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A randomized, controlled, multi-center clinical trial of post-extraction alveolar ridge preservation

Running Title: Dehisced extraction ridge preservation

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Abstract

Aim: To compare the effectiveness of two-ridge preservation treatments.

Materials and Methods: 40 subjects with extraction sockets exhibiting substantial buccal dehiscences were enrolled and randomized across 10 standardized centers. Treatments were demineralized allograft plus reconstituted and cross-linked collagen membrane (DFDBA+RECXC) or deproteinized bovine bone mineral with collagen plus native, bilayer collagen membrane (DBBMC+NBCM). Socket dimensions were recorded at baseline and 6-months. Wound closure and soft tissue inflammation were followed post-operatively, and biopsies were retrieved for histomorphometric analysis at 6-months.

Results: Primary endpoint: at 6-months extraction socket horizontal measures were significantly greater for DBBMC+NBCM (average 1.76 mm greater, p= 0.0256). Secondary and Exploratory endpoints: (1) lingual and buccal vertical bone changes were not significantly different between the two treatment modalities, (2) histomorphometric % new bone and % new bone+graft were not significantly different, but significantly more graft remnants remained for DBBMC; (3) at 1-month incision line gaps were significantly greater and more incision lines remained open for DFDBA+RECXC; (4) higher inflammation at 1-week tended to correlate with lower ridge preservation results; and (5) deeper socket morphologies with thinner bony walls correlated with better ridge preservation. 37 of 40 sites had sufficient ridge dimension for implant placement at 6-months; the remainder were DFDBA+RECXC sites.

Conclusion: DBBMC+NBCM provided better soft tissue healing and ridge preservation for implant placement. Deeper extraction sockets with higher and more intact bony walls responded more favorably to ridge preservation therapy. Further investigation of implant integration and long-term survival is warranted. clinicaltrials.gov identifier NCT02330523.

Clinical Relevance

Scientific Rationale

There are a wide variety of guided bone regeneration techniques and biomaterials available for ridge preservation. Bony dehiscence extraction sockets provide a critical test of their effectiveness.

Principal Findings

In a multi-center, private practice network investigation of dehisced extraction sockets, deproteinized bovine bone mineral with 10 % collagen plus native bilayer collagen membrane (DBBMC+NBCM) provided significantly more ridge width compared with demineralized allograft plus cross-linked collagen membrane (DFDBA+RECXC). During healing there were more and larger incision line gaps with DFDBA+RECXC.

Practical Applications

DBBMC+NBCM provided better wound healing and ridge preservation than DFDBA+RECXC. Extraction sockets with space-maintaining morphologies appear to respond more favorably to ridge preservation therapy.

Introduction

Since the development of guided tissue regeneration therapy for teeth, and later, its adjunct guided bone regeneration (GBR) for dental implants, researchers have tested a variety of surgical techniques and biomaterials. Early investigations focused on using regenerative membranes alone; however, research with bone grafts in periodontal defects led investigators to explore the utility of membranes in combination with bone grafts (Simion et al. 1994). Today GBR is generally performed as a combination procedure involving membranes and a supporting bone substitute. Within this context, some researchers have employed demineralized, malleable, rapidly resorbed and reportedly osteoinductive allografts (Bowers et al. 1985 and Wood 2012), while others have employed mineralized, more rigid, minimally resorbing and reportedly osteoconductive grafts (Berglundh & Lindhe 1997 and Araújo 2015).

At present there are a variety of GBR biomaterials, including demineralized and mineralized bone grafts and membranes of various stiffness and degradation characteristics. Two contrasting GBR approaches include: (1) longer lasting and stiffer, cross-linked collagen membranes combined with demineralized allografts, and (2) shorter lasting, more flexible and non-cross-linked collagen membranes combined with mineralized grafts. In order to better understand which approach might be more effective for ridge preservation, the two techniques were compared in a randomized, multi-center, ridge preservation study of extraction sockets exhibiting substantial buccal dehiscences.

Materials and Methods

Ethical Aspects

Subjects were enrolled by assuring verbal understanding and obtaining written informed consents, which, along with the study protocol, were reviewed by an ethical review board in accordance with the Declaration of Helsinki. An independent clinical research organization monitored the study progress and results, and the study was registered through clinicaltrials.gov, identifier NCT02330523.

Subjects

Forty subjects, 18 –70 years of age, intended for extraction and subsequent implant placement, were enrolled with ten study sites. Only posterior (1st premolar to 1st molar) extraction sockets, with intact adjacent teeth or dental implants (for measurement stent indexing) and with substantial buccal wall dehiscences of at least 1/3 the overall socket height and width were included (Class II/ III, Elian 2007).

Exclusion Criteria

Subjects were excluded if they had a history of tobacco use within the last six-months, healing disorders, i.e., diabetes mellitus, cancer, HIV, bone metabolic diseases, or had received systemic corticosteroids, immunosuppressive agents, radiation therapy, and/ or chemotherapy within the past two-months. Subjects taking intramuscular or intravenous bisphosphonates or who had allergies or sensitivity to alginate, latex, collagen or acrylic

were also excluded, as were pregnant, lactating or intending to become pregnant women, or those participating in other clinical intervention studies.

Test Materials and Randomization

The ridge preservation control therapy was demineralized allograft (Oragraft® DGC, LifeNet Health, Inc., Virginia Beach, VA, USA) plus reconstituted and cross-linked bovine collagen membrane (Biomend® Extend, Zimmer Dental, Inc., Carlsbad, CA, USA) - DFDBA+RECXC. The ridge preservation test therapy was deproteinized bovine bone mineral with collagen binder plus extracted, native porcine, bilayer collagen membrane (Bio-Oss® Collagen plus Bio-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland) - DBBMC+NBCM. Subjects were randomly assigned to either the control or test therapy in a block 1:1 ratio, first by the 10 investigators and then overall for all 40 subjects, so that each center might have equal numbers of test and control subjects, with a minimum of one and a maximum of three test and control patients per investigator. Investigators received randomization instructions only after enrolling a subject and immediately prior to surgery.

Primary and secondary endpoints and exploratory measures were as follows:

- Primary Outcome Variable
    - Bone ridge dimension preservation horizontal change from baseline to 6-months, as measured using indexed stents, from the outside of the stent, at the apical extent of the buccal dehiscence, bucco-lingually to the interior bony wall.
- Secondary Outcome Variables
    - Bone ridge dimension preservation vertical changes from baseline to 6-months, apico-coronally from the outside of the stent to the apical extent of the lingual and buccal walls.
- Exploratory Variables
    - Histomorphometric % new bone, % graft and % connective tissue/ bone marrow components from mid-section bone core biopsies at 6-mos,

including:

- % New Bone in Contact with Graft
- % Graft in Contact with New Bone

- Wound closure (incision line gap) at all time points.
- Soft tissue inflammation
- Baseline bone measurements - extraction socket morphology measured directly at baseline surgery
  - Baseline extraction socket dimensions, measured as the mesial-distal distance of the dehiscence at the crest and the coronal-apical distance from the crest to most apical extent of the buccal dehiscence, and also as the overall socket dimensions, mesial-distal at crest and coronal-apical from crest to the apical extent of the extraction socket.
  - Bony wall thickness, measured horizontally at the coronal tip of the most apical extent of the dehiscences of the buccal and, if present, lingual bony walls.

Prior to surgery, alginate impressions were obtained for fabrication of indexed measuring stents (see Figure 1) using vacuum formed 0.020" thermoplastic on stone models. Medical and dental histories and demographic data were recorded, oral exams and dental cleanings were performed.

Surgical Procedures and Follow-up

Following administration of antibiotics, extractions were performed with flap reflection, periotomes, elevators and forceps. To ensure that appropriate biopsy specimens were obtained, i.e., new bone and graft and not old host bone in the cores, furcation bone was eliminated in molar sites. Extraction socket morphology dimensions were recorded through direct measures, and baseline extraction socket buccal-lingual and vertical dimensions were recorded using indexed stents, as described above and shown in Figure 1, using either UNC 15 periodontal probes or Abou-Rass explorers (Hu-Friedy Mfg. Co., Chicago, IL, USA), depending on the dimension of the defect. The measurement methods were similar to those employed by Wood and Mealey (Wood 2012).

The control and test biomaterials were placed according to randomization codes. Bone defects were filled with particulate bone grafts confined to the existing alveolar ridge dimensions, making no attempt to go outside the confines of ridge, and then covered with absorbable collagen membranes placed to cover the grafts and extend slightly 2-3 mm beyond bone defect margins. Soft tissue was approximated only. Periosteal releasing incisions were not employed, and incision lines were not required to be closed primarily so that mucogingival junction dimensions might be preserved. Remaining soft tissue incision gaps, if any, were recorded both mesio-distally and buccal-lingually.

Photos were taken before, during and after the surgical procedure.

Subjects were instructed to take Ibuprofen 800 mg or hydrocodone/ paracetamol up to three times a day for pain, as needed. Subjects were instructed to use chlorhexidine (0.12%) mouth rinse for 30 seconds twice daily and to avoid excessive muscle tractioning or trauma and not to brush the study areas for the first two weeks.

Amoxicillin was provided 875mg BID for 10-14 days (with clindamycin 300 mg qid for 7-10 days for subjects with penicillin allergies).

After 2-weeks of healing, subjects were instructed in a brushing technique creating minimal apically directed trauma to the treatment area. At 4-weeks the subjects were instructed to stop chlorhexidine rinses and resume normal oral hygiene practices.

At 1-week post-surgery, photos of the test sites were taken, and clinical measurements of inflammation and remaining incision line gaps (if any) were assessed.
Categorical inflammation scores were:

- **0** Normal, (absence of inflammation)
- **1** Mild inflammation of any portion of the marginal slight changes in color)
- **2** Mild inflammation of entire gingival unit (but no edema)
- **3** Moderate inflammation (moderate glazing, redness, edema and/or hypertrophy)

4 Severe inflammation (marked redness and edema/hypertrophy, spontaneous bleeding, or ulceration)

Further follow-up evaluations occurred at 4-weeks, 3-months and 6-months post-surgery. Photos of the test sites and clinical measurements of remaining incision line gaps (if any) were obtained, and inflammation was assessed. Oral hygiene instructions were reviewed throughout the study.

At 1, 3 and 6-months dental cleanings were performed. Throughout the study, any changes in medications or adverse events were noted.

At 6-months post-surgery, the test sites were re-entered for implant placement. Prior to implant placement, vertical and horizontal ridge dimensions were recorded using the indexed measuring stents. 3 mm trephines (Ace Surgical Supply, Bur trephine 35 TL 2ID 2.8OD TI 18.5CL CA marks 8-10-13-15-18) were used to retrieve biopsies approximately 8-10 mm in length. Biopsies were retrieved from the implant sites, unless an implant was placed in original, non-grafted bone, in which case the biopsy was retrieved from the grafted area immediately adjacent to the implant, as indicated by the indexed stent. Biopsies were retained within their respective trephines, wrapped with lint-free gauze (the open ends of the trephines serving as the apical orientation reference) and preserved in 10% buffered formalin solution.

Histomorphometric Analysis

Specimens were delivered to the Cell Tissue Analysis laboratory at the Medical Center - University of Freiburg, Department of Oral and Maxillofacial Surgery, Freiburg, Germany. Trephines together with biopsies were fixed in 4% formalin for 5 - 7 days, dehydrated in serial steps of ethanol (70%, 80%, 90%, 100%), remaining for one day in each concentration, and degreased for one day in xylene. Specimens were then infiltrated, embedded and polymerized (Technovit 9100Heraeus Kulzer, Wehrheim, Germany). After polymerization, samples were hemisected and cut in 500 µm sections using a precision cutting machine (Secotom 50, Stuers, Ballerup, Denmark), so that two sections

were obtained per biopsy. The sections were mounted onto opacified acrylic-slides and ground to a final thickness of approximately 60 µm on a rotating grinding plate (Stuers, Ballerup, Denmark). Specimens were subsequently stained with azure II and pararosaniline (Axio Imager M1 and AxioCam HRc, Carl Zeiss, Göttingen, Germany). Histologic evaluation was performed with a microscope equipped with a digital analyzer (Axio Imager M1 and AxioCam HRc, Carl Zeiss, Göttingen, Germany).

Histomorphometric analyses were performed with imaging software (analySIS FIVE – Soft Imaging System, Münster, Germany) on composite overview scans. The area of new healing (*versus* old/ original bony tissues) was demarcated in each section. Within this area, the percentage contributions of each tissue type were computed.

Measurement Parameters

Prior to initiation of the study, using an indexed stent, stone cast model of an extraction socket exhibiting a substantial buccal dehiscence and a UNC 15 periodontal probe (Hu-Friedy Mfg. Co., Chicago, IL, USA), all investigators were calibrated (± 1 mm) to the principal investigator (ETS). All measures were rounded down to the nearest 0.5 mm.

Statistical Analysis

The first step in the analysis was to determine if the randomization resulted in balanced groups. Age, gender, race, ethnicity, BMI and all baseline clinical variables were tested to determine if the two groups were balanced. The analyses were based on the variable types, with dichotomous variables tested using Fisher' exact test and categorical variables using chi-square test and T-tests for continuous variables.

The primary hypothesis was that DBBMC+NBCM (test) was not inferior to DFDBA+RECXC (control) in the preservation of ridge volume as measured by the difference in the horizontal distance from stents, bucco-lingually to the interior (lingual) bony wall. The non-inferiority margin was 1.5 mm, as obtained from the approximate deviation in ridge dimension observed by Wood and Mealey.

Two-sample t-tests were used to evaluate non-inferiority, such that:

$H0 : \mu_{ck} - \mu_{ob} \leq \mu_o - \delta$

$Ha : \mu_{ck} - \mu_{ob} > \mu_o - \delta$

Where,

μ_{ck} = the mean change in ridge volume for DBBMC+ NBCM from baseline to 6 months

μ_{ob} = the mean change in ridge volume for DFDBA+RECXC from baseline to 6 months

μ_o = hypothesized mean difference = 0.0 mm

δ = non-inferiority margin = 1.5 mm

α = 0.05, one-sided

$1-\beta$ = 0.90

If the test for non-inferiority was statistically significant, then a two-sample t-test for superiority was subsequently conducted, as detailed in the methodology of Morikawa & Yoshida (1995).

The secondary statistical objectives of the study - treatment differences in the vertical ridge preservation changes (baseline to 6-months), measured apico-coronally from the outside of the stent to the apical extent of the lingual and buccal walls - were also evaluated using the non-inferiority method listed above.

Differences in histomorphometric variables were tested with two-sample t-test for superiority due to the exploit nature of the variables at 6 months. Due to multiple testing for the five histomorphometic variables, the significance level had to be less than 0.05/6 = .008 (Bonferroni). For histomorphometric differences to be clinically significant a margin of at least 15.0% was needed based on the work of Wood and Mealey.

The behavior of incision line closure over time was an additional goal of the analysis, with evaluations at one week, one month and at three months. The data were evaluated by testing for superiority at each time point. Inferiority testing could not be done due to a lack of historical data and the potential time dependence of the data. In addition to mean

differences tested with t-tests, the frequency of closed incisions was tested at each time point with Fisher's exact test.

Power Calculation

Sample size was predicated on assuring that the primary study objective had adequate power to assess the non-inferiority hypothesis. Under these assumptions, PROC POWER (SAS Version 9.2) required a sample size of 18 evaluable subjects in each treatment group. Therefore, a sample size of 40 subjects was considered sufficient to meet the primary objective of this study. Although the study was powered for the primary outcome variable, the secondary outcome variables each retained 90% power to demonstrate non-inferiority, assuming that the treatments were, in truth, equal.

Results

The study was conducted from November 2013 to February 2015. Forty subjects were enrolled and treated as per protocol, though one randomization code was misinterpreted so that there were 21 DFDBA+RECXC and 19 DBBMC+NBCM cases. The misinterpreted randomization code patient results were within the range of results reported. In all cases, grafting and soft tissue management were accomplished as directed by protocol (Figure 2).

At 6-months, of the 40 sites treated, all yielded areas that could be biopsied. 37 sites were deemed, according to the treating investigators, to have sufficient ridge preservation for implant placement. The 3 sites with insufficient ridge volume occurred at 3 different investigation centers and were all from the DFDBA+RECXC treatment group. These sites were all successfully re-grafted for later implant placement.

Baseline extraction socket defect measures were similar with no significant difference between DFDBA+RECXC and DBBMC+NBCM treatment sites. Soft tissue was approximated only, which left 36 of 40 sites open for secondary healing. Note: investigator center effects were also examined and did not influence results or change

significance levels. A list of teeth treated according to investigation center is provided in Table 1. As required by protocol, all extraction sites included buccal dehiscences greater than ⅓ the vertical and mesial-distal extraction socket dimensions. In fact, average dehiscences were approximately ¾ of the extraction socket vertical and mesial-distal dimensions. (Table 2.)

Age, gender, race, ethnicity and BMI were also comparable, though the DBBMC+NBCM treatment group had more lifetime tobacco use (10 of 19 subjects *vs.* 4 of 21 DFDBA+RECXC subjects). There was no difference in dental histories, though one of the DFDBA+RECXC subjects had a history of diabetes, which was deemed "controlled." There was no significant difference between treatment group subject oral hygiene compliance at any time point, though two DFDBA+RECXC subjects were recorded as not compliant at 1-week and one at 1-mo, with all DBBMC+NBCM subjects compliant throughout the study.

Primary Outcome – Horizontal Bony Ridge Dimension

Horizontal changes from baseline to 6 months, as measured using stents, were significantly different between the two modalities, with DBBMC+NBCM sites, on average, providing more bony width (1.76 mm).

Secondary Outcomes – Change in Vertical Bony Ridge Dimension, Soft tissue Inflammation/ Incision Line Gap and Histomorphometrics

From baseline to 6-months, vertical bone changes, measured at both buccal and lingual walls, were not significantly different between the two treatment modalities, although DBBMC+NBCM sites, on average, achieved more vertical ridge preservation (Figure 3).

There was no significant difference in inflammation between treatment modalities at any postoperative time point. However, inflammation was higher at 1-week compared with later time points, and, on average, was higher for DFDBA+RECXC, with more "2" and "3" inflammation levels recorded (13 DFDBA+RECXC sties *vs.* 8 DBBMC+NBCM sites). At 1-month, though overall inflammation scores decreased for both therapies,

DFDBA+RECXC still involved more mild, localized inflammation than DBBMC+NBCM (13 *vs.* 7). By 3-months, inflammation was ranked as "normal" for approximately 90% of both treatment modalities. Inflammation at 1-week was nearly significantly correlated (Spearman correlation 0.0573 *p*-value) with horizontal ridge change over 6-mos, in an inverse relationship, i.e., more inflammation tended to produce poorer ridge preservation results.

At baseline (surgical closure) incision line gaps were not significantly different between the two treatments, but at 1-week the buccal-lingual gaps were, on average, 1.82 mm greater for DFDBA+RECXC sites. By 1-month gap differences were statistically and significantly different (approximately 1.5 mm difference - Table 3). Additionally, at one month the frequency of closed incision gaps was significantly higher for the DBBMC+NBCM group (14 out of 19) *vs* the DFDBA+RECXC (8 out 21) p =0.0309. The frequency of closed incision lines was not different at either one week (most still open in both groups) or three months (most closed in both groups).

Histomorphometric Analysis

All biopsies (n=40) were successfully retrieved, processed, sectioned and digitally labeled for histomorphometry. Representative histological sections are depicted in Figure 3. Graft remnants were embedded either in bone marrow/ connective tissue or juxtaposed with new bone trabeculae. In the lower (apical) portions of the sections, graft remnants were most often directly encompassed with or in contact with new bone. In the coronal portion of the sections, connective tissue/ bone marrow tended to be more prevalent. This tissue was well vascularized and free of inflammation.

DFDBA grafting biomaterial observed prior to implantation appeared to be a combination of both mineralized and demineralized bone, including possible nuclear material. After 6-months, DFDBA biopsies tended to show signs of remineralization emanating from the mineralized portions of the grafts, and around this remineralization zone, osteocyte lacunae sometimes appeared to be the nidi for remineralization "islands." Occasionally osteoclasts could be seen resorbing the graft mineral – a phenomenon not

observed with DBBMC grafts.

DBBMC granules, which were more prevalent than DFDBA remnants, tended to form a dense trabecular network with new bone (Figure 4). Circular and elliptical outlines indicated endothelial structures of vascularization in both the apical and coronal portions of the sections, with the coronal portions also containing osteoid and osteoblasts. Osteon-like structures were sometimes, but rarely, seen within the DBBMC granules.

Mean and standard deviation values for the percentages of each tissue type and graft remnants are presented in Table 4. Only tissues (and graft) within the extraction socket defect were taken into account; older original bone was excluded. Accordingly, the sum of new bone, connective tissue/ bone marrow and graft equaled the entire area measured, or 100%.

The percentage of new bone formed was not significantly different between treatment modalities, though there were significantly more graft remnants within the DBBMC sites, and new bone in contact with graft remnants was, accordingly, greater for DFDBA. Overall there were no statistical differences between the two therapies either in the percentage of graft remnants in contact with new bone or in the overall percentage of trabecular network formed by the combination of new bone and grafts.

Additional Exploratory Variables

There was no statistical difference in baseline bony wall thicknesses between test and control therapies, but both the buccal and lingual baseline wall thicknesses were related to the amount of ridge preserved vertically and horizontally at 6-months, inversely: smaller bony wall thicknesses were related to better ridge preservation results. Both were significant (buccal $p \approx 0.02$; lingual $p \approx 0.01$).

There was no significant relationship found between the size of the bony wall dehiscences at baseline and the ridge preservation achieved at 6-months. However, the

baseline extraction socket measurement "Crest to Apical Extent of Socket " (which was also *not* statistically different between test and control therapies) was related to the 6-month ridge area changes, *p*-value ≈ 0.03. The relationship was positive, i.e., deeper baseline defects were associated with better ridge preservation at 6-months.

Discussion

This investigation compared two ridge preservation methodologies in substantial buccal wall dehiscence defects. Since bone remodeling and soft tissue collapse and their interference with bony healing are common in these defects, they provide a critical methodological test. One technique (DFDBA+REXC) was derived from the history of demineralized allograft use and the belief that such allografts might be osteoinductive and produce bone formation. DFDBA was combined with a reconstituted and cross-linked collagen membrane thought to better preserve (through stiffness) the volume intended for regeneration, and for a longer time than non-cross-linked collagen membranes. The other technique (DBBMC+NBCM) was derived from a history of mineralized xenogeneic graft use and the belief that such grafts might be osteoconductive, giving rise to bone integration, and yet resorb slowly to preserve the volume intended for regeneration. DBBMC was combined with an extracted and non-cross-linked collagen membrane thought to encourage trans-membrane vascularity and rapid tissue integration, with the membrane degrading over a time period thought sufficient for bone regeneration.

For the primary outcome of ridge preservation, horizontal ridge preservation was significantly greater for DBBMC+NBCM. There was only a trend, and not statistically significant, for more vertical bone preservation with DBBMC+NBCM. As a clinical consequence, implants could be placed in all but three treatment sites, which were DFDBA+RECXC sites.

Both treatment groups provided a degree of ridge preservation. In the review by Araujo et al. (2015), he postulated that, following tooth extraction, "… (i) up to 50% reduction of the original ridge width will occur; (ii) the amount of bone resorption will be greater at

the buccal aspect than at its lingual/palatal counterpart; and (iii) a larger amount of alveolar bone reduction will take place in the molar regions." In this review of clinical studies with various biomaterials, it was concluded that ridge contraction following tooth extraction can be minimized with socket grafts and/or the use of mechanical barriers. While grafts alone might be sufficient for space-maintaining sockets, others have found grafts *plus* membranes to be advantageous, particularly for limited space-maintaining defects like the dehiscence defects studied herein (Perelman-Karmon et al. 2012, Kim et al. 2008 & Sanz-Sanchez et al. 2015).

The secondary outcomes, based on histomorphometry, incision line gap closure and degree of inflammation, were included in the hope they might help explain the primary outcome of ridge preservation. Indeed, the percentage of new bone seen in the biopsy sections was not statistically different between the two therapies studied. However, the percentages of remaining graft mineral were significantly greater for DBBMC. This, in turn, may support the premise that minimal DBBMC resorption provided space-maintenance for improved ridge volume (Buser et al. 2013, Testori et al. 2013 & Galindo-Moreno 2014). Moreover, there was no difference in the percentage of trabecular structure (new bone plus graft) observed between the two therapies.

DBBMC biopsies showed little to no signs of osteoclastic resorption and graft remodeling; rather, DBBMC was intimately incorporated into new bone trabeculae. However, over time, particularly in the coronal regions where bone formation appeared less advanced than in apical regions, studies of DBBMC in the sinus have indicated woven bone turns into lamellar bone, along with further bone formation, integration and maturation (Sartori et al. 2003 & Traini et al. 2007). DFDBA biopsies depicted robust bone formation with islands of new bone that might be interpreted as evidence of bone induction; but in contrast to DBBMC, DFDBA appeared to be in a more active state of turnover and replacement. This bone-remodeling phenomenon has also been observed in the maxillary sinus (Soardi et al. 2013) – a phenomenon that might help explain the difference in ridge volume preservation observed between the two therapies in the present study.

Examining soft tissue healing, the degree of inflammation appeared to have negatively affected ridge preservation. The Spearman correlation, *p*-value of 0.0573, just on the edge of significance, was a strong *p*-value, considering inflammation was a categorical and subjective variable. More inflammation was observed, by count, at DFDBA+RECXC sites. Of the 18 sites with incision line gaps still open at 1-month, 13 were DFDBA+RECXC. Early vascularization and soft tissue integration of NBCM membranes, as compared with cross-linked collagen membranes, may have improved soft tissue healing and underlying bony tissue regeneration results, with NBCM membranes degrading over a time period reported as sufficient for guided regeneration (Schwarz et al. 2008 & Bornstein et al. 2007).

Following the hypothesis that GBR was simply a consequence of tissue separation and that the volume created by the membranes determined the volume of bone that could be regenerated, stiffer, titanium reinforced and shapeable expanded polytetrafluoroethylene (ePTFE) membranes were developed (Schenk et al. 1994). These membranes were designed for defects in which there was an absence of residual bony walls, i.e., the defects themselves were not able to prevent membrane collapse into the area intended for GBR. However, ePTFE membrane shortcomings included soft tissue dehiscences, along with potential inflammation and infection, recognized as clinical complications that diminished regenerative therapy outcomes (Machtei et al. 1994). Accordingly, biodegradable collagen membranes were designed to integrate with the healing tissues but also to degrade over time, particularly when exposed to the oral environment. Still, the quest for space-maintaining membranes and the uncertainty over what might be a suitable time period for degradable membranes to remain intact led to investigations of stiffer and more slowly degrading collagen barriers (Bunyaratavej & Wang 2001). Despite this history, and even though the historical suggested removal time for ePTFE in GBR procedures was after several months of healing, today some researchers speculate that the regenerative disposition of tissues may occur over the course of a few weeks (Susin et al. 2015).

Membrane integration and duration of resorption, which could be associated with the degree of soft tissue inflammation and incision line gapping reported in the study herein, might help explain the differences observed between the two therapies compared. Regardless, given the four biomaterials tested in this study (2 grafts and 2 membranes), it was not possible to isolate cause and effect for any single biomaterial. Only the combined effects of the grafts and their respective membranes could be evaluated.

Other potential design limitations included evaluation of implant integration and survival, the number of investigators and the variety of defect morphologies tested. Success of implant integration and long-term survival were not reported herein but will be provided in a subsequent report. Despite the potential variability inherent with a large group of investigators, statistically significant differences were detected between the two therapies. In this regard, the number of investigators may have provided a more poignant finding, with results that might better represent the clinical community at large. Defect morphologies treated ranged from “keyhole” to wide buccal dehiscences. Baseline extraction bony wall thickness and extraction socket depth (crest to apex) were related to ridge preservation outcomes, and case photos appeared to indicate both of these phenomena involved the regenerative potential of the baseline defects, i.e., remaining bony walls (measured as “thin” because of their coronal extension and “deep” in extent) were more space-maintaining.

In future studies, extraction defect morphologies should be further apportioned for more definitive analyses. In addition, studies should be performed in the anterior region, where esthetics, labial bone loss and soft tissue management are more critical. Finally, we recommend, as reported herein, that future ridge preservation studies include substantial, buccal wall dehiscence defects as critical tests of biomaterials, surgical techniques and, ultimately, patient outcomes.

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Table 1

Investigation Center	Tooth Number	Test or Control	Multi (M) vs. Single (S) Rooted Teeth
01-01	30	T	
01-02	19	C	
01-03	3	C	
01-04	14	T	
01-05	30	C	5 M
02-01	12	T	
02-02	4	C	
02-03	12	T	
02-04	12	C	4 S
03-01	19	C	
03-02	19	T	2 M

04-01	5	T	
04-02	30	C	
04-03	3	T	
04-04	19	T	3 M: 1 S
05-01	19	C	
05-02	3	T	
05-03	4	C	2 M: 1 S
06-01	19	T	
06-02	3	C	
06-03	3	C	3 M
07-01	30	T	
07-02	19	C	
07-03	19	C	
07-04	30	T	
07-05	30	C	
07-06	19	T	6 M
08-01	12	C	
08-02	5	T	
08-03	29	T	
08-04	20	C	4 S
09-01	30	C	
09-02	19	T	
09-03	19	C	
09-04	14	T	
09-05	30	C	5 M
10-01	5	T	
10-02	4	C	
10-03	30	T	
10-04	3	C	2 M: 2 S
Total			28 M: 12 S

Table 2

BASELINE DEFECT (mm ± SD)			
Direct Measure/ No Stents			
	DBBMC+NBCM (test)	**DFDBA+RECXC (control)**	***p* Value**
Number of Subjects	19	21	
Socket Crest to Apical Extent of Socket (mm)			
Mean ± SD	11.00 ± 2.89	11.43 ± 2.65	
Range	(6.0, 15.0)	(7.0, 15.0)	
T-Test			**0.6273**
Socket Mesial-Distal @ Crest (mm)			
Mean ± SD	8.63 ± 2.49	8.69 ± 2.83	
Range	(4.5, 14.0)	(5.0, 15.0)	
T-Test			**0.9450**
Vertical Dehiscence - Crest to Buccal Apex (mm)			
Mean ± SD	8.05 ± 2.58	8.60 ± 2.15	
Range	(3.5, 14.0)	(5.0, 13.0)	
T-Test			**0.4728**
Mesial-Distal Dehiscence @ Crest (mm)			
Mean ± SD	6.26 ± 2.57	6.62 ± 2.60	
Range	(2.0, 12.0)	(3.0, 13.0)	
T-Test			**0.6663**
BASELINE STENT MEASURES (mm ± SD)			
Horizontal (Buccal-Lingual)			

Mean ± SD	11.84 ± 2.61	11.52 ± 1.76	
Range	(7.5, 18.5)	(8, 15)	
T-Test			**0.6506**
Vertical (Lingual)			
Mean ± SD	11.05 ± 2.85	10.88 ± 4.25	
Range	(4, 19)	(7, 27)	
T-Test			**0.8827**
Vertical (Buccal)			
Mean ± SD	17.32 ± 3.36	17.83 ± 4.13	
Range	(13, 26.5)	(12, 18)	
T-Test			**0.6659**
SIX MONTH STENT MEASURES (mm ± SD)			
Horizontal (Buccal-Lingual)			
Mean ± SD	5.13 ± 1.79	6.57 ± 2.75	
Range	(2, 9)	(2, 14.5)	
T-Test			**0.060**
Vertical (Lingual)			
Mean ± SD	10.45 ± 2.11	10.95 ± 2.04	
Range	(5, 14)	(8, 15)	
T-Test			**0.4459**
Vertical (Buccal)			
Mean ± SD	11.08 ± 2.54	12.55 ± 2.85	
Range	(5, 16)	(8, 18)	
T-Test			**0.0948**
DEFECT CHANGE – STENT MEASURES BASELINE TO 6-MOS (mm ± SD)			
Horizontal (Buccal-Lingual) Δ Baseline to 6-mos (mm)			
Mean ± SD	6.71 ± 2.07	4.95 ± 2.65	

Range	(3.0, 10.5)	(0.0, 11.0)	
Mean difference between groups (90% C. I)	1.76 (0.48, 3.03)		
As the primary response variable tested non-inferiority. Lower C. I. 0.48, was greater than – $\delta = -1.5$			
T-Test Superiority (95% C. I) difference between groups	1.76 (0.02, 3.29)		**0.0256**
Buccal Vertical Δ Baseline to 6-mos (mm)			
Mean ± SD	6.24 ± 2.98	5.29 ± 3.73	
Range	(2.0, 12.0)	(0.0, 12.5)	
T-Test Superiority (95% C. I) difference between groups	0.95 (-1.22, 3.22)		**0.3818**
Lingual Vertical Δ Baseline to 6-mos (mm)			
Mean ± SD	0.60 ± 2.68	-0.07 ± 3.15	
Range	(-5.0, 7.0)	(-4.0, 12.0)	
T-Test Superiority (95% C. I) difference between groups	0 .67(-1.21,2.56)		**0.4714**

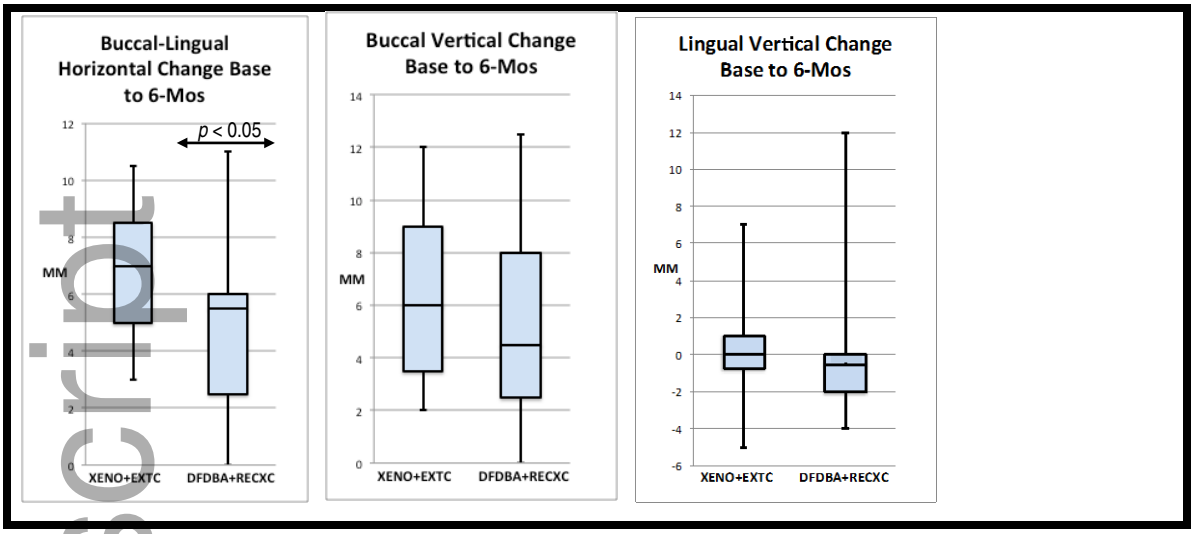
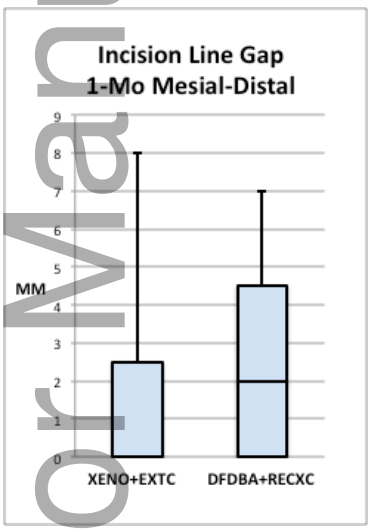


Table 3

	INCISION LINE GAP		
	DBBMC+NBCM	**DFDBA+RECXC**	***p* Value**
Number of Subjects	19	21	
Incision Line Gap Baseline: Buccal-Lingual (mm)			
Mean ± SD	4.84 ± 2.78	5.21 ± 3.22	
Range	(0.0, 9.5)	(0.0, 10.0)	
T-Test			**0.6992**
Incision Line Gap @ 1-Week: Buccal-Lingual (mm)			
Mean ± SD	3.16 ± 2.22	4.98 ± 3.49	
Range	(0.0, 8.0)	(0.0, 15.0)	
T-Test Superiority (95% C. I) difference between groups	1.82(-0.043, 3.68)		**0.0596**
Incision Line Gap @ 1-Mo: Buccal-Lingual (mm)			

Mean ± SD	0.79 ± 1.55	2.26 ± 2.68	
Range	(0.0, 5.0)	(0.0, 8.5)	
T-Test Superiority (95% C. I) difference between groups	1.47(0.079, 2.87)		**0.0427**
Incision Line Gap @ 1-Mo: Buccal-Lingual (mm)			
Mean ± SD	0.79 ± 1.55	2.26 ± 2.68	
Range	(0.0, 5.0)	(0.0, 8.5)	
T-Test Superiority (95% C. I) difference between groups	1.47(0.079, 2.87)		**0.0427**



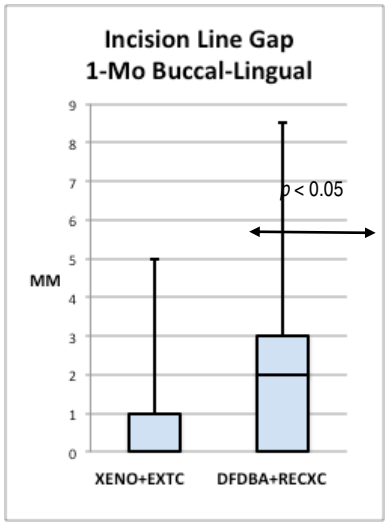


Table 4

Mean% ± SD	**New Bone**	**Connective Tissue/ Bone Marrow**	**Graft**	**New Bone Mineral in Contact w/ Graft**	**Graft in Contact with New Bone Mineral**	**New Bone + Graft**
DBBM-C	29.81 ±	50.77 ±	19.40 ±	16.26 ±	9.08 ±	49.21 ±

DFDBA	9.03 33.36 ± 11.09	8.26 53.66 ±7.62	10.99 12.78 ± 6.60	11.48 28.90 ± 11.47	8.05 9.61 ± 4.95	8.27 46.14 ±7.66
Mean Difference	-3.55	-2.89	6.60	-12.64	-0.53	3.07
95 % C. I.	-9.79, 2.69	-7.83, 2.05	0.91, 12.29	-19.76, -5.52	-4.72, 3.66	-1.89, 8.03
t-test, *p* value	0.1749	0.2569	0.0249	0.0013	0.8012	0.2304

Table and Figure Legends

Table 1 – Investigator Centers and Tooth Sites Evaluated

Table 2 – Baseline defect measures were comparable (not significantly different) between the two therapies. Vertical ridge preservation changes from baseline to 6-mos were also not significantly different; however, horizontal changes were significantly different. Boxplots reveal the trend in bony ridge preservation differences between therapies, with DBBMC+NBCM providing median, first and third quartile values 1-3 mm greater in all dimensions. However, given the wide range of results obtained (see whiskers), only buccal-lingual ridge preservation results were statistically different.

Table 3 – Though starting out with no significant difference (baseline, following surgical closure), incision line gaps were significantly greater by 1-month for DFDBA+RECXC. Median values were approximately 1.5 -2 mm greater for DFDBA+RECXC in both mesial-distal and buccal-lingual dimensions, though there was a wide range (boxplot whiskers) of gap measures for both therapies.

Table 4 - Relative Area of Biopsy Section Tissue Components (%), N=40

Figure 1 – (a) Baseline extraction socket measures insured that buccal wall mesial-distal and vertical dehiscences were at least 1/3 of the overall extraction socket dimensions. (b & c) Measuring stents were fabricated from 0.020" thermoplastic and registered on adjacent teeth. The stents included three indexing holes for measuring ridge buccal-lingual width, vertical height to the lingual wall and vertical height to the buccal wall.

Figure 1 – Left column DFDBA+RECXC and right column DBBMC+NBCM. Top to bottom; original extraction socket illustrating extent of vertical and mesial-distal dehiscences, graft placement, membrane coverage, closure, soft tissue appearance at 6-months, ridge preservation at 6-months.

Figure 3 – Complete trephine biopsy sections (original magnification 50x, azure II and pararosaniline) for DFDBA+RECXC (left pair) and DBBMC+NBCM (right pair), showing both original staining and digital labeling for histomorphometry. (Note that the split in the DBBMC+NBCM section was artifactual.) Yellow lines in the lateral regions delineate old bone (OB) from new bone (NB) and define the healing area of the defects, which were further labeled for the following tissue types: (1) red for NB *not* in contact with graft, (2) pink for NB in contact with graft. Dark blue for DFDBA *not* in contact with NB, and light blue for DFDBA in contact with NB. Light green for DBBMC *not* in contact with NB, and dark green for DBBMC in contact with NB (composite overview scans, individual microphotographs original magnification x50).

Figure 4 - (a) DFDBA graft prior to implantation showing different degrees of mineralization within the "virgin" grafting material: fully mineralized bone (mDB), partially demineralized bone (pDB), and almost completely demineralized bone (dDB), including osteocyte lacunae (OL) empty or filled with organic material. (b) 6-month biopsy showing remineralization of DFDBA: demineralized DFDBA (dDB), mineralized DFDBA (mDB), remineralized DFDBA (rDB), and "island"-like calcified structures (I) in the remineralization zone. (c) Resorption (R) of DFDBA by osteoclast (OC) – a phenomenon not observed with DBBMC. (d) Original, native bone (OB) with DBBMC (BB) embedded in connective tissue (CT) or in newly formed bone (NB). (e) A possible

vascular channel in DBBMC within the coronal portion of the biopsy. (f) Woven new bone (wNB) with tightly integrated DBBMC (BB) granules forms a dense trabecular network; loose connective tissue is free of inflammation and densely vascularized.

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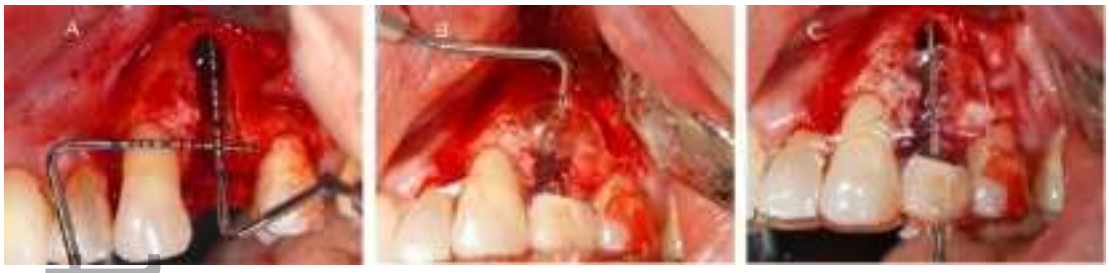

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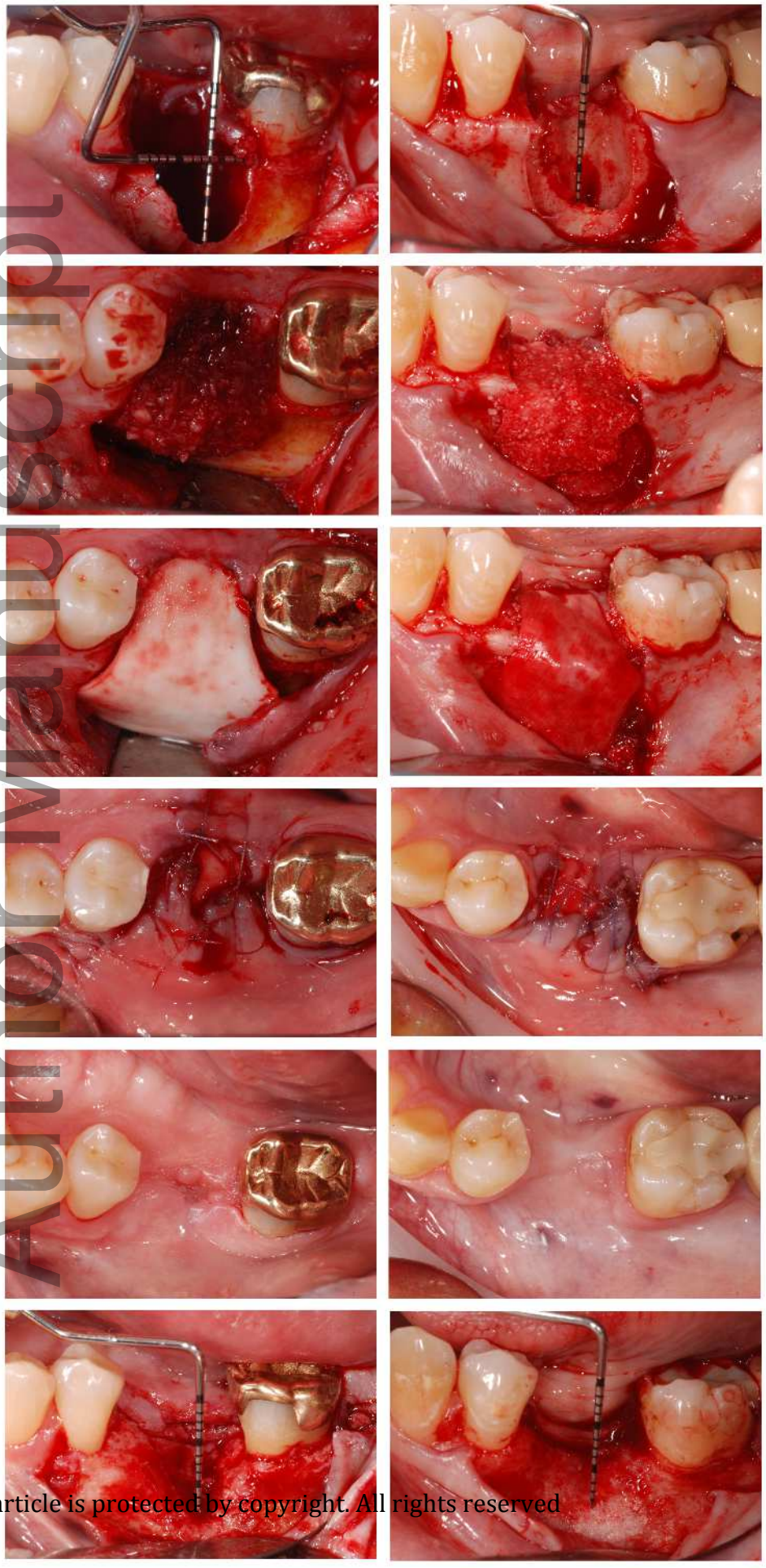

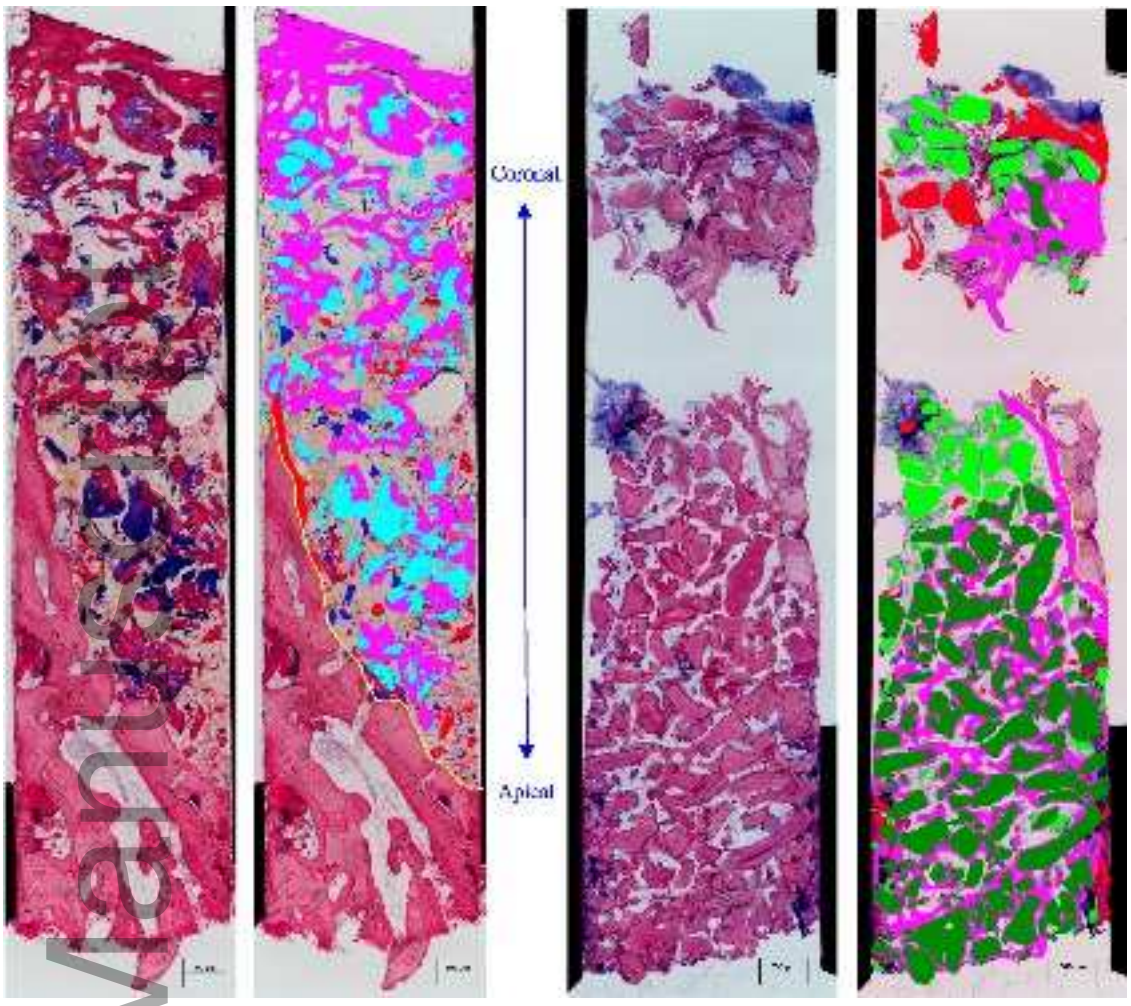

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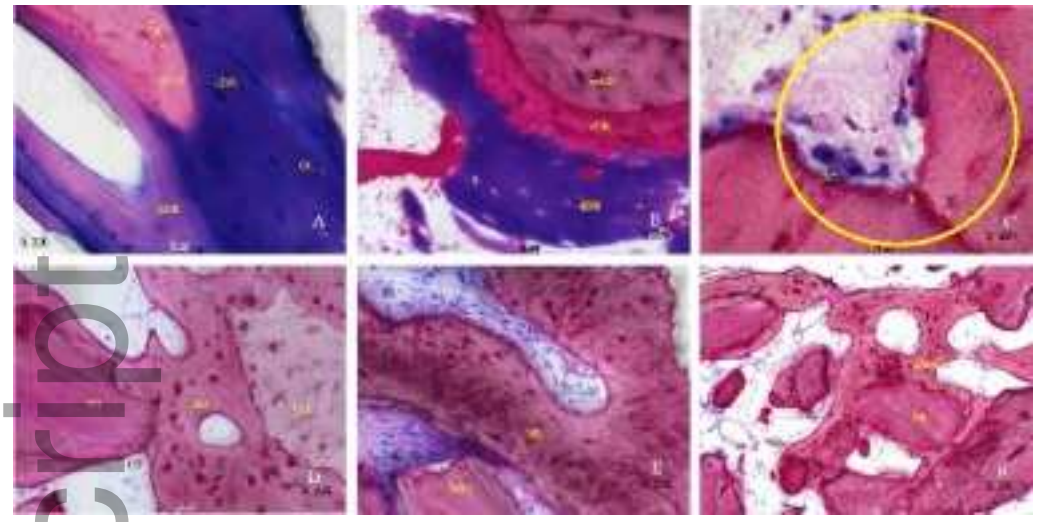

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