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Enamel matrix derivative promotes new bone formation in xenograft assisted maxillary anterior ridge preservation – a randomized controlled clinical trial.

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Abstract

Objectives: To compare the effectiveness of deproteinized bovine bone mineral with 10% collagen alone (DBBMC) or with enamel matrix derivative (DBBMC-EMD) in ridge preservation.

Methods: 42 maxillary anterior teeth were extracted and received either a DBBMC (control) or DBBMC-EMD (test) treatment protocol. CBCT taken before and 4 months after the extraction procedure was used to measure changes in alveolar ridge width (RW), buccal bone height (BH) and palatal bone height (PH). Bone cores were harvested during implant osteotomy preparation and the samples processed histomorphometrically to assess the fraction of new bone (%NB), residual graft (%RG), and soft tissue matrix (%STM).

Results: Overall, both treatment groups showed significant reductions in mean RW from baseline to 4 months after extraction, but no significant change in either mean BH or PH over this time. When CBCT measurements were analysed according to the initial thickness of the buccal wall (BT <1 mm vs. BT ≥1 mm), significant reductions in all ridge dimensions (RW, BH and PH) were noted in the <1 mm BT group. Histomorphometrically, the DBBMC-EMD test group showed significantly increased new bone formation (%NB): (control = $16.5 \pm 6.9\%$ cf.; test = $45.1 \pm 8.8\%$) with less residual graft (%RG): (control = $36.8 \pm 8.8\%$ cf.; test = $20.3 \pm 7.2\%$) compared to the DBBMC control group.

Conclusions: DBBMC alone and DBBMC-EMD treated sites 4 months after extraction both lost RW but showed no significant change in BH or PH. Irrespective of treatment, maxillary anterior teeth with thick initial buccal walls (≥1 mm) exhibited less alveolar ridge reduction 4 months after treatment. The addition of EMD to DBBMC stimulated bone formation in the test group.

Introduction

Extraction and replacement of maxillary anterior teeth is one of the most challenging tasks in oral rehabilitation. Healing of extraction sockets involves physiological resorption and remodeling resulting in three-dimensional changes affecting alveolar ridge height and width (Van der Weijden et al 2009, Araujo et al 2005, Cardaropoli et al 2003). The disruption of blood supply from the periodontal ligament after tooth extraction and increase in local osteoclastic activity in the area initiates the bone resorptive process (Araujo et al 2005, Cardaropoli et al 2003) resulting in an average of 1.5 - 3 mm vertical and 3 - 4.5 mm horizontal alveolar bone loss (Araujo et al 2005, Iasella et al 2003, Lekovic et al 1998). Most of these dimensional changes in the alveolar bone morphology take place in the first 3 months following tooth extraction (Araujo et al 2005, Schropp et al 2003). In the anterior maxilla, these three-dimensional changes represent the key causative factor for esthetic implant complications and failures (Belser et al 2009, Chen and Buser 2009).

Variable outcomes have been obtained in numerous ridge preservation studies using different osteoconductive particulate grafting materials to maintain post-extraction ridge dimensions in the anterior maxilla (Kassim et al 2014, Avila-Ortiz G. et al 2014, Macbeth N et al 2017, Darby I et al 2009). Generally, favourable outcomes have been obtained with slowly resorbing materials, such as deproteinized bovine bone mineral (DBBM) and DBBM stabilized with 10% Collagen (DBBMC) (Iorio-Siciliano et al 2017). However, these slowly resorbing materials also interfere with new bone formation in the healing socket (Araujo et al 2009), which may compromise the osseointegration of implants subsequently placed in these sites. Hence, biologically active materials, such as platelet-rich plasma (Strauss et al 2018, Cheah et al 2014), platelet-rich fibrin (Marenzi et al 2015) and recombinant bone morphogenetic protein-2 (Lim et al 2018) have been utilized to improve the performance of osteoconductive materials with mixed results.

Enamel matrix derivative (EMD) is an insoluble matrix derived from the extract of naturally occurring enamel matrix proteins (EMPS), which are formed during amelogenesis by ameloblasts in Hertwig's epithelial root sheath (HERS) during tooth formation. HERS

regulates the formation of the periodontal attachment apparatus, particularly the maturation of acellular extrinsic fibre cementum producing cementoblasts from progenitor cells (Gestrelius et al 1997, Heijl L. 1997, Hammarstrom L. 1997). Emdogain®, a regenerative product introduced in the 1990s, is a gel product extracted from porcine tooth buds which contains mainly amelogenins and propylene glycol alginate (PGA) as carriers.

A recent study explored for the first time the effect of incorporating EMD with DBBMC for ridge preservation in the anterior maxilla, reporting no beneficial effect on ridge dimensional outcomes (Lee et al 2020), but the osteogenic effect of EMD was not explored. Therefore, the aims of this prospective randomized controlled clinical study were 1) To assess dimensional changes of grafted extraction sockets using either deproteinized bovine bone mineral with 10% collagen alone (DBBMC) or DBBMC with enamel matrix derivative (DBBMC-EMD) in preservation of the maxillary anterior ridge. 2) To assess the osteogenic potential of EMD by assessing histomorphometrically a tissue biopsy harvested from the treated alveolar ridge.

Materials and Methods

Ethics and consent

The Griffith University Human Research Ethics Committee provided ethical approval for this project (GU Ref No: 924). The study was also registered in the Australia and New Zealand Clinical Trial Registry (ACTRN1269000062123). The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. The reporting of the results of the study complies with the EQUATOR (<http://www.equator-network.org>) guidelines for randomised trials (CONSORT).

The surgical protocol was discussed with the patients including all of the risks and benefits of the procedure. 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.

To be considered eligible for the study, the following inclusion criteria were applied:

- Non-smoker, over 25 years of age at the time of the surgery and needing extraction of one of the maxillary anterior teeth (13, 12, 11, 21, 22 and 23).
- Systemically healthy and able to undergo oral surgical procedures under local anaesthesia and oral sedation.
- Any presenting periodontitis was treated and stabilized (no probing depth >4 mm) and enrolled in a supportive periodontal program, with Full Mouth Plaque Score (FMPS) and Full Mouth Bleeding Score (FMBS) less than 20% at the time of surgery.
- Adequate mesio-distal restorative space (minimum 7 mm) for dental implant.
- Tooth root in ideal position for future implant placement so as to ensure the trephined tissue could be taken mainly from the grafted site.
- Absence of significant peri-apical pathology (peri-apical pathology <2 mm).
- Absence of acute infection/suppurative at the time of extraction.
- Buccal dehiscence ≤ 1 mm present at the time of extraction, no palatal defect.

Exclusion criteria

Patients were considered ineligible for the study if any of the following conditions were present:

- All inclusion criteria were not met.
- Pregnant or lactating patients.
- Systemic condition that would prevent successful healing after extractions and implant placement (immunosuppressive medications, anti-resorptive medications, uncontrolled diabetes mellitus, auto-immune diseases).
- Mucosal diseases such as erosive Lichen Planus.
- Severe bruxism and parafunctional habits.
- Previous guided bone regeneration (GBR) or dental implant rehabilitation in the anterior part of the maxilla.
- Benign or malignant neoplasms at the anterior part of the maxilla.

Subject Enrolment Protocol

Fifty-three consecutive patients referred to a private periodontal practice (Western Sydney, NSW Australia) specifically for extraction of failed maxillary anterior tooth and replacement with dental implants (from Jan 2014 to June 2018) were recruited for the study. Eleven patients were excluded from the study because of a) complex medical history such as uncontrolled diabetes mellitus and prescription of bisphosphonates medications (2 patients), b) 9 patients were excluded because of the presence of buccal and palatal defects greater than 1 mm.

It was calculated that a sample size of 16 patients per group was required to detect a 10% difference in the means of the primary parameter (%NB), based on 95% confidence level, 80% power and a predicted variance of 100 (10% deviation from the mean) (Alayan et al 2016). Accounting for a potential dropout rate of 20%, a total of 42 patients were enrolled (42 teeth, 21 in each test and control groups). The indications for extractions of maxillary anterior teeth were a) 70% failed root canal treatment, b) 30% tooth fracture and un-restorable or failed full crown coverage. All the recruited patients completed the study from initial examination, extraction and dental implant rehabilitation.

Randomization and allocation

At the start of the study, 42 opaque envelopes were sealed with a piece of paper indicating one of the two groups (Test or Control). Following extraction and curettage of the socket and before the bone grafting procedure, randomization was accomplished by selection of one of these envelopes. Thus patients were randomly assigned to one of the following groups:

- Test - for central incisors and canine teeth: 250 mg block of DBBMC (Bio-Oss Collagen®, Geistlich Switzerland) mixed with 0.7 ml of EMD (Emdogain®, Straumann, Basel, Switzerland), for lateral incisors: 100 mg block of DBBMC (Bio-Oss Collagen®, Geistlich Switzerland) mixed with 0.35 ml of EMD (Emdogain®, Straumann, Basel, Switzerland).
- Control – for central incisors and canine teeth: 250 mg block of DBBMC only (Bio-Oss Collagen®, Geistlich Switzerland), for lateral incisors: 100 mg block of DBBMC only (Bio-Oss Collagen®, Geistlich Switzerland).

Presurgical procedures

Full-mouth debridement consisting of 1 - 2 sessions of periodontal debridement with selective local anaesthesia was performed using an ultrasonic scaler and hand curettes as required. The goal was to achieve a FMBS and FMPS of less than 20% at 8 - 12 weeks after initial examination, in preparation for the extraction and ridge preservation procedures and subsequent dental implant placement four months later. Supportive periodontal therapy was subsequently performed at 3 - 4 monthly intervals from tooth extraction and throughout the study.

Surgical Procedures – extraction and socket management (Figure 1)

A 0.2% chlorhexidine mouthwash was utilized for 60 seconds before the patient received local anesthesia in preparation for the extraction procedures. All surgical procedures were performed by the same experienced periodontist (F.M.). While blinding of the clinician for the materials used was not possible, blinding was possible during the data collection, CBCT and histology analyses. One hour prior to the surgical appointment, the patient took 5 - 10 mg of Diazepam orally. After local anesthesia (Lidocaine HCL 2% with 1:100,000 epinephrine, Septodont, Australia), atraumatic extraction of the tooth was achieved, using a 15C blade (Swann-Morton, Sheffield England), by performing an intra-sulcular incision around the affected tooth extending 2 - 3 mm below the gingival margin. Once a clear circumferential incision was made, proximal force was introduced on the tooth involved to gain some mobility using a periotome (Nobel Biocare, Kloten, Switzerland). Once significant mobility was achieved, a universal maxillary anterior forcep (Aesculap Anatomica, Center Valley, PA, USA) was used to deliver the tooth, applying a slight rotary motion avoiding any buccal movement, in order to preserve buccal bone. The extraction socket was curetted and irrigated with sterile saline for 10 minutes. The extraction socket was examined for any dehiscence or defect (no palatal defect, no buccal defect greater than 1 mm of the entire buccal wall). Once the socket was fully examined and all the inclusion criteria were met, the patient was randomized into the Test group (DBBMC-EMD) or Control group (DBBMC only).

Following extraction and randomization, socket management was performed using either DBBMC-EMD (Test) or DBBMC only (Control). For the test group, EMD (0.7 ml for central

incisors and canine and 0.35 ml for lateral incisors) was added to DBBMC xenograft (250 mg for central incisors and canines, 100 mg for lateral incisors) in a sterile dappen dish for 15 minutes. The materials were then crushed together to create a uniform putty consistency and were placed and plugged in the extraction socket using a Stricker-DX55R applicator (Aesculap, Center Valley, PA, USA) to ensure uniformity and even placement throughout the socket. No grafting material was placed outside the socket. For the control group, DBBMC was moistened with sterile water to achieve a putty consistency and was placed in the extraction socket using the same carrier. All the sockets were filled to the bony rim.

Using the sterile foil packaging of the suture material packaging (4-0, Vicryl-Rapide, Johnson and Johnson, Diegem, Belgium), the size of the extraction socket circumference was copied and used as a pattern for free gingival graft harvesting. The anterior area of the hard palate (palatal area 2 mm below the gingival margin of teeth 15 - 13) on the same side as the extracted tooth was anesthetized (Lidocaine HCL 2% with 1:100,000 epinephrine, Septodont, Australia), and using a 15-C surgical blade (Swann-Morton, Sheffield England) a free gingival graft with the size and shape of the foil pattern was harvested (8 - 10 mm diameter free-gingival graft, FGG). A haemostatic agent (Surgicel absorbable haemostat, Ethicon, LLC USA) was applied firmly on the rounded donor site and the haemostatic agent was secured using 2 - 3 single interrupted sutures crossing the mesio-distal border and the antero-posterior border of the donor sites using a resorbable suture (4-O, Vicryl®, Johnson and Johnson, Diegem, Belgium). The free gingival graft was secured over the extraction socket with 4-5 single interrupted sutures using the same suture material (4-O, Vicryl®, Johnson and Johnson, Diegem, Belgium). All of the donor site healed well 2 - 3 weeks after the free-gingival graft harvesting with no complications such as profuse bleeding or sloughing.

Postoperative Care

Patients were instructed not to brush the surgical sites for 2 weeks. All patients were issued with 20 tablets of analgesic (combined Paracetamol 500 mg and Ibuprofen 150 mg) and advised to take them as needed. All patients were given a post-operative kit containing 0.12% chlorhexidine digluconate mouthwash, surgical brush and 0.12%

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. The patients were recalled after 1 week, 3 weeks, and 5 weeks and then at the 4th month after extractions.

Surgical procedure – trephine biopsy and dental implant placement

A pre-implant surgery CBCT scan was taken 4 months after the extraction and ridge management procedure. This CT scan was used for examination of the healed ridge and for implant size determination. One hour prior to the surgical appointment, the patient took 5 - 10 mg of Diazepam orally. A 0.2% chlorhexidine mouthwash was utilized for 60 seconds before the patient received local anesthesia. The maxillary anterior area was anesthetized (Lidocaine HCL 2% with 1:100,000 epinephrine, Septodont Australia). Using a 15-C blade (Swann-Morton, Sheffield England), a full thickness mucoperiosteal flap was outlined and elevated using a mucoperiosteal elevator to expose enough of the healed alveolar crest and implant bed. A single core biopsy was harvested from each participant during the initial osteotomy preparation using a standard trephine bur (internal/external diameter 2.0/3.0, length 11 mm). The osteotomy preparation was completed following the manufacturer's standard protocol using a surgical guide and the implant was inserted. Three different kinds of implants were placed depending on the preference of the referring dentists (40% Straumann, 30% Nobel Biocare, 30% Astra Dentsply) The mucoperiosteal flap was then closed using resorbable sutures (4-O, Vicryl®, Johnson and Johnson, Diegem, Belgium). The bone core biopsies were immediately stored in paraformaldehyde until further histological processing.

All patients received the planned dental implants at least 4 months after the extraction and ridge management according to the patient's convenience and time schedule. All of the post-extraction CBCTs were taken within a week of the fourth month anniversary of the tooth extraction. The dimension of the implants used was based on site-specific measurements according to the dimensions obtained using a NobelClinician computer software (NobelBiocare, Gotheberg, Sweden). All patients were issued 20 tablets of analgesic (combined Paracetamol 500 mg and Ibuprofen 150 mg) and were advised to take it as needed. All patients were given a post-operative kit containing 0.12%

chlorhexidine digluconate mouthwash, surgical brush and 0.12% 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. The patients were recalled after 1 week, 3 weeks, 5 weeks, 3rd months and 4th months after implant placement.

CBCT Measurements (Fig. 2a-2b)

Each patient had a CT scan taken before extraction. The dimension of the alveolar ridge was measured using a computerized implant treatment planning software (NobelClinician, NobelBiocare, Gotheberg, Sweden). Blinding during CT scan analysis was achieved by numerical coding of each sample (number 1 - 42). The CT scan was analyzed (FM) and a second blinded clinician who was not involved in the surgical procedure also analyzed the CT scan data separately. A calibrating exercise was performed to achieve intra and inter-examiner reproducibility: CT scans of the same five patients were measured at weekly intervals for 3 consecutive weeks. Calibration was confirmed when the measurements were consistently accurate to 0.5 mm. There was a 98% agreement on the CT scan values measured by the two-blinded examiners (F.M. and A.A.).

The thickness of the buccal wall was measured at a distance that was 2 mm apical (point b) from the most coronal part of the buccal wall (point a) (Fig. 3). The perpendicular distance from point b to the buccal root surface of the concerned tooth (point c) was designated as the thickness of the buccal wall (BT= b to c). The height of the buccal wall was measured from point a to the floor of the nasal cavity/spine, point d (Buccal Bone Height, BH = point a to point d). The thickness of the palatal wall was also measured at a level (point f) that was 2mm apical from the highest point of the palatal crest (point e). The perpendicular distance from point f to the palatal root surface of the involved tooth (point g) was designated as the thickness of the palatal wall (PT = f to g). The height of the palatal wall (PH) was measured from point e to the nasal floor, point h (PH = e to h). The thickness/width of the alveolar ridge is the measurement from point b to point f (Ridge Width, RW = b to f). These measurement protocols were repeated in the pre-implant CT scan taken 4 months after tooth extraction and ridge management. The differences in the

measurements of RW, BH and PH before and after extractions/ridge managements were analyzed.

Histological Processing

Blinding during histological preparation of bone core biopsies was achieved by numerical coding each sample (number 1 - 42) and using different operators for the surgery (FM) and histomorphometry (CV). The technique that was used to prepare bone core biopsies into undecalcified tissue resin sections has been previously described (Alayan et al. 2016, Schmitt et al 2015). Briefly, each bone core biopsy was fixed in 4% paraformaldehyde phosphate-buffered formalin immediately after harvesting from the patient. The samples were cut and dehydrated in a graded series of ethanol and resin infiltrate (Methyl Methacrylate/Glycol Methacrylate, Tecknovit 7200, Heraeus Kulzer, Hanau Germany). The cured resin blocks were ground using an EXAKT400 CS micro-grinding system to expose the samples. The samples were glued onto a glass slide and sectioned to 150µm using the EXAKT300 cutting system (Exact Apparatebau GmbH, Norderstedt, Germany). The sample slides were polished down to 20 µm thickness using the EXAKT 400 CS micro-grinding system and were then stained with 0.1% toluidine blue (Sigma-Aldrich, St Louis, MO, USA) for light microscopy analysis.

Histomorphometric Measurements (Fig.3)

The histological slides were scanned and digitized using a digital slide scanner (Aperio Technologies Inc, Nussloch, Germany). Histomorphometric analysis was carried out using the scanner's software program (Aperio Imagescope version 12.1, Aperio Technologies Inc, Nussloch, Germany). During the examination of each sample, the examiner (FM) was blinded with regard to the treatment group. Three area fractions (percentage components) were identified in each sample core using magnification of up to 40x to achieve accurate delineation of each of the components:

- 1) % New Bone (NB)
- 2) % Residual Graft (RG)
- 3) % Soft tissue and marrow spaces (STM)

New bone (NB) was identified as lighter stained mineralized tissue with osteocytes in lacunae, while residual graft (RG) material (DBBMC) was mineralized tissue with empty lacunae. The toluidene blue staining allowed differentiation between NB and RG. The remaining area fraction not marked as RG and NB was designated as % STM (Fig 3a, 3b).

Statistical analyses

Statistical analysis was performed using commercially available software (SPSS Statistics for Windows version 21.0, IBM Corporation 2012, Armonk, NY, USA). Mean values and standard deviations (mean $\pm$ SD) from CT scan measurements were calculated for each treatment at two different time points (baseline and 4 months). The a priori significance of the difference within each group and between groups before and after treatment was analyzed using a two-sample, two-tailed student *t*-test after assessing the normality of the data. Data from histomorphometry scores (%RG, %NB and %STM) were analyzed using Mann-Whitney U-tests. The null hypothesis was that there was no statistically significant difference between control and test groups for all CT scan and histomorphometric parameters. The primary outcome variable was % NB. Secondary variable outcomes were differences in RW and BH, %RG, differences in PH and %STM. A *p* value of ≤ 0.05 was considered to represent statistically significant differences between test and control groups.

Results

A total of forty-two patients (42 teeth) participated in the study and underwent maxillary anterior tooth extraction and ridge management procedures. The mean age in the control group was 51.4 ± 11.3 years with 71% of the patients female. The mean age in the test group was 53.6 ± 10.7 years with 66% of the patients female. There was no statistically significant difference in the age range between the two groups (*p* = 0.26, total population mean age was 52.5 ± 10.8 years, 69% female). There were no significant intra or post-operative complications and all the patients completed their implant rehabilitation, including implant restoration four to six months after extraction and ridge management (Table 1).

In the control group 57.1% of the extracted teeth were maxillary central incisors, 33.3% lateral incisors and 9.5% canines. In the test group 52.3% of the extracted teeth were central incisors, 38.1% were lateral incisors and 9.5% were canines. In the total population 54.8% of the extracted teeth were central incisors, 35.7% were lateral incisors and 9.5% were canines.

CBCT analyses

At baseline there were no statistically significant differences in the mean CBCT values between the two treatment groups. The baseline ridge measurements for the control group were: RW = 7.7 ± 0.96 mm, BH = 17.6 ± 3.3 mm and PH = 21 ± 4.0 mm respectively. For the test group, the baseline ridge dimensions were: RW = 7.2 ± 1.0 mm, BH = 17.4 ± 1.9 mm and PH = 22.4 ± 2.0 mm (Table 2). Similarly, at the 4th month measurement, there were no statistically significant differences when comparing the mean RW, BH and PH between test and control groups (control 4th month RW = 6.8 ± 1.3 mm, BH = 16.5 ± 3.7 mm, PH = 20.3 ± 3.7 mm; test group 4th month RW = 6.6 ± 1.1 mm, BH = 16.7 ± 2.2 mm, PH = 21.4 ± 2.7 mm, Table 2).

Following extraction and ridge management, there was a statistically significant reduction in mean RW in both the control and test groups from baseline to the 4th month: (control baseline RW = 7.7 ± 0.96 mm decreased to 6.8 ± 1.3 mm, $p < 0.005$, while test baseline RW = 7.2 ± 1.0 mm decreased to 6.6 ± 1.1 mm, $p < 0.04$) by the 4th month. Small changes in mean BH and PH dimensions four months after extraction and ridge management were not significant (Table 2).

However, when these changes in the test and control group ridge dimensions over time were considered as a percentage dimensional change (Table 3), there were no statistically significant differences in any of the ridge dimensions between test and control groups 4 months after ridge management procedures (control group: % change in RW = -7.7 ± 12.8 , % change in BH = -6.4 ± 7.7 , % change in PH = -3.9 ± 4.7 ; test group: % change in RW = -5.3 ± 9 , % change in BH = -3.2 ± 4.8 , % change in PH = -3.4 ± 5.6).

Further analyses of the patients' data were subsequently done stratifying the patients according to whether their baseline buccal wall thickness (BT) was <1 mm or ≥1 mm instead of the ridge management treatment performed (Table 3). This showed there was a significantly greater percentage reduction in ridge dimensions RW, BH and PH in the <1 mm BT group when compared to the ≥ 1 mm BT group (% change in RW: <1 mm group = 14 ± 10 vs. ≥1 mm group = 5.4 ± 12.4 , $p < 0.01$; % change in BH: <1 mm group = -6.9 ± 6.7 vs. ≥1 mm group = -2.1 ± 5.4 , $p < 0.009$; % change in PH: <1 mm group = -5.8 ± 5.4 vs. ≥1 mm group = -2.5 ± 5.3 , $p < 0.02$).

Gross histological features

The three types of tissues filling the socket (NB, RG and STM) were readily identifiable in each ground section from the 10x to 40x magnification of the image slides. The biomaterial (DBBMC) was readily identifiable as structures with empty Haversian canal system and with a much more intense blue staining than the surrounding bone and soft tissue matrix (Fig 3b, c). New bone (NB) with varying degrees of maturity (woven and lamellar bone) was identified as mineralized tissue with osteocytes in lacunae and slightly paler blue/pinkish staining compared to the DBBMC counterpart (Fig 3b, c). At higher magnification the NB was observed to be composed of woven bone and mature lamellar bone with concentric lamellae (Fig 3c).

Histomorphometric analysis

In the control group, $16.5 \pm 6.9\%$ NB filled the socket, which was significantly lower than the $45.1 \pm 8.8\%$ NB found in the test group ($p < 0.00001$). In the control group, the socket was occupied by $36.8 \pm 8.8\%$ RG, which was significantly higher than the $20.3 \pm 7.2\%$ found in the test group ($p < 0.00001$). There was also significantly higher %STM in the control group versus the test group (%STM control = 46.5 ± 10.4 , %STM test = 34.6 ± 13.8 , $p < 0.003$) (Figure 4).

Discussion

This study demonstrated that the application of DBBMC and DBBMC-EMD into extraction sockets covered with a free gingival graft (FGG) resulted in no significant changes to either buccal bone height (BH) or palatal bone height (PH) in both treatment groups 4

months after tooth extraction. These results confirm the relative effectiveness of this technique in minimizing vertical ridge height reduction post-tooth extraction. These results are consistent with an RCT where the application of DBBMC covered with free gingival graft resulted in less vertical changes of the alveolar ridge compared with sockets that had spontaneous healing and sockets filled with β -TCP (Jung et al 2013). The -0.3 mm to -1.4 mm reduction in the BH and PH in the DBBMC- gingival graft group in the Jung et al (2013) study is also within the range of -0.8 mm to -1.2 mm reduction in the BH and PH of the similar material in the control group of the present study. These results are also comparable to a study where the socket was filled with DBBMC or DBBM then sealed with collagen membrane (Nart et al 2017), which showed that DBBM and DBBMC demonstrated similar behavior histologically and in terms of minimizing ridge resorption after tooth extraction. The -0.98 ± 1.28 mm BH change and -0.82 ± 0.61 mm PH change in the Nart et al (2017) study is also comparable to the corresponding findings in the present study. Taken together, these findings demonstrate the reproducibility of minimizing the post-extraction buccal and palatal wall height resorption to a range of 1 - 1.5 mm when utilizing DBBMC. Indeed, the 1.0 - 1.5 mm (-10% to -18%) reduction in the ridge dimensions of the 4-walled socket in the present study when DBBMC was used was superior to the 6.0 mm (-77%) ridge reduction reported with the use of β -TCP (Jung et al 2013). A limitation of the present study was the lack of a negative control. However, the effectiveness of the ridge preservation procedures employed in this study can be confirmed by comparisons with clinical trials on unassisted extraction socket healing (Jung et al 2013, Barone et al 2008) and a systematic review on alveolar bone dimensional changes of post-extraction sockets (der Weijden et al 2009), which reported an average clinical loss in ridge dimension of 1.7 - 4.0 mm, a range that is significantly greater than the BH, RW and PH of the present study.

The use of EMD as an adjunct to DBBMC in this study did not minimize volumetric ridge reduction after tooth extraction. Although the percentage reduction in BH and PH after ridge management is smaller in the test group, where EMD was added with DBBMC, this did not reach statistical significance. In this context, the relatively high standard deviation is worth noting, especially when the results are presented as percentage change. However, it should be noted that the percentage difference in BH reduction (6.4 ± 7.7 in

the control group vs 3.2 ± 4.8 in the test group) almost reached statistical significance ($p=0.06$). Although statistical significance may be achieved if the number of samples in each group was increased, the clinical significance of this level of difference ($<5\%$) in BH in the aesthetic zone is questionable, and may warrant further investigation in studies with increased participant numbers. Notably, a recently published study which compared ridge preservation using DBBMC with and without EMD, similarly did not report a difference in ridge dimensional outcomes (Lee et al 2020).

When the patients' data were analyzed according to the initial buccal wall thickness (<1 mm vs. ≥ 1 mm buccal wall thickness BT) instead of the allocated treatment protocols, significant differences in the ridge dimensions were noted after the 4 months healing period. Patients with <1 mm BT sustained ridge reduction of 1.0 to -1.5 mm (or -5.8% to -14%) ridge reduction (RW, BH, PH) compared to the minimal ridge reduction of 0.17 to -0.4 mm (or -2% to -5.4%) when the initial buccal wall thickness was ≥ 1 mm. It has been reported that the thickness of the buccal bone crest significantly influences the amount of vertical crestal resorption after tooth extraction (Ferrus et al 2010, Braut et al 2011, Nevins et al 2006). In a study where 93 subjects had extractions and implants immediately placed in the maxillary anterior area, it was demonstrated that patients with thin buccal wall (≤ 1 mm, 43% ridge reduction) sustained greater percentage ridge reduction than patients with thick buccal wall (>1 mm, 21% ridge reduction) 16 weeks after implant placement (Ferrus et al 2010). Therefore, although our study shows that the ridge preservation outcomes were improved in the presence of thicker buccal walls, when taken in the context of the increased resorption associated with thin buccal walls in the absence of ridge preservation (Ferrus et al 2010), the results show that patients with a thin buccal wall (<1 mm) may benefit as much, if not more, from the socket management technique. It has been reported that thin buccal walls (<1 mm) have a prevalence of 50-80% in the anterior maxilla, which is more common than thick buccal walls (>1 mm) that have a prevalence of 10-12% (Huynh-Ba et al 2010, Braut et al 2011, Januario et al 2011). Considering the large prevalence of patients susceptible to significant ridge resorption after extraction of maxillary anterior and posterior teeth after examination of pre-extraction radiographic analysis of the buccal bone wall using CBCT it may mean that

in most clinical situations encountered, augmentation procedures and guided bone regeneration may be needed prior to implant rehabilitation (Huyhn-Ba et al 2010).

The histomorphometric analysis showed readily identifiable components of NB, RG and STM. The observation that most of the xenograft particles were surrounded by bone of varying maturity with no associated inflammatory reaction corroborates the excellent biocompatibility of DBBMC (Bio-Oss collagen®, Geistlich Pharma AG, Switzerland) that has been reported in the literature (Alayan et al 2016). In terms of histologic appearance, DBBMC was much more intensely stained with toluidine blue than the surrounding tissues (Alayan et al 2016), which may indicate the presence of organic material such as endogenous protein surrounding the xenograft, which can enhance this material's biocompatibility (Bosshardt DD 2014). The intensely blue staining of DBBMC in the present study can also be attributed to the exogenous coating of EMD onto DBBMC in the test group of the present study. This ability of DBBM to be pre-coated with exogenous proteins has been demonstrated in vitro whereby DBBM has been shown to retain EMD (Emdogain®, Straumann AG, Switzerland) (Miron et al 2012).

In relation to the osteogenic potential of EMD, a number of in-vitro and in-vivo studies have demonstrated the osteopromotive potential of EMD (Stout et al 2014, Miron et al 2016, Grandin et al 2012, Galli et al 2006, Fawzy El-Sayed et al 2014). It has been shown that the low-molecular-weight protein content (7 to 17 kDA) within EMD has greater osteoconductive potential than the full commercially available EMD formulation, and that its effect on BMP signalling and increased osterix and VEGF-A expression are responsible for this increased osteogenic potential (Stout et al 2014). EMD has also been reported to upregulate osteogenic gene expression of progenitor cells (Miron et al 2016b, Grandin et al 2012, Galli et al 2006, Fawzy El-Sayed et al 2014). It has also been reported that EMD inhibits bone resorption by affecting osteoclast activities through increased osteoprotegerin (OPG) release and decreased release of receptor activator of nuclear factor kappa B ligand (RANKL) by 50% (Galli et al 2006). Furthermore, it was reported that EMD affects proliferation of progenitor and osteogenic cells, an effect that decreases with cell maturation and differentiation (Miron et al 2016, Grandin et al 2012).

The histomorphometric analysis showed statistically significant differences in terms of percentage of new bone, residual graft and soft tissue and marrow spaces between the test and control groups. The test group had significantly increased % NB ($45.1 \pm 8.8\%$), less %RG ($20.3 \pm 7.2\%$) and less %STM (34.6 ± 13.8) when compared to the control group (%NB 6.5 ± 6.9 , %RG 36.8 ± 8.8 , %STM 46.5 ± 1.4). The composition of the three components in increasing order was RG: STM: NB in the test group, and NB: RG: STM in the control group. This composition of the control group was consistent with similar studies where the extraction socket was filled with DBBM and closed with porcine-derived non-cross-linked collagen matrix, where the socket was mostly filled with residual graft and soft tissue instead of new bone 6 months post-extraction (Maiorana et al 2017). The increased amount of NB in the test group shows that the addition of EMD with DBBMC had increased the osteogenic potential of this biomaterial. Evidence to support the increased osteogenic potential of EMD has been reported in *in vivo* studies showing that ameloblastin degradation products stimulated cementum formation, bone growth and craniofacial bone formation (Brookes et al 2001, Robinson et al 1998, Grandin et al 2012, Tamburstuen et al 2010). The increase in expression of a known angiogenic growth factor, Vascular Endothelial Growth Factor (VEGF) in the presence of EMD may also contribute to tissue healing by faster renewal of blood supply within the wound (Johnson et al 2009, Schlueter et al 2007).

It was also reported that increased healing time could contribute to the formation of more new vital bone (Whetman and Mealey 2016). In this study, where demineralized freeze-dried bone allograft was placed in forty-four extraction sockets, it was shown that higher percentage (47, 41%) of new vital bone was observed in cone biopsies harvested at 18 - 20 week versus 32.63% new vital bone observed at core biopsy harvested after 8 - 10 weeks. Since histological analysis was only carried out at one point in the present study, it is unknown if the addition of a biologic factor such as EMD can facilitate accelerated formation of new bone and hence allow for a shorter healing time.

Furthermore, combining EMD with absorbable collagen sponge (ACS) influenced the activity of induced pluripotent stem cells (iPSCs) by upregulating the expression of bone

sialoprotein and osteopontin and increasing the levels of osteoblastic differentiation and mineralization, when compared to ACS alone (Hisanaga et al 2018).

Another possible effect of EMD in the present study is that it may promote resorption of the residual graft particles. This hypothesis is supported by studies that showed the ability of EMD to induce osteoclast formation in mouse bone marrow cells via the RANK-OPG-RANKL pathway in vitro (Gruber et al 2014), and in vitro evidence that a purified EMD fraction enhanced osteoclast activity and bone resorption in the monocytic cell line RAW 264.7 (Itoh et al 2006). Whether EMD increased the formation of new bone or whether it increased the rate of resorption of the xenograft cannot be elucidated from the present study.

The positive effect of EMD on wound healing is well established, via its effect on macrophages, neutrophils and lymphocytes, and by its downregulation in MMP-1 and MMP-8 (Okuda et al 2001) and upregulation of TGF- β 1 (Agrali et al 2016) expression. It was also shown in an in vivo angiogenesis assay that recombinant porcine amelogenin induced significant pro-angiogenesis activity, suggesting another mechanism of this protein as an adjunct to accelerate wound healing (Thoma et al 2011). Because of the broad effects of EMD on various host cells and their functions, and not just exclusively on osteoblast and osteogenic activity, EMD has been described as osteopromotive rather than osteoinductive (Boyan et al 2000). Numerous subsequent studies eventually supported this broad spectrum of biofunctionality of EMD (Miron et al 2016b, Grandin et al 2012, Bosshardt et al 2008). Indeed, the general pro-wound healing effects of EMD are reflected in its established clinical effectiveness in periodontal regeneration (Esposito et al 2009), root overage procedures (Mercado et al 2020a, Mercado et al 2020b) and the management of peri-implantitis (Mercado et al 2018, Isehmed et al 2018). Furthermore, a number of other human histological studies have confirmed the periodontal regenerative potential of EMD (Rasperini et al 2000, Roman et al 2013, Sculean et al 2000, Cochran et al 2003).

To the best of our knowledge, this is the first clinical study to show more %NB formation in healing extraction sockets by the addition of EMD to DBBMC. The finding of the

present study, where more %NB was noted 4 months after extraction in the test group, may translate to earlier implant placement, enhanced implant osseointegration due to the presence of more bone and possibly improved long-term implant outcomes. The use of biologically active materials as an adjunct to osteo-conductive materials in preserving jawbone for future implant rehabilitation should be examined in larger multi-center clinical trials, whereby the long-term outcome of implant placement in these sites is also evaluated.

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Conflict of Interest and source of funding statement

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Table 1. Patient and Teeth Distribution

	Control Group (DBBMC-only)	Test Group (DBBMC with EMD)	Total/Mean for both groups
Patient Distribution			
Patient Numbers	21	21	42
Mean Age	51.4 ± 11.3	53.6 ± 10.7	52.5 ± 10.8
Female	71%	66%	69%
Teeth Distribution			
Number of teeth / group	21	21	42
Central Incisor	12 (57.14%)	11 (52.38%)	23 (54.76%)
Lateral Incisor	7 (33.33%)	8 (38.09%)	15 (35.71%)
Canine	2 (9.52%)	2 (9.52%)	4 (9.52%)

Table 2. Baseline and 4th month CT scan measurements, Control and Test groups (mean $\pm$ SD), Buccal bone thickness (BT), Ridge Width (RW), Buccal bone height (BH) and Palatal bone height (PH).

		Control BT (1.06 $\pm$ 0.49 mm)	Test BT (1.03 $\pm$ 0.47 mm)	p value
Ridge Width (RW)	Baseline	7.7 $\pm$ 0.96	7.2 $\pm$ 1.0	0.07
	4 th month	6.8 $\pm$ 1.3	6.6 $\pm$ 1.1	0.34
	p value	*0.005	*0.04	
Buccal Wall Height (BH)	Baseline	17.6 $\pm$ 3.3	17.4 $\pm$ 1.9	0.06
	4 th month	16.5 $\pm$ 3.7	16.7 $\pm$ 2.2	0.30
	p value	0.17	0.18	
Palatal Wall Height (PH)	Baseline	21 $\pm$ 4.0	22.4 $\pm$ 2.0	0.06
	4 th month	20.3 $\pm$ 3.7	21.4 $\pm$ 2.7	0.13
	p value	0.24	0.10	

*p value <0.05

Table 3. Changes in ridge dimension (mm and % change) of Ridge Width (RW), Buccal Bone Height (BH), Palatal Bone Height (PH).

Group	Δ RW mm	Δ RW %	Δ BH mm	Δ BH %	Δ PH mm	Δ PH %
Control (BT=1.06 ± 0.49mm)	-0.37 ± 1.1	-7.7 ± 12.8	-1 ± 1.2	-6.4 ± 7.7	-0.8 ± 1.0	-3.9 ± 4.7
Test (BT = 1.03 ± 0.47mm)	-0.40 ± 0.5	-5.3 ± 9	-0.62 ± 0.9	-3.2 ± 4.8	-1.0 ± 1.2	-3.4 ± 5.6
p value	0.37	0.25	0.11	0.06	0.35	0.47
Buccal Wall Thickness (BT)	Δ RW mm	Δ RW %	Δ BH mm	Δ BH %	Δ PH mm	Δ PH %
<1 mm (BT = 0.63 ± 0.1 mm)	-1 ± 0.8	-14 ± 10	-1.2 ± 1.0	-6.9 ± 6.7	-1.2 ± 1.0	-5.8 ± 5.4
≥1 mm (BT = 1.5 ± 0.3 mm)	-0.17 ± 1.1	-5.4 ± 12.4	-0.4 ± 0.9	-2.1 ± 5.4	0.4 ± 1.5	-2.5 ± 5.3
p value	0.003*	0.01*	0.003*	0.009*	0.03*	0.02*

*p value <0.05

Table 4. Proportion (%) of three different tissues in test and control groups; % New bone (NB), % Residual graft (RG) and % Soft tissue and marrow spaces (STM).

Group	% NB		% RG		% STM	
	Mean $\pm$ s.d	median (range)	Mean $\pm$ s.d	median (range)	Mean $\pm$ s.d	median (range)
Control	16.5 $\pm$ 6.9	17 (5 - 28.3)	36.8 $\pm$ 8.8	39 (20.6 - 53.3)	46.5 $\pm$ 10.4	48 (20.1 - 67.4)
Test	45.1 $\pm$ 8.8	45 (21 - 57.7)	20.3 $\pm$ 7.2	20.6 (7 - 31.8)	34.6 $\pm$ 13.8	36 (15.2 - 72)
p value	<0.00001*		<0.00001*		<0.003*	

*p value <0.05

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clr_13742_f3.png

clr_13742_f4.png