beneficial bacterial sRNA may suppress, causing only a short-term (pulse-type) disturbance of the resident population.

Keywords: Regenerative Surgical Debridement; Human Jawbone Microbiome in Disease and Health; Biofilm Population Shift; Enduring Osseointegration

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Introduction

The most important clinical variable in determining enduring implant outcomes in human jawbone is the pre-existing microbial ecological health (or disease) status of the bone bed.

We have previously published data obtained from samples of human jawbone, and we used DNA 454 pyrosequencing curation and ordination, which specifically look for the presence of bacteria in biofilm communities, rather than "culture methodology" which detects planktonic bacteria only. We found no sterile sites. Using NMDS ordination and curation of this data, the jawbone samples revealed a universal presence of resident polymicrobial biofilm assemblages, and a 20 phyla jawbone microbiome, across a site-specific spectrum of natural (healthy) and pathogenic ecosystems.

The paradigm shift from a sterile bone model to our "permanently resident, living, bacterial biofilm model", is that disease in the human jaw must firstly be identified (sclerosis/lysis or both) and if identified, the resident community must be returned to health through population-shift, back to a homeostatic microbial ecosystem. A more diverse, resident, homeostatic biofilm population (characterised by higher numbers and complexity of beneficial bacteria) provides ecologic stability through microbial resistance and resilience.

We have shown that a site-specific ecologic health recovery, of higher diversity of the resident microbial population, could be achieved using a process of surgical intervention, adapted from the successful treatment of orthopaedic osteomyelitis, which we named Regenerative Surgical Debridement (RSD). We were able to show that higher ecologic diversity in the dental implant bone bed did translate into improved functional stability, supporting improved osseointegration outcomes.

The biofilm science used to recover health ecology and internal histologic osseous architecture included: surgically removing pathologically altered necrotic and devitalised tissues, reconnecting the healthy capillary microvasculature with avascular areas, reconnecting the stem-cell-rich periosteum to regenerate new bone, reconnecting and achieving convergence of resident beneficial health microbiota from the adjacent healthy tissue, decompress raised intraosseous pressure to prevent further capillary compression lysis, and lastly, perfuse dense sclerosis to assist penetration of immune cells and serum antibiotics.

We have previously published the first "debridement beyond sclerosis YES/NO study" to record a medium effect size, after establishing a statistically significant association between surgical debridement and the success of the implant ($\chi^2 = 14.13$, df = 1, $p < 0.001$) and the achieved power is 96%, indicating confidence in the statistical validity of this result. The clinical effect of RSD is population shift, resulting in a quantitative difference between the ordination of pre- and post-debridement curated pyrosequencing, measured against our ecologic health control.

Over several years, we have investigated the nature of jawbone cellular homeostasis and pathologic disturbance using a combination of microbiological culture techniques and histopathology, and ultimately, using methods specific to the detection of biofilms [1]. These methods initially involved scanning electron microscopy (SEM) and later, culture-independent DNA molecular 454 pyrosequencing, with titanium reagents (bTEFAP). The advent of 454 sequencing [2] permitted the sequencing analysis of a specific 16S rRNA gene reference sequence, amplified from the community DNA. Microbial identity was considered accurate for genera level and above, and this provided estimates to study the microbial diversity, community structure and composition of microbial assemblages that were revealed in the jawbone samples. Pyrosequencing liberated us from the constraints of "culture-microbiology", which could be associated with the unacceptable diagnosis of "culture negative disease", when pathologically altered host-tissue had an aetiologic origin and persistence in the presence of undetected, indolent biofilm infection. This provided us with "a unified theory of bacterial growth and disease pathogenesis" [3].

We have shown, using NMDS multivariate ordination, that "that population shift could be a partial, or a complete ecologic recovery" [4]. Depending on the internal osseous architectural recovery, partial recovery may require additional surgical intervention to return the bone-bed to ecological health and anatomical recovery, sufficient to sustain enduring osseointegration. Debriding beyond the sclerotic pathologic confinement to reconnect microvasculature, regenerative stem cells and the surrounding resident health microbiota, may seem a simple surgical objective, but the success of the procedure can be frustrated by chronic, diffuse and severe sclerosis [5]. Infected medical devices are often marsupialised, so that they are isolated from unaffected tissues. Similarly, biofilm niduses in the peritoneum and the alveolar areas of the lung are "walled off" by fibrosis and subsequent calcification [4].

Siclari et al. showed that cortical bone forming a skeletal envelope had higher numbers of stem cells, which were also more metabolically active, versus spongy, trabecular bone [6]. This is logical when cortex is 90% mineralised, as compared to just 30% mineralised, in trabecular bone. We measured much higher alpha diversity in Exponential H community analysis for cortical bone, and this supports bi-cortical debridement, if possible, and ultimately, at the time of implant installation, bi-cortical anchorage, where anatomically feasible. Ecological diversity and functional stability are provided by the associated ecologic resistance (ability to resist pathogen invasion) and resilience (the ability

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to recover pre-disturbance ecologic stability after a short [pulse-type] disturbance) [7].

The sterile bone model cannot embrace RSD as a treatment option, as it does not understand the resulting beneficial population shift from a pathologic biofilm population to less, or non-pathologic biofilm [4,8]. Schultz et al., stated that debridement could not be used alone as it did not remove all of the bacteria from chronic wounds, a statement deeply rooted in sterile bone theory [9]. Normalising the biome-supported regeneration of the internal histologic architecture is the ultimate outcome in normalisation of the wound. It is this symbiotic ecologic recovery of both biome and osseous histologic regeneration with trabecular connectivity, that must be achieved before implant installation, in order to ensure enduring osseointegration on inert and smooth (<1micron) abiotic biomaterial surfaces, which do not incite immunomodulation. Native bacteria recovered in beneficial microbial ecological health homeostasis support passive, regenerative osteoblastic anchorage. An example of stable, post-RSD, enduring osseointegration, is seen in Figure 1.

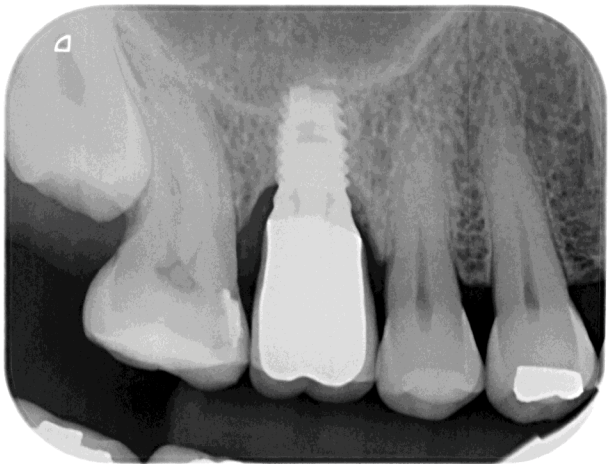

Figure 1: An example of enduring osseointegration taken 9 years after RSD. Note the connected trabecular mesh pattern, with internal architecture consistent with health around both the implant and natural healthy teeth, following RSD of an infected socket with root fracture. Implant placed after 4 months of post-RSD healing, following blood-fill and closure with a mucoperiosteal flap advancement, optimising wound exposure to the periosteum and the associated pluripotent stem cells. Eradication of biofilm disease and population shift before implant installation is a universal biofilm-based clinical protocol, regardless of implant choice.

Current orthopaedic literature has established the fact that necrotic bone areas can be regenerated if continuous contact with healthy, vascularised bone can be re-established. This will reinstitute the microvasculature and eliminate microsequestrae which otherwise may maintain long-term persister cells in chronic biofilms. Even in dormancy, persister-cell biofilm will, after an early period of non-replication, slowly disperse low numbers of pathogenic planktonic cells, producing chronic local inflammation which may progressively devascularise the bone adjacent to the implant surface by both direct toxin lysis and increased intraosseous pressure, with compression lysis of the capillaries [10,11]. Compromised vascular nutrition increases the risk for infection, as the bony surfaces progressively become non-viable and defenceless. In the absence of debridement, infection might be suppressed, but may recur years later, as cell-to-cell quorum sensing communication reactivates a phenotypic Type 4 dormant persister-cell biofilm relapse-infection [12,13].

Regenerative Surgical Debridement – The Development of a Clinical Protocol

RSD was developed as a clinical protocol based on the successful orthopaedic experience of surgically debriding chronic osteomyelitis in long bones, recovering diseased bone to health. If the resection was adequate [14] the outcome could be consistent with Costerton [15] who stated that "meticulous removal of all affected bone is demonstrably effective in the treatment of all forms of osteomyelitis". The reactive sclerotic confinement of a residual pathologic biofilm nidus creates a mechanism for biofilm persistence where microsequestrae, present as necrotic bone particles containing bacteria [16] are secluded from the host's defences [17].

Not all human jawbone needs to be "recovered" to health following tooth loss. Congenital tooth absence, traumatic tooth avulsion, tooth extraction for reasons not associated with periapical infection will leave a bone bed that supports a healthy, homeostatic microbial biome, and implant installation can be carried out without the need for RSD. However, in many cases the reason for tooth extraction is death of the tooth pulp, with an associated periapical infection of the surrounding tissues, or endodontic failure, often seen as a dual osteolytic/osteosclerotic lesion which has a high potential to persist in post-extraction edentulism as a sclerotically circumscribed pathologic biofilm nidus. This asymptomatic lesion may be potentiated during subsequent clinical manipulation to produce refractory sequelae during implant installation, should it be allowed to remain *in situ*.

Until relatively recently, it has been assumed that the removal of an infected tooth results in spontaneous resolution of all associated infection, especially in the supporting hard tissues; that is, remove the infected tooth and within 3 to 6 months, the bone will spontaneously heal, and normal trabecular anatomy will return, with a return to sterility.

Our research does not support the sterile bone model. Of 153 samples taken from human jawbone, all tested positive for the presence of bacterial biofilms, and the communities ranged from health to disease, as referenced against our health controls [18].

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As discussed in the orthopaedic literature, diseased bone requires a surgical intervention to return it to health, through circumvention of the pathologic population, to less pathologic or non-pathologic communities [8]. It was not until Simpson, Deakin & Latham showed that resection of biofilm-infected osteomyelitic bone required a resection of at least 5mm past (into) what he perceived to be the health margin [19]. Identification of the health margin was often a difficult discretionary decision during surgery, but indicators such as punctate-point bleeding of Haversian canals in the cortex served as a predictable indicator. Simpson et al. stated that "the remaining bone was clearly viable with good punctate bleeding". Importantly, his successful debridement extended through the radiographic sclerotic zone into the vascularised tissue.

When Simpson et al. performed local eradication of all necrotic and devitalised bone with 5mm or more of clearance into his perceived in-surgery health margin, this level of resection produced no recurrence in review, averaging 26.2 months (12-48 months). They had created a benchmark for the pre-implant-installation eradication of biofilm disease in deep tissue and the recovery of a resident health ecology, where biomaterial implantation may defy refractory sequelae (Figure 2).

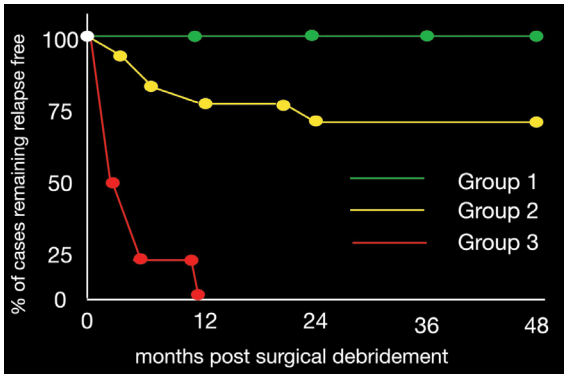

Figure 2: This graphic representation summarises the results of Simpson et. al. (2001) and shows the outcomes of surgical resection, using one of three protocols: (i) debridement at least 5mm into the surgeon-perceived health margin (Group 1), (ii) surgical debridement up to the perceived health margin (often the debridement was limited by anatomical structures) (Group 2), and (iii) Group 3, where only intralesional debulking was undertaken, with no regard to a health margin. Importantly, there was no recurrence in Group 1 over 48 months, but relapse infection occurred in 28% of cases (within 24 months) in Group 2 and in 100% of cases (within 12 months) in Group 3.

Translating the above orthopaedic groups to dental groups:

(i) Group 1: RSD as is described in this paper.

(ii) Group 2: Aggressive curettage of periapical fibrotic encapsulation, with the removal of some, but not all sclerotic confinement

(iii) Group 3: intralesional debulking is equivalent to curettage of periapical fibrotic encapsulation of a radiolucent/radio-opaque endodontic lesion within the persistent osteolytic/osteosclerotic encapsulation and is essentially an "intralesional biopsy" of the periapical granuloma, leaving a bone space that is disconnected from microvasculature and the regenerative periosteal stem cells by an undisturbed sclerotic confinement.

Wallenkamp suggested that the most difficult cases to treat include chronic osteomyelitis which had caused severe sclerotic changes, often with irregular cavities and bone bridges which we have observed in edentulous jawbone [20]. This is described as D5 bone in the Nelson and Viljoen bone quality index [21].

NMDS ordination of our curated pyrosequencing bone samples, taken from a bone-bed that we describe as Debrided Apparently Healed Bone (DAHB), produced two distinct ecologic groups, DAHB Gp1 and DAHB Gp2 as seen in figure 3. Both categories had been population shifted by our debridement, but DAHB 2 ordinated much closer to our ecologic health control. Thus, DAHB Gp1 represented a partial shift that could potentially be pushed further along an ecologic recovery trajectory by repeating the debridement. DAHB Gp2 represented a complete return to ecological health.

We noted that RSD in different bone beds resulted in differing degrees of shift, which could be differentiated according to pathologic chronicity. We observed that the longer the duration of the pathology, the more dense and severe the sclerosis, and the harder the forage drilling and reaming became [5,20].

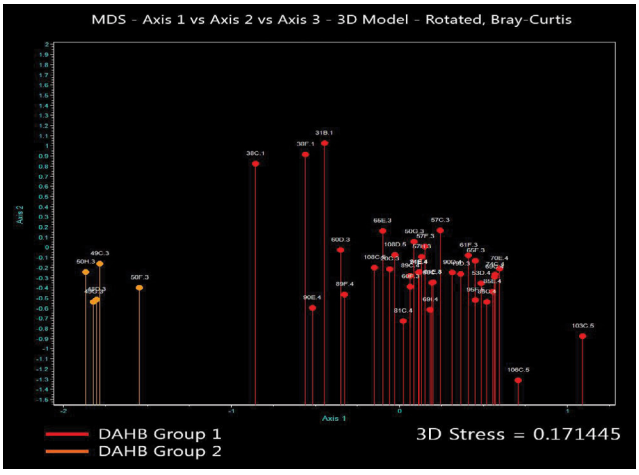

Figure 3: Results of an NMDS ordination for Debrided Apparently Healed Bone (DAHB), which separated into two microbially distinct groups, DAHB Group 1 and DAHB Group 2. DAHB Gp 1 ordinates more distant to our health control, indicating only a partial ecological recovery, and further debridement could be required, depending on radiographic and clinical observations, whilst DAHB Gp2 ordinates close to CAB 1 (the health control).

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The post-debridement anatomic and radiographic regeneration of the bone bed reflected the success of the debridement (or debridements) in bioremediated microbial ecology, which aimed at returning the bone bed to H2/H3 bone quality, as shown in figure 4. This OPG radiograph, taken 6 months after the removal of the 4 remaining infected canines/first premolars, clearly shows that spontaneous healing has not occurred, and there is radiographic evidence of lytic areas, confined by dense sclerosis.

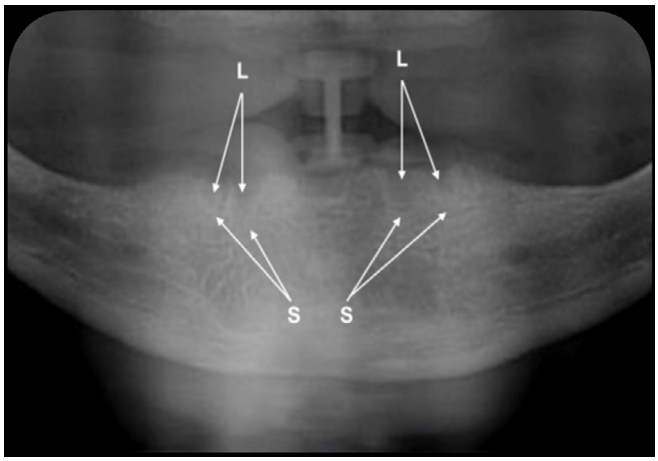

Figure 4: OPG of mandible 6 months post-extraction. Note areas of lysis (L), confined by sclerotic borders (S). Spontaneous healing has not occurred.

Based on the radiographic evidence seen in figure 4, we elected, with patient understanding and consent, to debride this bone-bed through the confining sclerosis to a viable health-margin, prior to implant installation.

A full thickness, inter-foraminal crestal flap was raised, and incomplete cortical plate healing was noted. The bone in the so-called "healed" extraction sockets was soft and appeared fibrotic. RSD was carried out which involved using a No. 8 round bur to forage (penetrate) into the areas of soft, fibrotic bone, through the confining sclerosis and into the surrounding healthy bone tissue. The initial forage osteotomies were 5mm apart (figure 5a), and engagement with a health margin was confirmed by bleeding (the confined "soft/fibrotic" bone had a poor blood supply with little bleeding). Once the initial vertical "forage" penetration holes had been prepared, they were joined together using a round surgical flute bur, creating a trough in the trabecular bone bed, but preserving buccal and lingual cortical (crestal) bone height [22].

The defect was then allowed to fill with blood, and the mucoperiosteal flap soft tissues approximated and closed. No graft materials were used, nor were membranes placed; simply "blood-fill and close" [23], maximizing wound exposure to the stem-cell-rich periosteum, which can generate all layers of bone [24]. After 4 months of healing, radiographic recovery of normal bone anatomy was achieved as seen in figure 6.

During the RSD process multiple fragments of bone, with an aggregate diameter of 7mm were harvested and sent to a pathology lab for histological examination and report, which stated: *"multiple levels of this bony curetting were examined. It shows necrotic areas with fibrosis of the marrow spaces and areas of chronic inflammatory infiltrate comprising predominantly of plasma cells. There is no evidence of malignancy. The overall features favour chronic osteomyelitis. Correlation with radiological and clinical history is recommended"*. The H+E histopathology of "apparently healed" edentulous mandible before RSD confirmed that persistent pathologic lytic/sclerotic bone ridge supported microscopic presence of fibrosis, necrosis

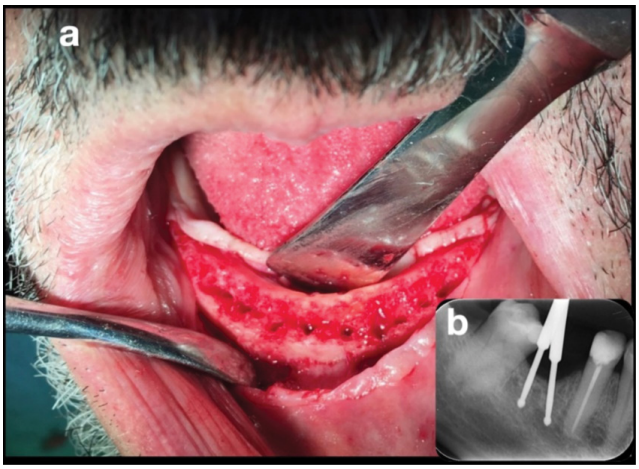

Figure 5: (a) Initial preparation of future implant bone-bed using a No. 8 round bur, which penetrates through the confining sclerosis and into healthy bone tissue. These vertical forage channels were then joined together to form a trough within the trabecular bone while conserving cortical ridge height and confirming punctate point Haversian canal bleeding in the cortical envelope (b) radiograph of a single tooth site preparation, using an implant round bur to prepare the initial forage sites. The mesial round bur is removing lytic zone of deep space intraosseous granulation tissue from the periapical area of the former 46 mesial periapical area.

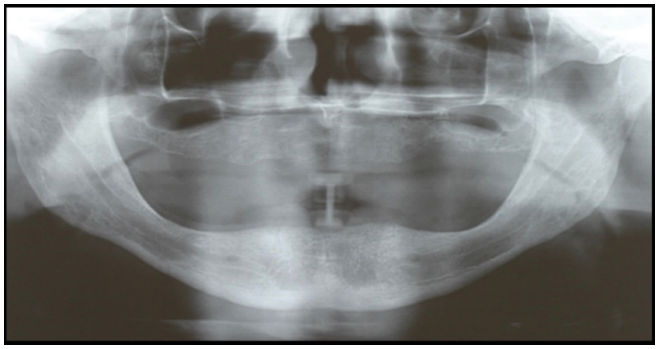

Figure 6: OPG radiograph taken 4 months post-RSD. Note clearly defined superior mandibular cortical plate, even and regular trabeculation, and no evidence of lysis or confining sclerosis. Normal, healthy bony architecture supporting a healthy microbiome and no evidence of any sclerotic confinement.

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and plasma cells providing indirect evidence for chronic osteomyelitis and contraindicating implant installation before RSD and recovery.

Regenerative Surgical Debridement - Clinical Case Examples

Clinical case 1: Complete population shift

This case is an example of a complete population shift, as validated in the NMDS ordination plot in figure 9. Bone quality was shifted from D5 to H3, as referenced against the Nelson & Viljoen bone quality index (figure 7).

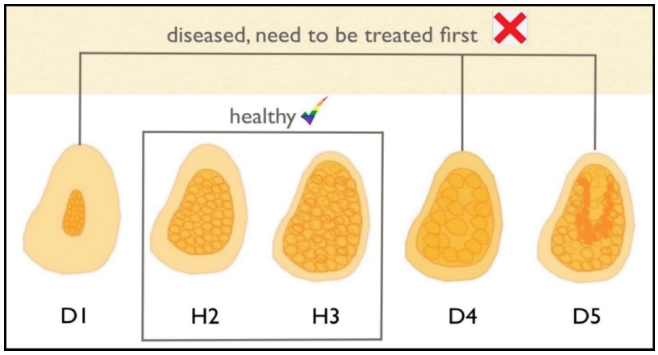

Figure 7: The Nelson & Viljoen classification of bone health and disease, based on a non-sterile, biofilm supporting bone bed. D1 = diseased sclerotic bone with a compromised capillary blood supply, increased cortical thickening and trabecular densification, supporting a pathologic microbiome that ordinates away from that of the health-control; H2 = normal healthy bone exhibiting robust cortical and trabecular bone supporting a stable and diverse microbiome (mostly anterior mandible); H3 = thinner cortex but similar trabecular bone to H2, with a stable and diverse microbiome (mostly anterior maxilla); D4 = diseased bone exhibiting trabecular disruption and lytic areas, supporting a pathologic microbiome; D5 = diseased bone exhibiting areas of dense or diffuse sclerosis surrounding lytic areas (often post-extraction) with trabecular disruption, in which the microbiome ordinates away from the health-control [21].

In this first case, the bone bed at the time of tooth extraction ordinated as a single genus pathology (Streptococcus), however, after RSD and four months of healing, the osteotomy preparation ordinated in the DAHB Gp 2, close to the health control (CAB 1). The bony architecture had returned radiographically to normal and supported a rich, diverse, even, and healthy microbial flora. Successful osseointegration was the outcome (figure 8c).

All of our results suggests that bacterial residency is normal and constant.

This case shows population shift from dominance by a single genus disease biomarker Streptococcus, to 42 genera with most dominant abundance by health biomarker genus Rheinheimera at 29%, with rich, even and diverse assemblages. Enduring osseointegration is supported

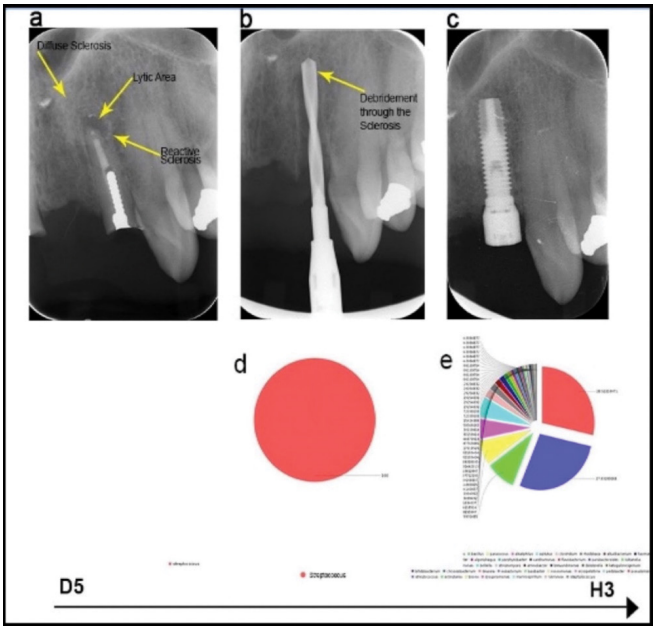

Figure 8: RSD of an infected extraction socket, with both cortical and diffuse sclerotic confinement of a lytic area. Quality D5 bone. Debridement was carried out through the sclerosis, into healthy bone. This case went from a single genera pathology (Streptococcus) to polymicrobial bone health. a) tooth root with chronic periapical pathology showing dual osteolytic/osteosclerotic D5 lesion. There is both circumscribed osteosclerotic demarcation of the osteolytic lesion and diffuse sclerosis superiorly and laterally to the nasal cortex b) following forceps extraction of tooth root and spoon curettage of granulomatous tissue, and axial walls of socket, a labio-lingual mucoperiosteal flap was raised, sufficient to visually check for labial plate defects and to allow for advancement of the flap at closure, following RSD. The socket should not be debrided laterally into the alveolar bone proper, as this is a specialised cortical plate with multipotent mesenchymal stem-cell lineage, essential in the subsequent bone and connective tissue regeneration [25]. Debride within the socket and vertically, through and beyond the tooth apex, and through the sclerosis, using a 2mm twist drill in the extraction socket with profuse, cool sterile saline irrigation, penetrating into the diffuse sclerosis, at not above 2,000 rpm. In this case, the diffuse periapical sclerosis resisted further penetration to nasal cortex to complete a bicortical debridement, but we appeared to achieve perfusion of the wider bone segment. Allow to blood fill, and then close [16,23]. c) implant at 7 months post-installation showing good osseointegration d) a pie-chart of the ordination of the bone bed at the time of tooth removal, showing a single genus biofilm, confirming that not all chronic biofilm pathologies are polymicrobial [9]. e) ordination of the resident genera at the time of the osteotomy preparation and implant installation – note a rich, diverse, even community, with dominance of the health biomarker Rheinheimera, conducive to long-term bone health and resistance and resilience to pathological bacterial invasion, ensuring enduring osseointegration.

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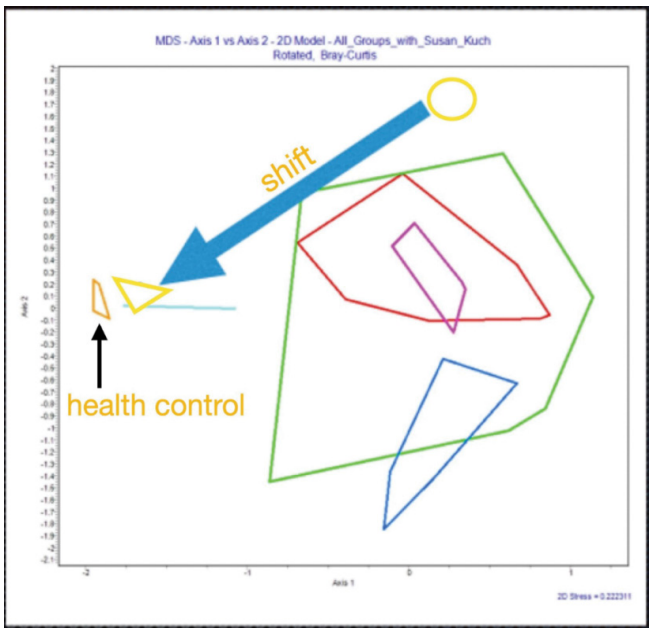

Figure 9: NMDS ordination of samples from the bone bed which underwent RSD 4 months previously. The ecological groups identified are; red = inherited apparently healed bone (IAHB); green = debrided apparently healed bone (DAHB) Group 1 (partial ecologic recovery); turquoise = DAHB Group 2 (complete ecologic recovery); pink = apparently healed soft bone subgroup; orange = congenitally absent bone CAB 1 health control; blue = CAB 2 health control. Yellow represents the community prior to and after the RSD. Note that following RSD, the community ordinates close to the CAB 1 health control.

by symbiont colonisation of health biofilms on smooth (<1 micron), inert implant surfaces and smooth and uniform healthy bone cells.

We have previously published the results of a clinical audit which addressed the RSD outcome question: "was there debridement beyond the sclerosis YES/NO", and "was there implant success YES/NO" [18].

We found a statistically significant association between bone beds that had been surgically debrided before implant installation and the success of the implant (χ^2 = 14.13, df = 1, $p < 0.001$). With a sample size of n = 330, and setting the alpha or p value threshold at 0.05, the association here has a small to medium effect size (w = 0.20), and the achieved power is 96% (indicating that you can be confident in the statistical validity of this result).

The quantitative effect of RSD was population shift, not a return to sterility [26], with a return of normal bone anatomy (qualitative effect). RSD beyond sclerosis acts as the causal event to deliver a press (prolonged) disturbance sufficient to population shift chronic biofilm pathology, with additions and extinctions of phyla along an ecological recovery trajectory in the bone bed. Chronic, persistent biofilm niduses are walled off by osteosclerotic bony encapsulation when the infection has surpassed one month's duration, making the use of antimicrobials ineffective [27].

Clinical case 2: Partial population shift

This case shows a shift from bone quality D5 to H3. The area being treated was the lower left second molar area which had been extracted 12 months previously (figure 10).

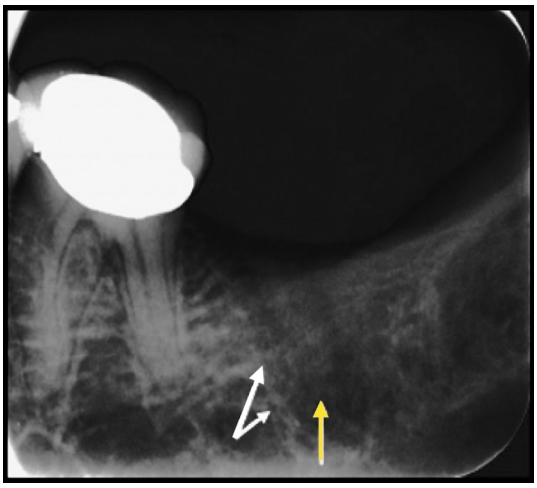

Figure 10: Pre-operative radiograph of mandibular ridge 12 months post-extraction of tooth 37. Note the lytic area with anatomical trabecular disruption (yellow arrow) which is confined by sclerosis (white arrows). This is an example of soft, diseased D5 bone, which proved to be poorly vascularised at the time of surgery. Additionally, cutting torque was very low at the time of the RSD surgery.

Pre- RSD, the patient was placed on a 5-day course of antibiotics which was started the day before surgery (Penicillin V 500mg bid, or Keflex 500mg bid if penicillin allergic).

Following administration of local anaesthetic, a central ridge incision with a bucco-distal relieving incision was made and a full mucoperiosteal flap raised. The crestal cortical plate was then carefully examined, and any residual granulation/fibroconnective tissue curetted with excavators, Rongeurs, scalpels or all three. A countersink drill along with cool sterile saline can also be used to debride the crestal cortex where there are crestal bone defects, after removing any soft tissue. Other instruments used in the debridement process include an implant surgical round drill and different widths of spiral twist drills, a large round surgical flute bur, Rongeurs and scalpels.

Once the crestal ridge has been cleared of all fibro-connective soft tissue, a small round implant drill was used to drill a vertical "forage" hole into the ridge [22], in approximately the lower left 3rd molar area, again, using cool sterile saline and not exceeding 2000 rpm, to avoid overheating the bone. An example of forage holes is seen in figure 5a & b.

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The forage holes allow us to check for vascularity. In this case, the forage hole was avascular. Obviously, understanding and avoidance of the inferior alveolar nerve (IAN) is mandatory, and it is this anatomical structure that will limit the depth of the forage preparation. We then proceeded anteriorly by 5mm and drilled a second vertical forage hole with the round drill. This preparation too was avascular. Next, we proceeded a further 5mm anteriorly and drilled third vertical forage hole, where there was bleeding. A final forage site was then made into the trabecular bone just distal to the root of the 36, and anterior to the vertical section of the confining sclerosis, and this too produced bleeding.

The bleeding forage sites were then joined to the avascular forage sites, with a decorticated crestal trough. This removed devitalised and necrotic tissue and maximised revascularisation and re-connection to the stem-cell-rich periosteum when the wound was closed. The trough was created using a large round surgical flute bur, being prudent about the position of the IAN (if there is any doubt about the proximity of the IAN, a radiograph can be taken with the drill in a forage hole, and the depth referenced against the depth markings on the drill (Figure 5b)). Rongeurs can also be used to remove soft crestal and intra-trough pathologic bone to normal punctate point bleeding in healthy cortical bone. Following trough preparation, the bone was irrigated profusely with cool sterile saline, as was the bony surfaces below the mucoperiosteal flap, then visually inspected and allowed to fill with blood. The periosteal flap was then closed, preferably with a non-resorbable suture material, which is removed after 10 days. Wound healing by primary intent is the objective which was achieved in this case. Failure to achieve wound closure by primary intent may require further suturing.

After 3 to 4 months of healing, the trabecular bone connectivity and architectural regeneration with cortical closure can be reviewed radiographically. If the return to normal architecture is inadequate, a second or even third debridement may be required. The patient needs to be informed prior to commencing a RSD treatment plan, that more than one surgical intervention may be required.

Following the 3-4 month healing period, should the bone-bed appear radiographically normalised and should the patient decide to proceed with implant installation, then further confirmation of bone bed health can be made during the osteotomy preparation, by noting (i) uniform cutting torque, consistent with H2/H3 bone quality (ii) punctate bleeding rather than avascularity or excessive haemorrhage, (iii) lack of any granulation or fibroconnective tissue, and (iv) a closed, regenerated crestal cortex. If any of these four categories of clinical recovery are noted to be absent during implant osteotomy preparation, the implant installation may need to be deferred, and the surgery continues as a "second debridement", with the patient having given prior informed consent to further RSD surgery (if needed) and a further healing period, prior to implant installation, which will only occur once unambiguous bone bed regeneration is noted clinically.

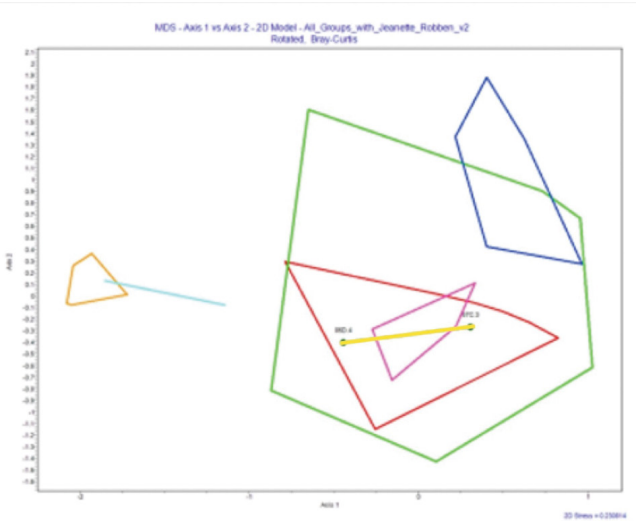

Figure 11: NMDS ordination of samples from the bone bed which underwent RSD 4 months previously. The ecological groups are identified as: red = inherited apparently healed bone (IAHB); green = debrided apparently healed bone (DAHB) Group 1 (partial ecologic recovery); turquoise = DAHB Group 2 (complete ecologic recovery); pink = apparently healed soft bone subgroup; orange = congenitally absent bone (CAB) 1 health control; blue = CAB 2 health control. Yellow represents the community after the RSD and it ordinates within the confines of DAHB Gp 1, thus in this case there was only a partial return to ecological health, although there was a sufficiently good improvement in bony architectural anatomy to support an implant.

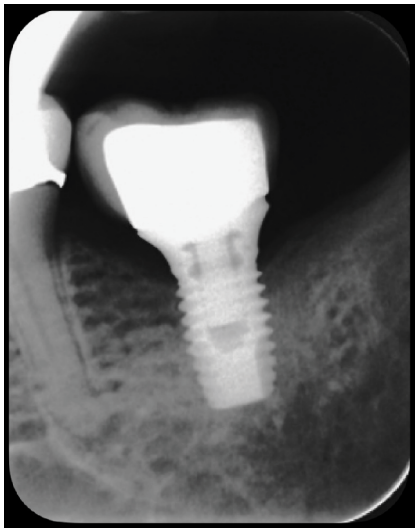

Figure 12: Implant success in a bone-bed that originally presented as poorly vascularized, with disrupted trabeculation and mixed sclerotic/lytic areas. Following RSD there was a return to normal trabeculation resulting in stable and enduring osseointegration.

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Of note is that following the RSD, there was a doubling of the abundance percentage of genera in phylum Firmicutes from 41.81% to 80.52%. This is a phylum at high abundance in the ecologic health control, and this shift that may sustain ecologic homeostasis and enduring osseointegration, as seen in figure 12. With recovery of connected trabeculation, elimination of the mesial sclerotic border of 37 socket and bone up to thread one.

Bone is a regenerative tissue but does not spontaneously regenerate to the pre-disturbance ecology and architecture, as the host cannot overcome biofilm pathosis without debridement and population shift [4,28]. RSD should be repeated until connected trabeculation is achieved, validating the recovery of microbial ecologic health, with concordant internal architectural regeneration. This aims to ensure that enduring, passive osteoblastic osseointegration is supported by diverse, resident bacterial ecologic health and stability, where the native beneficial microbial residency preserves an inert implant surface in symbiotic colonization of abiotic and biotic surfaces, not recognized by the immunobiological system.

Discussion

Man's understanding of bacteria, their preferred life-forms, and their ubiquitous and pervasive nature has grown rapidly over the past few decades. Brånemark, the "father" of implant dentistry, was not aware that human jawbone supported permanent, native, living microbiological ecosystems. However, he clearly understood that the bone-bed had to be "healthy" before implant installation, that is, free of any resident pathogenic bacteria [29].

Bacteria minimize the probability of their extinction by avoiding change in community make-up, and by persisting. Aspects of alpha diversity, such as species richness and evenness, have been shown to enhance the functional resilience and resistance of bacterial communities, as well as the functional resilience of communities of larger organisms [7].

"Resilience" in the microbiome sense means to "spring back "or "rebound", after attempted foreign microbial invasion. Ecological stability, which Botton et al. [7] defined as "the ability of a system to return to a state of equilibrium after a temporary (pulse-type) disturbance" and resilience are, in an ecological sense, inseparable. This means that an "alternative stable state", as described by Shade et al. [30] may emerge from the pre-disturbance condition with an ecological status of lower diversity and stability, more distant or dissimilar to "ecological health" in compositional structure, and therefore, more susceptible to future and further disturbance and even pathologic relapse/recurrence [26].

A resistant, resilient, diverse, and stable biofilm community in bone will foster enduring, host-tissue health and homeostasis in osseointegration. Where a full return to health homeostasis has not been achieved (DAHB Gp1), these alternative stable states of lowered diversity (which are ecologically distinct from their pre-disturbance condition), may be difficult to return to complete health recovery, clinically [7]. Additionally, the "apparent stability" of altered states may be of finite duration, not enduring, which means that the long-term outcomes of these bone-beds and their supported implants may be marred by recurrent (latent) pathology of persister cell biofilm dormancy [30-32] where cell-to-cell quorum sensing communication may resuscitate dormancy [12] to deliver a phenotypic Type IV persister-cell biofilm relapse infection [13] and implant failure. Without dental clinicians embracing a biofilm-based, non-sterile human jawbone model, "periimplantitis", (referred to extensively in the literature), will remain a condition of debated and mis-understood aetiology, will never be fully understood, and will remain without definitive treatment protocols.

The best outcome for an alternative stable state may be the longest relapse-free period, and recurrence may prevail, especially where the compositional population-structure is closer to the pathologic end of the spectrum, where adequate resection into a vascularised health margin was not achieved [19,20].

Our findings support the ecological literature which suggests that recovery of health ecology, supports a microbial community of higher diversity. We have shown that high diversity has a significant clinical value in a comparative context, in sustaining health and colonisation resistance. Our more diverse communities with higher Exponential H plotted closer to our ecological health controls.

Diversity may be the outcome of ecological processes, such as surgical debridement, recovering habitat fitness [33] and not an ecological process in itself [34]. Ecological diversity expressed in the stability of richness and evenness as Exponential H, is greater in bone beds surgically debrided to a vascularised health margin, beyond the confining sclerosis. Diversity defines ecological stability and colonisation resistance and resilience [7]. Nothing can demonstrate the clinical importance of recovering communities with higher diversity, which are more functionally stable, than the inverse fact that implant failure records the lowest Exponential H alpha diversity of any of our ordinated community entities [34]. Of the ecological distinct groupings in this study, the failed, infected implant had lowest Exp H and the most genera distinct from the health control. A biofilm infection of bone and implant surface ordinated as the most significant pathogenicity.

Debridement of extraction sockets showed increased number of genera three to four months after debridement (from average pooled results of 142 genera to 313 genera (DAHB), compared to 200 in inherited apparently healed

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bone (IAHB) where sockets had been left to spontaneously heal, without surgical intervention. In addition, the constituent genera of DAHB contained all the 51 genera in our ecological health control.

The need to debride implant bone-beds which support pathologic communities, arises from the fact that bacteria primarily have a biofilm-mode of life. The sessile biofilm life cycle has a mature final stage which includes production and release of differentiated planktonic dispersal cells [35]. Madhok, Vowden & Vowden [36] stated that "debridement is a crucial and necessary component of wound management". The method of debridement must be adapted and customised to the anatomical location, the width of resection required, and an understanding of the wound histopathology. The latter may reveal current status of the bone-bed as a non-healing site that may require multiple debridements, or the use of more than one type of debridement.

RSD lays a solid foundation for bone bed preparation, prior to implant installation. It plays the principal role in bacterial community management by creating the opportunity for the synergistic coaggregation of different planktonic subsets beyond the pre-existing pathogenic profile of the wound microenvironment. Normalising the wound (the extraction site) microbiota may be consistent with regeneration of a health habitat in human jawbone [35-39].

Conclusion

Debridement of human jawbone is not intended to return bone to sterility. Rather, debridement is intended to circumvent pathologic biofilm by inducing a population shift, to communities which are either less pathologic (in partial recovery), or non-pathologic (in complete recovery), as measured against an ecologic health control. Multiple RSD may be required to drive recovery closer to ecologic health.

We have demonstrated live biofilm population shift in longitudinal same-case NMDS analysis of curated pyrosequencing. The magnitude of the difference between "no debridement" and "debridement" is the scale or quantitative effect through population shift. This confirmed and measured the clinical effect of population shift and the quantitative and qualitative (radiographic) difference between the ordination of pre- and post-debridement curated pyrosequencing, measured against our ecologic health control.

We identified the genera lost in extinction, and the genera acquired by habitat fitness, during resident population shift in deep bone-space. Implants should only be installed in quality H2/H3 bone. Once we accept a biofilm-based bone model, and biofilm-based osseointegration, we can continue to develop implant surfaces that evaded pathogen adhesion, as well as surgical protocols, perhaps routinely undertaken at the time of tooth extraction, to definitively eradicate residual biofilm infection before implant installation, improving ecological diversity, stability, bone quality and enduring osseointegration. The established statistical clinical effect of RSD in population shift confirmed that the comparative increase in microbial diversity did deliver more functional stability in the face of disturbance, and improved osseointegration outcomes.

The authors acknowledge that further research into the nature of human jawbone microbiome, the bacterial relationship with bone health and disease, as well as bone quality and anatomy, and the role that the implant surface topography may play, is required.

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Author's contributions

Stephen Nelson, Honghua Hu, Georgina Luscombe 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 Viljoen: Analysis and interpretation of data, drafting, editing and critical reviewed for significant intellectual content. Final approval was given for submission.

Ethics

The study was conducted in accordance with the declaration of Helsinki and approved by the University of Sydney Human Research Ethics Committee (HREC) (reference 07-2007/9962).

Informed consent statement

Written information about the study was provided to all participants and written, informed consent obtained.

Data availability statement

Data are available from corresponding author on request.

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