Regenerative surgical therapy for peri-implantitis using deproteinized bovine bone mineral with 10% collagen, enamel matrix derivative and doxycycline - a prospective 3-year cohort study.

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Running Title: Enamel matrix derivative with xenograft for peri-implantitis treatment.

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ABSTRACT

Background: There is limited evidence regarding the long-term efficacy of regenerative treatment for peri-implantitis. The aim of this study was to evaluate a combination therapy of deproteinized bovine bone mineral with 10% collagen (DBBMC), enamel matrix derivative (EMD) and doxycycline in the regeneration of bone defects associated with peri-implantitis.

Methods: 30 patients diagnosed with peri-implantitis (BoP/suppuration, probing depth greater than 4mm, minimum radiographic bone loss of 20%, at least 2 years in function) were enrolled in the study. Clinical measurements included probing depths, recession, radiographic bone fill, gingival inflammation and bleeding on probing/suppuration. Following surgical access and debridement, the implant surfaces were decontaminated with 24% EDTA for 2 minutes and the bone defects were filled with a combined mixture of DBBMC, EMD and doxycycline powder. The defects were covered with connective tissue grafts where necessary. Clinical measurements were recorded after 12, 24 and 36 months.

Results: The mean probing depth and bone loss at the initial visit was 8.9 mm (± 1.9) and 6.92 mm (± 1.26) respectively. Both mean probing depth and bone loss reduced significantly from baseline to 3.55 mm (± 0.50) and 2.85 mm (± 0.73) at 12 months, 3.50 (± 0.50) and 2.62 mm (± 0.80) at 24 months and 3.50 mm (± 0.50) and 2.60 mm (± 0.73) at 36 months. 56.6% of the implants were considered successfully treated (according to Successful Treatment Outcome Criterion: PD < 5mm, no further bone loss >10%, no BoP/suppuration, no recession > 0.5 mm for anterior implants and > 1.5 mm for posterior implants) after 36 months.

Conclusion: Regenerative treatment of peri-implantitis using a combined mixture of DBBMC, EMD and doxycycline achieved promising results. The benefits of this protocol incorporating EMD should be tested in randomized clinical trials.

Introduction

Peri-implantitis is defined as an inflammatory process affecting the surrounding peri-implant tissues, resulting in loss of supporting bone (Zitzmann and Berglundh 2008). The reported prevalence of peri-implantitis varies depending on the defining criteria used, ranging from 6.6% over a 9-10 year observation period (Mombelli et al 2012, Roos-Jansåker et al 2006) to 36.6% after an average of 8 years of implant loading (Koldslund et al 2010, Atieh et al 2013). Given the increasing numbers of implants being placed, a predictable and effective treatment for peri-implantitis is urgently required.

Laboratory and clinical studies point to a similar pathogenesis between periodontitis and peri-implantitis, and hence similar approaches to management have been advocated (Heitz-Mayfield and Lang 2010, Heitz-Mayfield LJ. 2008, Meffert RM. 1996). However, human biopsy results show that the apical extension of the inflammatory infiltrate relative to the alveolar bone crest is more pronounced in peri-implantitis than in periodontitis, suggesting that peri-implantitis may progress faster than periodontitis and/or the lesion may be less contained (Carcuac and Berglundh 2014, Berglundh et al 2011; Heitz-Mayfield and Lang 2010). The potentially accelerated nature of the disease progression around implants suggests the need for a more 'aggressive' treatment approach to peri-implantitis than periodontitis. Indeed, it was concluded at the Sixth European Workshop on Periodontology that non-surgical therapy alone seems to be insufficient in the treatment of most cases of peri-implantitis and surgical therapy is often required (Lindhe and Meyle 2008).

The available literature on the management of peri-implantitis focuses on anti-infective treatments which involve non-surgical or surgical debridement and decontamination followed by ongoing supportive therapy (Heitz-Mayfield and Mombelli 2014, Heitz-

Mayfield et al 2016, Heitz-Mayfield et al 2012) or regeneration of the peri-implant bone defect (Heitz-Mayfield and Mombelli 2014, Esposito et al 2012). However, the details of the protocol in relation to the implant surface decontamination approach and the regenerative materials used, are still open to debate. Numerous implant regenerative surgical therapy studies, including the use of bone grafts/bone substitutes with and without membranes, have reported clinical and radiographic improvements for at least 3 years (Schwarz et al 2017, Froum et al 2015, Froum et al 2012, Schwarz et al 2013, Roos-Jansåker et al 2011, Schwarz et al 2009, Behneke et al 2000). These clinical studies used different grafting materials, thus it was difficult to draw conclusions in regards to which method is superior (Esposito et al 2012), but no currently available methods are able to achieve predictably successful outcomes and hence further research is required in relation to the regenerative treatment of peri-implantitis (Esposito et al 2012).

There are limited studies that report on the use of biologically active materials such as enamel matrix derivative (EMD) to improve the performance of osteoconductive bone grafts (Froum et al 2015, Isehmed et al 2016). The purpose of the present clinical case series was to evaluate the 3-year clinical and radiographic results of combined osteoconductive deproteinized bovine bone mineral with 10% collagen (DBBMC), osteoinductive EMD and topical antibiotic doxycycline for surgical regenerative therapy of peri-implantitis.

MATERIALS AND METHODS

This study was designed as a prospective cohort study in compliance with the STROBE checklist.

Patient Selection

30 consecutive patients (age range 20-70) referred for peri-implantitis were recruited to participate in the study. The inclusion criteria were:

- 1) systemically healthy, non-smokers,
- 2) adequate oral home care
- 3) patients who completed the pre-surgical phase of periodontal treatment which involved full-mouth ultrasonic debridement and manual curettage which resulted in FMBS below 20%.
- 4) Only one implant affected with peri-implantitis, exhibiting a “crater-like” or circumferential defect, a bleeding/suppurating pocket of > 4 mm, minimum 20% alveolar bone loss and in function for at least 2 years.

Exclusion criteria were:

- 1) patients with uncontrolled diabetes,
- 2) taking bisphosphonate medications
- 3) pregnant or lactating females.

Ethics Approval

All patients received a detailed explanation of the surgical procedures and objectives of the study and signed an informed consent form prior to being included in the study. The Griffith University Human Research Ethics Committee provided ethical approval for this project (GU Ref No: 924). The project complies with the provisions contained in the National Statement on Ethical Conduct in Research Involving Humans and

complies with the regulations governing experimentation on humans. The reporting of the results of the study complies with the EQUATOR (<http://www.equator-network.org>) guidelines for cohort studies (STROBE).

Pre-surgical phase

All patients received full mouth ultrasonic debridement and manual curettage as needed (with or without local anaesthesia) 4-6 weeks before the surgical protocol was performed. All patients reached full mouth plaque score (FMPS) and full mouth bleeding scores (FMBS) below 20% before proceeding with the surgical treatment.

Preparation of Combined Grafting Material (Figure 1a -1d)

In a sterile dish, deproteinized bovine bone mineral with 10% collagen (DBBMC) (Bio-oss Collagen, Geistlich, Switzerland) was mixed with 0.35 ml of enamel matrix derivative (EMD) (EMDOGAIN, Straumann, Basel, Switzerland) for 15 minutes. One capsule of Doxycycline 100 mg was added to the DBBMC and EMD and the cocktail of materials were mixed until a homogenous material was formed. The rationale for using this treatment cocktail was to harness the reported beneficial effects of each of these materials in the management of peri-implantitis - the osteogenic and space maintenance properties of DBBMC (Roccuzzo et al 2016), the wound healing enhancement capacity of EMD (Miron et al 2010; Isehed et al 2016) and the antibiotic and immunomodulatory effects of Doxycycline (Golub et al 2016).

Surgical Phase (Fig 2a-Fig2d)

All surgical procedures were performed under local anesthesia (Lidocaine HCL 2% with 1:100,000 epinephrine) and oral sedation (Diazepam 5-10 mg, one hour prior to

procedure) by the same experienced periodontist (F.M.). An intra-sulcular incision at the affected implant was made using a 15C blade (Swann-Morton, Sheffield England) and the flap was extended distally, with a vertical releasing incision made one to two teeth away from the affected implant. After flap elevation and removal of granulation tissue, the exposed implant threads were debrided using a fine tip low power ultrasonic scaler (Cavitron SPS, Dentsply, York, PA, USA). The implant surface was dried with gauze and 24% ethylenediaminetetraacetic acid (EDTA) (Prefgel, Switzerland) was applied to all exposed threads for 2 minutes. The surfaces were then washed with saline solution. The prepared cocktail of materials was applied to all the exposed threads. Primary closure was performed by using a coronally advanced flap (CAF) and a connective tissue graft was added if necessary, such as when there was less than 1 mm of keratinized gingiva surrounding the fixture or if the defect was in the aesthetic zone (anterior maxilla). Six maxillary anterior implants and two mandibular posterior implants received connective tissue grafts as part of the regenerative therapy. Resorbable sutures were exclusively used (3-0, Vicryl[®], Johnson and Johnson, Diegem Belgium).

Clinical Measurements

The following clinical parameters were measured with a periodontal probe (Hu-Friedy[®], Hu-Friedy Mfg. Co, LLC, USA) at baseline, 12, 24 and 36 months:

- Periodontal recession (REC): distance between the implant-abutment and gingival margin (GM) at the mid-buccal aspect of the implant.
- Probing depth (PD): distance between the GM and the base of the deepest pocket of the implant.

- Buccal Keratinized Tissue (KT): distance between GM and mucogingival junction at the mid-buccal aspect of the implant.

A second clinician who was not involved in the surgical procedure recorded all clinical parameters using 3.5x magnification. A calibrating exercise was performed to achieve intra-examiner reproducibility: Full mouth charting of the same five patients was carried out at a weekly interval for 3 consecutive weeks. Calibration was confirmed when the measurements were consistently accurate to 0.5 mm.

Radiographic measurement

Radiographs using a standardized long cone paralleling technique x-ray holder (XCP-DS® x-ray holder, Chicago, USA) were taken preoperatively, 12, 24 and 36 months after treatment (Figure 3a-3d). The same x-ray machine (Kodak 2200 intra-oral X-ray unit, Trophy, Cedex 2 France, operated at 70 kVp, 15 mA, .14 sec.) was used as the source of radiation for all images. The change in bone level (mm and % bone level) as a result of the treatment was measured by comparing the distance between the abutment-implant junction and the bone level at the mesial and distal surfaces pre-operatively with that at 12, 24 and 36 months post-regenerative therapy. The radiographic measurements were also carried out by the clinician who was not involved in the surgical procedure.

Postoperative Care

Patients were instructed not to brush the surgical sites for 2 weeks. All patients were advised to take an analgesic (combined Paracetamol 500 mg and Ibuprofen 150 mg), as needed. All patients were given a postoperative kit containing .12% chlorhexidine digluconate mouthwash, surgical brush and .12% chlorhexidine gel. The mouthwash

was used twice a day for the first week, and the surgical brush and chlorhexidine gel were applied to the treated area during the second and third weeks post-operatively.

Supportive Periodontal Therapy

The implants were reviewed and supportive periodontal therapy (SPT) was provided at 3, 6, and 12 months, and then every 4 months thereafter. The SPT protocol included full mouth ultrasonic debridement (Cavitron SPS, Dentsply, York, PA, USA) and prophylactic clean and polish with rubber cap and pumice (Nupro®, Dentsply, Victoria, Australia). Additional manual curettage (with or without local anesthesia) using Gracey curettes (Hu-Friedy^R, Chicago, USA) and ultrasonic instruments was performed on the affected implant or dentition if there was BoP/suppuration. The patients were recalled after 1 week, 3 weeks, the 3rd month, 6th month and every 4 months thereafter.

Successful Treatment Outcome Criterion (STOC)

A composite criterion to determine whether an implant was successfully treated and did not need further surgical intervention was followed (Heitz-Mayfield et al 2014). Specifically, the proposed success criterion at the 36th month were:

- 1) Implant with deepest PD of <5 mm.
- 2) No further bone loss >10%
- 3) No BoP or suppuration
- 4) Recession of < 0.5 mm for anterior implants (aesthetic criterion) and <1.5 mm for posterior implants (cleansability criterion).

Statistical Analyses

A paired t-test was used to assess the differences in the clinical parameters between baseline and 12, 24 and 36 months. A p-value less than 0.01 was considered to be significant.

RESULTS

Patient and Implant Attributes (Table 1)

Thirty consecutive systemically healthy patients (with 30 peri-implantitis affected implants) completed the study. The female to male ratio was 2:1 and the mean age of patients treated was 44.9 (± 11). The majority of the treated implants were located posteriorly with almost half located on the mandibular arch (40%). Most of the treated implants were micro-rough surface implants (Table 1). All patients enrolled in the study maintained a low FMPS and FMBS of less than 20%.

Implant Survival

All 30 implants that were treated in this study survived and were functioning at the 36 months post-surgical review. There were statistically and clinically significant improvements in bone level, probing depth and percentage BoP from baseline, which were maintained at 12, 24 and 36 months after surgical treatment.

Clinical Parameters (Table 2)

Bone Level Changes

The peri-implantitis treated in this study can be considered moderate to advanced with a mean bone loss prior to treatment of 6.92 mm (± 1.26) ($57\% \pm 16.5$). This was reduced significantly to 2.85 mm (± 7.3) ($15.5\% \pm 9.2$) at 12 months and maintained at 24 months (2.62 mm ± 0.80 mm, $14.8\% \pm 8.2$) and 36 months (2.60 mm ± 7.3 , $14.5\% \pm 7.3$). The difference in the percentage bone loss from pre-treatment to 12, 24 and 36 months is statistically significant ($p < 0.01$). There were no statistically significant changes between 12, 24 and 36 months.

Probing Depth

The mean depth of the deepest peri-implant pocket of each implant (PD) at the initial visit was 8.9 mm (± 1.9). This mean PD reduced significantly to 3.55mm (± 0.49) by 12 months post-treatment, and was maintained at 3.50 mm (± 0.50) at 24 and 36 months after the regenerative therapy. The difference in the probing depth from pre-treatment to 12, 24 and 36 months post-treatment was statistically significant ($p < 0.01$).

Recession

The mean recession at the initial visit was 0.16 mm (± 0.14). This recession remained stable at 0.15 mm (± 0.12) at 12 months and 24 months. After 36 months the mean recession levels slightly increased to 0.22 mm (± 0.19). The difference in the recession level from baseline to 12, 24 and 36 months was not statistically significant.

Bleeding on probing and suppuration (Figure 4)

All of the patients achieved a FMBS and FMPS of less than 20% at every review visit. In terms of bleeding/suppuration percentage at the treated implants, 100% of the implants treated had BoP at baseline. This dropped to 20% at the 3rd month review, and reduced further to 10% at the 6th month maintenance visit. The maintenance period was increased to 6 months because of the stability achieved after the first 6 months of healing. The percentage of implants with BOP/suppuration increased to 50% after an additional 6 months (12 month follow-up) without peri-implant maintenance. Subsequently, four monthly SPT was performed from the 12th to 36th month after surgical treatment. This led to a reduction of implants presenting with BOP

and suppuration (Figure 4), which was observed to be 20% at both the 24 and 36 month reviews.

Success Criterion (Table 3)

At 36 months after regenerative therapy, 56.6% of the implants treated were considered successful. On the other hand, 43.3% of the treated implants failed to achieve the success criterion. The most common cause of failure 36 months after treatment was persistence of BoP (6 implants, 20%) and radiographic evidence of interproximal bone loss (4 implants, 13%). Also contributing to the treatment failure rate were the anterior implants with gingival recession greater than 0.5 mm (2 implants, 6.66%) and posterior implants with greater than 1.5 mm gingival recession (1 implant, 3.33%).

DISCUSSION

This study reports on a cohort of 30 consecutive patients suffering from peri-implantitis who were treated using a regenerative surgical protocol utilizing commercially available products. The result of this study showed that the approach of using the combination of DBBMC-EMD-Doxycycline is effective in the regenerative treatment of moderate to advanced peri-implantitis, with maintenance of the positive outcomes for a 3-year period.

The present case series found statistically significant improvement in PD, BL and BoP % after 12, 24 and 36 months. This PD reduction is similar to Froum et al (2012), who combined an osteoconductive bone graft with biologically active material (EMD/PDGF) and reported a PD reduction of 5.10 mm (± 2.20). In the present study, it is possible that DBBMC acted as a delivery vehicle for longer or sustained release of EMD in the peri-implantitis defect, potentially enhancing its biological effectiveness in promoting healing within the peri-implant defect.

Published case series and RCTs that used bone graft alone without biologically active materials, such as xenograft (Schwarz et al 2017, Rocuzzo et al 2017, Rocuzzo et al 2016, Rocuzzo et al 2011, Schwarz et al 2009), phycogenic bone graft (Alipore, Friadent, Malmo, Sweden) (Roos-Jansåker et al 2014) and alloplast (Nanocrystalline hydroxyapatite (NHA), Ostim, Heraeus, Hanau Germany) (Schwarz et al 2009, Schwarz et al 2006) showed a PD reduction range of 1.5-3.2 mm. Rocuzzo et al (2016) used DBBMC without a membrane in 13 circumferential peri-implantitis defects and showed a PD reduction of 3.10 mm (± 2.69) after 12 months. These mean values are lower than the 5.4 mm (± 1.2) mean PD improvement in the present study. Further

follow-up of the same patients 7 years after surgical treatment with adequate SPT demonstrated that stable peri-implant tissue conditions were achieved in many cases, although some patients required further treatment and some implants were lost (Roccuzzo et al 2017).

A randomized clinical trial investigating the use of EMD for the management of peri-implantitis showed that the use of EMD did not result in improved PDs and BoP after 12 months (Ishida et al, 2016). This study however suggested a potential use of EMD in peri-implantitis management by reporting that the adjunctive use of EMD was associated with increased marginal bone level and increased prevalence of Gram +/- aerobic bacteria after 12 months of healing.

Osteogenic Properties of Enamel Matrix Derivative

The effectiveness of enamel matrix derivative (EMD) in the treatment of gingival recession and periodontal defects is widely reported (Parashis et al 2004), demonstrating a clear positive effect on soft tissue healing. However, the effectiveness of EMD in treating peri-implantitis, and in particular its role in peri-implant bone regeneration, is still unclear. It was demonstrated in an in vitro study that EMD significantly increased alkaline phosphatase activity and osteocalcin production in MG-63 cells grown on titanium discs, suggesting that EMD may have a positive effect on implant integration (Qu et al 2011). In another in vitro study, the capacity of ceramic coated with EMD to assist in the formation of hard tissue by mesenchymal and periodontal ligament fibroblasts was assessed (Mrozik et al 2012). The study showed up-regulation of several important bone-related genes suggesting that EMD may have a potent positive effect on the differentiation of mesenchymal cells to mature bone

cells in vitro (Mrozik et al 2012). In a study evaluating the effect of EMD on the attachment, proliferation and differentiation of osteoblasts on titanium in vitro, it was concluded that coating titanium with EMD enhances proliferation and differentiation of osteoblasts, suggesting the potential use of this product in bone regeneration, particularly on implants affected by peri-implantitis (Miron et al 2010).

Nevertheless, although our study suggests that EMD may be beneficial for bone formation within the peri-implant defect, the literature is unclear about the osteogenic potential of EMD. In support of our findings, a histomorphometric study in dogs showed that EMD, when combined with a membrane, positively influenced bone healing in dehiscence-type defects around implants when compared to EMD alone (Casati et al 2002). On the other hand, the use of EMD and deproteinized bovine bone mineral (DBBM) with GBR in comparison with GBR alone did not enhance the amount of bone formation in mandibular jaw defect in rats (Donos et al 2005), and did not contribute to bone formation around titanium implants in rabbit models (Franke et al 2003). Therefore, the pro-osteogenic effectiveness of EMD requires further investigation in pre-clinical and clinical models.

Implant Surface Debridement and Decontamination

Numerous implant surface decontamination techniques and materials have been suggested, with no clear superior protocol being supported by the available evidence (Persson et al 2004, Schou et al 2003). The debridement and decontamination protocol used in the present study involved the use of low-power ultrasonic instrumentation, EDTA surface treatment and the local administration of the antibiotic Doxycycline. Based on the favorable clinical outcomes reported, it appears that this

protocol is effective in decontaminating the implant surface and the associated defect, facilitating the resolution of the peri-implant inflammation.

Ultrasonic instrumentation was used in this study based on its widespread clinical availability, established effectiveness in biofilm removal around teeth, time-effectiveness and ability to access the diseased surfaces. It has been demonstrated that there were no significant differences in the clinical parameters measured following peri-implantitis treatment when titanium hand-instruments were compared with ultrasonic instrumentation (Renvert et al 2009). Further, the use of EDTA in our study is supported by the findings of Rocuzzo et al (2011) and Rocuzzo et al (2016) who showed a beneficial effect of the use of 24% EDTA as an implant surface decontaminant.

The local application of doxycycline, using the xenograft as the delivery method, may have helped improve surgical outcomes via its antimicrobial and anti-collagenase activity. The use of local antibiotic therapy has been advocated for the management of peri-implantitis, and it has been reported that non-surgical management of peri-implantitis with local delivery of micro-encapsulated minocycline resulted in improved clinical outcomes after 12 months (Salvi et al 2007).

Supportive Periodontal Therapy (SPT)

It has been demonstrated that when surgical and regenerative management of peri-implantitis is followed by regular SPT, a majority of the implants affected can be maintained over the long term (Heitz-Mayfield et al 2016). It was also demonstrated that the complete health and absence of bleeding at all sites of the treated implant is

harder to achieve in the long term (Heitz-Mayfield et al 2016). In the present study there was a tendency towards recurrence of bleeding on probing (50% of treated implants) when the SPT interval was set at 6 months, as was the case between the 6 and 12 month follow-up. The percentage of implants with bleeding on probing reduced to 20% when the SPT was subsequently reduced to a 4 monthly period. This suggests that the dental implants treated in the study are at a high risk of recurrence of inflammation if the supportive therapy is extended longer than 4 months. Furthermore, the same 20-30% of patients demonstrated persistent bleeding on probing throughout the 36 months period of the study suggesting a possible phenotype of patients or type of implants that are susceptible to inflammation recurrence after surgical therapy for peri-implantitis.

The probing depth reduction and bone fill was considerable with the 'cocktail' protocol used in the present study, showing the effectiveness of this approach for the regenerative therapy of peri-implantitis. Whether the radiographic bone fill reflects the establishment of new bone-implant contact cannot be ascertained from the present study. The potential of peri-implantitis affected implants to achieve re-osseointegration can only be inferred from available animal studies that showed the possibility of "re-integration" after peri-implantitis therapies (Persson et al 2001, Schou et al 2003). More recently, histological assessment of a single case has provided proof-of-concept evidence that re-osseointegration is possible in humans (Fletcher et al 2017).

This cohort study shows that the described protocol, using a combination of DBBMC, EMD and doxycycline, is effective in the regenerative therapy of peri-implantitis.

However, it only reports on the single treatment protocol without any comparison/control groups, and hence it is not possible to determine the relative contributions of the various 'cocktail' components on the treatment outcomes. This is a drawback of the study, and the relative contributions of the various components of the 'cocktail' need to be explored in controlled long-term randomized clinical trials

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Conflict of Interest and source of funding statement

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