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The Influence of Deproteinised Bovine Bone Mineral on Dimensional Changes of the Maxillary Second Incisor Socket

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Running Title: DBBM and healing of maxillary incisor sockets

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Abstract

Objectives: To investigate the dimensional changes following the extraction of maxillary second incisors and to evaluate the influence of deproteinised bovine bone mineral on the healing outcomes

Materials and Methods: The second maxillary incisors in nine dogs were extracted bilaterally in a minimally traumatic manner. Deproteinised bovine bone mineral with collagen (DBBMC) and a collagen matrix (CM) were placed in one socket with the contralateral socket left to heal naturally. After three months of healing, the dogs were sacrificed and the pre-maxilla resected. Cone beam computerised tomography scans (CBCT) were obtained and the specimens prepared for histological preparation and analysis. Surface scans of study models taken pre- and post-extraction were digitally subtracted to analyse volumetric changes.

Results: All dogs healed uneventfully without any complications. No inflammation was seen and DBBMC was well integrated into a network of mineralised tissues, bone marrow and connective tissue. The horizontal width of the buccal crest was found to be significantly greater in grafted sockets, but the vertical height to be similar. No significant difference was seen in regards to volumetric changes of sockets over three months post-extraction.

Conclusion: Maxillary second incisor sockets of dogs grafted with DBBMC post-extraction had significantly greater horizontal width at the ridge crest compared to ungrafted sockets. Volumetric analysis revealed no significant difference between grafted and non-grafted sockets suggesting possible soft tissue thickening post-extraction to counteract osseous resorption.

Key words: extraction socket, bone resorption, dimensional changes, wound healing, grafting, biomaterial, ridge preservation

Introduction

Missing teeth in the maxillary anterior zone often present significant challenges to clinicians. Dimensional changes post-extraction in the maxillary anterior region are of particular importance when it comes to implant therapy. The healing of the socket results in a loss of both height and width of the alveolar process with an overall reduction in bone volume and ridge dimensions (Amler et al., 1960; Cardaropoli et al., 2003; Evian et al., 1982; Atwood, 1963; Atwood & Coy, 1971; Johnson, 1967). Resorption patterns have shown dimensional changes to be more pronounced on the buccal compared to the palatal/lingual side (Pietrokovski, 1975; Schropp et al., 2003) with changes in the width of the socket being reported as much as three times greater than the height (Lekovic et al., 1997).

A number of preclinical and clinical studies have documented that the buccal bone wall of a socket undergoes significant vertical and horizontal resorption in the first weeks and months following tooth extraction (Araujo et al., 2008; Araujo & Lindhe, 2005, 2009a; Barone et al., 2008; Cardaropoli & Cardaropoli, 2008). The bundle bone concept has been proposed as an explanation for these early dimensional changes as it is a tooth-derived structure (Araujo & Lindhe, 2005). Following the extraction of a tooth, it undergoes resorption and removal as part of the physiological sequence of events. As the coronal region of the buccal socket wall is comprised of almost entirely bundle bone, a significant proportion of this region of the socket wall is resorbed. This leads to both the vertical and horizontal diminution of the buccal bone wall (Araujo et al., 2006; Covani et al., 2004; Farmer & Darby, 2014).

In order to minimise the dimensional changes that take place following an extraction, ridge preservation procedures have been recommended (Darby et al., 2009). Preclinical and clinical studies have documented that diminution of the ridge on the buccal cannot be prevented completely despite the use of a variety of different materials, primarily due to the resorption of the bundle bone (Araujo & Lindhe, 2009b; Araujo & Lindhe., 2005; Fickl et al., 2008; Lee et al., 2015; Lindhe et al., 2013; Shi et al., 2007, Artzi et al., 2000; Lekovic et al., 1998; Lekovic et al., 1997; Serino et al., 2003). These studies have established the principles of wound healing and the potential of different grafting materials and techniques to minimise the dimensional changes in particular deproteinised bovine bone mineral (DBBM). The majority of experimental studies in this field have employed the mandibular premolar socket in a canine model, which may not be an appropriate comparison with the anterior maxilla in humans.

Recently, a maxillary second incisor model in the canine was proposed (De Santis et al., 2011, Mellati et al., 2015). This model has advantages over the mandibular premolar socket model. Firstly, extraction of the maxillary second incisor teeth is more straightforward than the extraction of mandibular premolar teeth in the canine. The density of the alveolar bone in the mandible is very high, compared to the maxilla, which has much less density due to a high degree of trabeculation in the surrounding alveolus. Thus, extraction of the maxillary second incisors can be performed with relatively low trauma compared to the extraction of mandibular premolars. This minimises the impact of any trauma from the extraction that may occur on the subsequent resorption and dimensional changes of the socket walls. Secondly, the preparation of the extraction site to receive implants is very similar to the situation encountered when placing implants immediately into extraction sockets in the anterior maxilla in humans. Thirdly, the maxillary second incisor socket of greyhounds has been shown to be dimensionally similar to that seen in humans, allowing any morphometric findings to be somewhat more representative of a clinical scenario (Mellati et al. 2015).

There are no studies reporting on the outcomes of ridge preservation procedures using the canine maxillary second incisor model. This study therefore examined the dimensional changes that occur following extraction and effect of ridge preservation procedures using deproteinised bovine bone mineral (DBBMC) covered by a resorbable collagen matrix (CM) to attempt to counteract these dimensional changes.

Methods

The study was approved by the Animal Ethics Committee of the University of Melbourne (Ethics ID no. 1413317.1) and the study report written according to the ARRIVE guidelines (Kilkenny et al. 2010). Nine healthy greyhounds, both male and female, ranging in weight from 22.2Kg to 33.6Kg, aged older than 12 months were used in the study.

Clinical Procedures

All procedures were performed under general anaesthesia with bilateral infra-orbital nerve blocks. Thirty to sixty minutes prior to anaesthetic induction, sedation was achieved via subcutaneous administration of ACP® (0.1mg/kg, Acetylpromiazine, Delvet, Seven Hills NSW, Australia). A 20-gauge catheter was then placed into the cephalic vein to allow for the administration of anaesthesia, and to administer intravenous fluids. A general anaesthetic (Alfaxan®, Alfaxalone 10mg/ml, Jurox, Rutherford, NSW) was then administered via this IV pathway at 2mg/kg. Each greyhound was intubated with a cuffed endotracheal tube, and anaesthesia maintained with oxygen (1.5-2Litres/minute) and Isorrane® 1.5-2% (isoflourane, Baxter Healthcare, Qld Toongabbie, NSW, Australia) delivered through an out-of-circle vaporiser anaesthetic machine. Following induction, Hartmann's solution (compound sodium lactate) was administered intravenously via the cephalic catheter in order to maintain fluid hydration. This was delivered at 10ml/kg/hour, and able to be increased if the subject's blood pressure dropped below 90mmHg. Local anaesthesia was provided via infra-orbital nerve block at the sites of extraction via administration of Lignocaine®(Lignocaine HCL, 20mg/ml, Troy laboratories, Glendenning, NSW, Australia) with a 27-guage needle.

If plaque and calculus were detected prior to the commencement of the surgery, the maxillary incisors were scaled with an ultrasonic tip. Intrasulcular incisions were performed around both maxillary 2nd incisors. Without flap elevation, extraction of the maxillary 2nd incisors was completed using fine periotomes and extraction forceps, in a minimally traumatic manner (Figure 1A). Extreme care was taken not to damage the socket walls, particularly the buccal wall. Following successful delivery of the tooth, the sockets were then irrigated with saline to remove any debris and cleared of any soft tissue remnants. The sockets were then examined closely for the presence of any fenestration or dehiscence of the buccal bone. Using a Michigan O probe with Williams markings (Hu-Friedy, Chicago, IL, USA), the depth, and the bucco-lingual and mesio-distal widths of the sockets were measured at the level of the bone crest. The ridge width was also measured at the mucogingival margin using callipers. All measurements recorded were rounded to the nearest half a millimetre.

Sockets were randomised by coin toss to decide which was to be grafted and which was to be left to heal naturally (Figure 1B). A commercial preparation of 90% Deproteinised Bovine Bone Mineral and 10% collagen (100mg BioOss® Collagen, Geistlich Pharma, Wolhusen, Switzerland) was soaked in saline and then placed into the assigned maxillary 2nd incisor sockets. It was gently packed into the socket up to the mid-buccal bone crest (Figure 1C). Care was taken not to overfill the socket. A collagen matrix (8mm Mucograft Seal®, Geistlich Pharma, Wolhusen, Switzerland) was placed over the BioOss® Collagen at the opening of the socket. (Figure 1D). Resorbable sutures (Glycolon® 5.0, Resorba Medical GmbH, Nuremberg, Germany) were placed over the socket, and through the Mucograft Seal® to secure it in its place (Figure 1E & F).

The first two weeks after the extraction, the plaque control regimen included a daily application of Hexarinse® (chlorhexidine gluconate, with cetylpyridium chloride, and zinc gluconate, Virbac, Regents Park, NSW, Australia). After two weeks mechanical plaque control was initiated and performed every day for the next ten weeks using a soft toothbrush with toothpaste. The first week after surgery, the dogs were placed on a soft diet, followed by a normal diet thereafter for the duration of the experiment.

At three months, all nine dogs were sacrificed with an intravenous injection of Lethabarb® (pentobarbitone, Virbac, Regents Park, NSW, Australia) into the cephalic vein.

Histological Preparation

Following sacrifice, all pre-maxillae were resected en bloc and immediately placed in 10% buffered formalin (Orion, Balcatta, WA, Australia). The specimens were then delivered to the Cell Tissue Analysis Laboratory (Medical centre - University of Freiburg, Department of Oral and Maxillofacial Surgery, Freiburg, Germany) and fixed in 4% formalin for 5-7 days. They were dehydrated in a series of graded ethanol (70%, 80%, 90%, and 100%), remaining in each concentration for 2 days, and degreased for one day in xylene (Merck, Darmstadt, Germany). Specimens were then infiltrated, embedded, and polymerised in methyl methacrylate (Merck, Darmstadt, Germany). After polymerisation, specimens were orientated to ensure sectioning through the mid-buccal aspect, and long axis of the socket. Samples were then cut in a bucco-lingual direction using a low-speed rotary diamond saw (Sectocom 50, Stuers, Ballerup, Denmark) to 600μm sections. Three sections were obtained for each extraction socket. These were then mounted on opaque acrylic slides (Maertin, Freiburg, Germany), and ground and polished to a final thickness of approximately 60μm on a rotating grinding plate (Stuers, Ballerup, Denmark). The sections were then stained in Azure II for ten minutes, followed by parosanilline (Merck, Darmstadt, Germany) for two minutes. The section that best represented the midbuccal of the socket was selected for the analysis

(Figure 2).

Histomorphometric Analysis

Histological evaluation was performed with a light microscope equipped with a digital analyser (Axio Imager M1 and AxioCam HRc, Carl Zeiss, Gottingen, Germany). The following landmarks were identified on each slide (Figure 3):

- A: Apex of socket
- cP: palatal crest
- cB: buccal crest
- Outline of the socket
- COR: crest of the ridge, most coronal point of bone/DBBMC

Subsequently, the following measurements were performed:

- Vertical Difference in height between the buccal and palatal crests (Figure 4a)
- Bucco-palatal width of the ridge measured at the palatal crest, and at 1mm increments moving apically, up to 5mm (Figure 4b)

With the outline of the sockets identified, histomorphometric analysis was conducted to assess the presence of newly formed bone, connective tissue and bone marrow, and remaining BioOss Collagen® particles at fifty times magnification using an on screen digital ruler and Microsoft Powerpoint (Microsoft, Redlands, WA, USA).

Radiographic analysis

Prior to the pre-maxilla being processed for histological analysis, cone beam computerised tomography scans (90Kv, 4mA) were taken (Veraviewepocs 3D F40, Morita, California, USA). Para-axial sections at the mesio-distal mid-point of the ridge were obtained. The plane of the sections were coincident with the long axis of the socket and orthogonal to the line of the arch. Measurements were conducted to determine the distance from the buccal crest (cB) to the crest of the ridge or regenerated bone (COR), and the distance between the buccal crest (cB) and palatal crest (cP) (figure 5).

Volumetric Analysis

Using customised trays, a two- phase polyvinylsiloxane impression was taken (Elite HD+®, Zhermack, Italy) pre-extraction, and again after three months of healing. Models were then poured up in orthodontic stone. Using a laboratory model scanner (3Shape®, Copenhagen, Denmark), the models of each specimen were scanned in order to create a Standard Tessellation Language (.STL) file. The pre-extraction scan and post-extraction scans were then superimposed upon each other, using Geomagic Control®(333 three D Systems Circle, Rock Hill, South Carolina, USA). Using this software and Microsoft PowerPoint® (Redmond, Washington, USA), analysis was conducted in order to analyse the degree of post-extraction volumetric changes, and to visualise the exact nature of where these volumetric changes occurred.

Statistical analysis

The primary outcomes variables were the difference in the vertical and horizontal dimensions between the grafted and non-grafted groups. The histometric measurements, composition, CBCT measurements and the volumetric surface scan substraction data were analysed using a paired t-test, with significance at $p \leq 0.05$. One section was used from the grafted and non-grafted sites in each dog resulting in a total of 9 grafted and 9 non-grafted sites.

Results

At initial surgery, both 2nd incisors in each dog were extracted without incident. There were no differences in the dimensions of the sockets between groups (for all sites combined; apico-coronal from the buccal crest = 11.8 ± 1.47mm; mesio-distal at the crest = 3.9 ± 0.24; bucco-palatal at the crest = 5.9 ± 0.32mm). The buccal plate was found to be intact in all sockets. All dogs healed uneventfully, with complete socket closure achieved in all dogs three weeks after extraction. Grafted sockets overlayed with CM were found to take up to a week longer for complete socket closure. After three months the mucosa covering the edentulous space and around adjacent teeth was found to be clinically healthy and free of any inflammation.

Histology

Non-Grafted Sockets

A layer of keratinised epithelium was found covering the healed socket. The underlying connective tissue was characterised by the presence of a dense network of mature collagen fibres, arranged in an irregular fashion (Figure 6a). At the socket entrance, a convex-shaped dome of mineralized bone bridged the lingual and buccal crests. This was comprised primarily of woven bone, with a small amount of lamellar bone also noted (Figure 6b). Remodelling of the original buccal and lingual crests was evident by the presence of resorptive defects and osteoclasts, positioned adjacent to newly formed osteoid, and more mineralised juvenile woven bone (Figure 6c, 6d). The original alveolar bone was separated from newly deposited bone by cement lines (Figure 6e). The central areas of the socket were characterised by a dense trabecular network of newly formed bone (Figure 6f). Smaller amounts of lamellar bone were seen in the central aspects of the socket. The intervening spaces surrounding the trabecular network were made of up connective tissue and bone marrow, with some vascular structures also noted.

Grafted Sockets

Similar to the non-grafted sites, the sockets were found to be covered by a mucosal epithelium with an underlying coarse-fibred connective tissue. In some instances, unattached DBBMC particles were found embedded in the peripheral aspects of the connective tissue situated over the socket entrance (Figure 7a). All grafted sockets exhibited a convex shaped dome of new bone at the socket opening, connecting the original buccal and lingual crests. This was comprised primarily of woven bone, small amounts of lamellar bone, and DBBMC particles. The peripheries of the sockets were outlined predominately with newly deposited bone. The central aspects of the socket were characterised by a greater presence of DBBMC particles surrounded by newly deposited bone, both woven and lamellar, organised in a dense trabecular network (Figure 7b). Osteoblasts were found to line the advancing wave of newly deposited bone, giving rise to a provisional matrix (osteoid), which was seen to mature into newly mineralised woven bone (Figure 7c) On the rare occasion, formation of isolated osteons was seen within a DBBMC particle (Figure 7d)

Histometric Measurements

Grafted sites displayed a mean vertical difference in height of the buccal crest compared to the palatal crest of 1.08 ± 0.47mm (Range: 0.11:1.57mm). In the non-grafted site, this difference amounted to a mean of 1.50 ± 0.76mm (Range 0.65: 2.85mm), which was not statistically significant (Table 1). In all sockets, the dome-shaped osseous cap extended over the socket opening, coronal to the height of the remodelling palatal crests. Grafted sites demonstrated a mean 1.18 ± 0.46mm (Range: 0.59:2.14mm) of bone formation coronal to the palatal crest, compared to 0.96 ± 0.72mm (Range: 0.2: 1.21mm) in the non-grafted sites, which was not found to be statistically significant (Table 1). The comparison of the ridge width at 1mm increments (Table 2) revealed that the mean width of the ridge was greater in the grafted sites. However, only at the level of the palatal crest was this difference found to be statistically significant (p-value: 0.02).

Morphometric Measurements

The data regarding morphometric analysis of each specimen was pooled and presented in Table 3. The non-grafted sites exhibited a greater amount of mineralised bone, and connective tissue and bone marrow compared to the grafted sockets. The greatest difference was seen in regards to amount of new bone formation (non-grafted: 46.49% vs. grafted: 37.51%). The non-grafted sockets also demonstrated a higher concentration of connective tissue and bone marrow (53.31% vs. 43.28%). In the grafted sockets, it was found that DBBMC particles made up 19.21% of the socket composition.

Radiographic Analysis

The distance between the buccal crest (cB) and palatal bone crest (cP) in a vertical plane were found not to be significantly different between groups. Grafted sites measured 2.93 ± 1.08mm (range: 1.58:4.6mm), compared to 3.38 ± 1.20 (range: 1.84:5.13mm) in the non-grafted sites. The distance between the buccal crest (cB) and crest of the regenerated bone (COR) in a horizontal plane was found to be significantly greater in non-grafted sockets (3.51 ± 1.01mm; range: 1.74: 3.95mm) compared to grafted sockets (1.52 $\pm$ 0.40mm; range: 1.03: 2.34mm; p-value: 0.0001) indicating that the regenerated ridge crest was significantly more buccally positioned for grafted sockets (Table 4, Figure 8).

Volumetric Analysis

No significant difference was seen in regards to volumetric changes of sockets over three months post-extraction. The volumetric analysis incorporated soft tissue volume as well as the osseous aspect (Figure 9). Grafted sites showed a decrease in volume, which amounted to a difference of 0.20 $\pm$ 0.05 cm^3 (Range: 0.12: 0.27cm^3) after three months. Similarly, non-grafted sites reported a volumetric change of 0.22 $\pm$ 0.04 cm^3 (Range: 0.18: 0.3cm^3) (Table 5).

Discussion

Ridge preservation has been studied extensively in the dental literature. However, the majority of pre-clinical controlled trials have often been conducted in the mandibular pre-molar dog model. The ability to translate these results to the anterior segment, where dimensional changes are more critical, is difficult due to the inherent anatomical differences. Furthermore, post-extraction dimensional changes in the maxillary anterior zone can considerably alter the treatment plan and ability to provide optimal outcomes.

The present study showed that maxillary second incisors left to heal naturally without any intervention healed with marked dimensional changes after three months. Vertical changes were most notable on the buccal crest, which was always located apical to the palatal crest. Significant horizontal differences in the ridge width were also noted with the largest degree of difference seen in the coronal third. Similar findings were reported in a clinical study in the maxilla, where marked changes in the ridge width and height were seen after three months (Johnson, 1967). Recent clinical findings focusing on extraction sockets in the anterior maxilla supported findings in this current study that dimensional changes were more marked in the coronal aspect with a gradual decrease in the level of resorption seen apically (Misawa et al., 2016). They reported a shift towards a more triangular shaped ridge after one year. This pattern of resorption after extraction is also supported in other clinical studies (Jung et al., 2013; Nevins et al., 2006) and can be explained anatomically by the bundle bone theory (Araujo & Lindhe, 2005).

The present study was able to demonstrate that the use of DBBMC following extraction in a maxillary lateral incisor in a canine model resulted in a significantly greater horizontal ridge width after 3 months of healing. The findings are consistent with the literature in other pre-clinical studies (Araujo et al., 2008; Araujo & Lindhe, 2009b; Fickl et al., 2008). Fickl et al., (2008) using the mandibular premolar socket in beagle dogs was able to show that regardless of grafting with DBBMC, vertical resorption was inevitable. Similarly, horizontal ridge width at 1mm below the lingual crest was found to be significantly reduced in both the grafted and non-grafted sites (4.4 ± 0.3mm vs. 3.7 ± 0.3mm), highlighting that while the use of DBBMC was able to minimise the degree of horizontal ridge changes, it could not prevent it completely.

The use of a graft material made little difference to the extent of vertical resorption of the buccal bone crest with no significant difference compared to naturally healing sockets. However, horizontal resorption in the grafted sites was reduced by more than half, compared to the naturally healing socket. Similar clinical studies have supported the findings of the present study (Araujo et al., 2015; Barone et al., 2008; Mardas et al., 2010). Araujo et al., (2015), using a CBCT method of analysis, followed the use of DBBMC in the anterior maxilla for four months. Sockets were compared by their overall cross-sectional area as measured radiographically. They found that the graft material had no effect on the eventual degree of vertical resorption. However, non-grafted sockets reported an overall reduction in cross-section of 25%, compared to only 3% in those sites treated with DBBMC. The finding of this study confirms that in the horizontal plane, DBBMC can counteract the extent of osseous resorption post-extraction.

Pre- and post-extraction surface scans were super-imposed and subtracted in order to determine the overall volumetric change over the course of three months after extraction. The software was able to distinguish the areas with the greatest volumetric change. The coronal third and mid-buccal aspects of the sockets were consistently highlighted as the aspects of the extraction socket with the greatest dimensional change, regardless of grafting or not. This pattern of resorption is supported both in clinical (Farmer & Darby, 2014; Misawa et al., 2016; Pietrokovski, 1975) and pre-clinical studies (Araujo & Lindhe, 2005; Blanco et al., 2011). Farmer and Darby (2015) reported, that six to eight weeks after extraction in the anterior maxilla, buccal bone loss followed an inverted 'V' pattern. Araujo et al., (2008), after 3 and 6 months respectively, showed that post-extraction changes in both grafted and non-grafted sockets were confined exclusively to the coronal third of the socket (Araujo et al., 2008; Araujo & Lindhe, 2009). This was in contrast to the results of the present study in which resorptive changes were not only observed in the coronal third, but also along the entire apico-coronal extension of the socket. This difference can be likely explained by the difference in experimental sites. Araujo et al., (2008) conducted their research in the mandibular pre-molar site of a canine as opposed to the maxillary lateral incisor in this study.

Unlike the histologic and radiographic analysis, which focused on hard tissue alterations only, the volumetric analysis incorporated soft tissue as well. No significant difference was seen between the overall volumetric change between the grafted and non-grafted sockets despite greater hard tissue dimensional changes seen in the non-grafted sockets. This is likely due to a spontaneous thickening of the soft tissue in the areas where resorption has occurred masking the underlying osseous resorption. This concept was supported by a recent study (Chappuis et al., 2015), which compared soft tissue dimensional changes in the maxillary anterior region between sites with thick and thin phenotypes. Sockets were evaluated after 8 weeks using a similar volumetric subtraction technique. They found that in sockets with a thinner buccal bone a seven-fold increase in soft tissue thickness was seen. The post-extraction resorptive changes, which were more pronounced in the thinner phenotype, led to a non-contained defect, which allowed the soft-tissue to proliferate into the space. In thicker phenotypes, the horizontal resorptive changes were not as pronounced leading to far less soft tissue thickening.

Morphometric analysis of the sockets was conducted with a focus on the degree of mineralised tissue, bone marrow and connective tissue. A marked difference was seen between the non-grafted and grafted sockets after a healing phase of three months. The grafted sites were seen to have a reduced mineralised content and less bone marrow and connective tissue formation when compared to the non-grafted sites. This difference in composition was accounted for by the presence of the DBBM particles. The lower percentage of both mineralised bone and the surrounding provisional matrix in grafted sockets can be indicative of the delayed healing commonly associated with DBBM particles, as has been suggested in previous literature (Araujo et al., 2008; Carmagnola et al., 2003; Jensen et al., 2006; Lindhe et al., 2014). The current study reported 19.21% of DBBMC particles remaining after three months, which was similar to clinical results reported by Carmagnola et al., (2003), who reported 21.1% of BioOss® particles remaining. This current study concurs with other clinical and preclinical trials confirming that DBBM particles remain relatively unaltered over time (Norton et al., 2003; Lindgren et al., 2012; Araujo et al., 2010).

Animals models have been extensively utilised in the dental literature. The 8th European workshop of Periodontology (Berglundh et al., 2012) confirmed that dogs were the most commonly used experimental animals. They offer the advantages of being anatomically similar to humans with similar healing processes. The faster healing times in comparison to humans allows for studies to be completed in a shorter time frame (Giannobile et al., 2011). While in implant and ridge preservation studies the mandibular premolar was seen as ideal due to the ability to extract it without damaging the socket walls. However, De Santis et al., (2011) successfully utilised the maxillary second incisors in Labradors to study immediate implants, which was more recently followed by Mellati, et al., (2015). Both these studies were able to highlight several advantages associated with the use of maxillary second incisors as an experimental model over the mandibular pre-molar. Firstly, extraction of the incisors was more straightforward, with no sectioning required, and the maxilla was associated with a lower bone density compared to the mandible. Hence extraction could be carried out in a minimally traumatic manner. Secondly, Mellati et al., (2015) reported that the dimensions of the maxillary second incisor extraction socket were very similar to that seen in humans, with a mean width of 6mm and a mean depth of 11mm. Considering that implant placement and dimensional changes following extraction have critical importance in the anterior maxilla, the use of the maxillary second incisor provides a model with greater clinical relevance.

A limitation of this current study was the three-month healing period. It is possible that further dimensional changes may have occurred with an extended observation time. However, previous literature in both humans (Schropp et al., 2003) and canines (Araujo et al., 2005) has shown that the vast majority of changes occur in the first three months.

The clinical implications of the findings have a direct bearing on considerations of tooth replacement options within the maxillary anterior zone. Regardless of the use of any biomaterial, dimensional changes in the vertical plane remain inevitable. Conversely, ridge preservation using DBBMC xenograft seems to effectively minimise horizontal changes in the coronal aspect of the socket. Preserving osseous dimensions can facilitate the placement of appropriately sized implant in a restorative-driven optimal position. Further, by preventing these dimensional changes post-extraction, the need for significant and more complicated grafting procedures (e.g. block grafts) can be minimised. Additionally, without the use of any ridge preservation procedure, post-extraction dimensional changes could in some cases be so significant as to preclude the option of implant therapy.

In conclusion, grafting of extraction sockets with DBBMC significantly reduced the amount of horizontal resorption at the most coronal crest, but did not prevent vertical resorption. Volumetric analysis revealed no significant difference between grafted and non-grafted sockets, suggesting possible compensatory soft tissue thickening post-extraction to counteract osseous resorption. Further, this study highlighted that the canine second maxillary incisor site is a suitable model for the study of post-extraction healing.

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Conflicts of Interest:
The authors declare there are no conflicts of interest

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Table 1: Histometric Analysis – Vertical Dimensions. Outlines mean (SD) difference in buccal bone height (mm) and the mean (SD) extent of bone remodelling coronal to the palatal crest.

	Non-grafted		Grafted		
	Mean (SD)	Range (mm)	Mean (SD)	Range (mm)	
Histometric Analysis					p-value

Difference in height of the buccal crest compared to palatal crest (mm)	1.50 (0.76)	0.65-2.85	1.08 (0.47)	0.11-1.57	0.098
Bone remodelling coronal to the palatal crest (mm)	0.96 (0.72)	0.2-2.4	1.18 (0.46)	0.59-2.14	0.251

Table 2: Histometric Analysis – Horizontal Dimensions. Outlines the ridge width (RW) measured at the palatal crest, and at 1mm increments apically.

	Non-grafted		Grafted		
	Mean (SD)	Range (mm)	Mean (SD)	Range (mm)	
Histometric Analysis (mm)					
Ridge Width (RW) at the palatal crest	4.20 (1.46)	1.35-6.40	5.66 (0.88)	4.16-6.74	p =0.02
RW 1mm below the palatal crest	7.08 (1.04)	5.45-8.90	7.27 (0.64)	6.39-8.00	0.64
RW 2mm below the palatal crest	7.98 (0.65)	7.00-9.00	8.33 (0.71)	7.38-9.29	0.30
RW 3mm below the palatal crest	8.48 (0.60)	7.75-9.29	8.57(0.97)	7.05-10.14	0.82
RW 4mm below the palatal crest	8.79 (0.53)	8.00-9.40	8.99 (0.85)	8.00-10.43	0.57
RW 5mm below the palatal crest	8.89 (0.73)	7.85-10.15	9.26 (0.75)	8.27-10.36	0.31

Table 3: Mean composition of newly formed tissue within the extraction socket after three months of healing

	Non-Grafted		Grafted	
	Mean (%)	Range (%)	Mean (%)	Range (%)
Mean Composition of Extraction Sockets at 3 months				

	Non-grafted		Grafted	
New Bone	46.49	37.4-60.0	37.51	25.4-46.6
Connective tissue & Bone Marrow	53.31	40.0-62.6	43.28	35.2-48.6
DBBMC			19.21	10.7-27.6

Table 4: Comparison of the mean distance from the buccal crest to the buccal aspect of the regenerated bone crest, and the distance between the buccal crest and palatal crest, as analysed by CBCT radiographs

	Non-grafted		Grafted		
	Mean (SD)	Range (mm)	Mean (SD)	Range (mm)	
cB – cP (mm)	3.38 (1.20)	1.84-5.13	2.93 (1.08)	1.58-4.6	P = 0.076
cB – COR (mm)	3.51 (1.01)	1.74-4.6	1.52 (0.40)	1.03-2.34	P = 0.0001

cB - cP is the distance between the buccal crest (cB) and palatal crest (cP) in a vertical plane

cB-COR is the distance from the buccal crest (cB) to the buccal aspect of the regenerated bone crest (COR) in a horizontal plane

Table 5: Change in Volume between grafted and non-grafted sockets, analysed by surface scan subtraction

	Mean (SD)	Range (mm)	Mean (SD)	Range (mm)	
Volumetric change by surface scan subtraction					
Volumetric reduction in ridge dimension on the buccal aspect (cm^3)	0.22 (0.04)	0.18-0.30	0.20 (0.05)	0.12-0.27	p = 0.084

Figure Legends

Figure 1. Clinical Photographs of the surgical procedure.

Figure 2: Histological Images. A: non-grafted socket, B: socket grafted with DBBMC. Newly formed bone is stained dark magenta, older bone and DBBMC light magenta and soft tissue blue (undecalcified ground sections; stain azure II / pararosaniline, magnification x 50)

Figure 3: Histomorphometric Analysis: A: non –grafted socket, B: Socket grafted with DBBMC. Histometric Landmarks: A: Apex of Socket, cP: Palatal Crest, cB: Buccal Crest, COR: most coronal aspect of bone/DBBMC. Labelling for histomorphometric purposes: Green: DBBMC granules, Red: Newly formed bone, Yellow: denotes region of interest

Figure 4: Diagrammatic representation of histologic analysis. a: Vertical morphometric measurements. b: Horizontal morphometric measurements. cB buccal crest, cP palatal crest, COR most coronal part of the bone, A socket apex.

Figure 5: Diagrammatic representation of the radiographic analysis. The plane of the cross-section at the mesio-distal midpoint of the ridge is coincident with the long axis of the socket and orthogonal to the line of the arch. The distances cB to cP, and cB to COR were recorded. A - long axis of the socket, cB - buccal crest, cP - palatal crest, COR - crest of the regenerated ridge.

Figure 6: Detailed histology of non-grafted sockets. A: Epithelium and connective tissue overlying socket opening, mag. X 50. B: NB= new bone, NB formation at socket

opening, mag. X 50. C: new bone forming adjacent to original crestal bone (OB), with resorptive pattern (R) noted, mag. X 50. D: closer magnification of resorption area in image 4c. Shows presence of osteoclasts (OCL) resorbing original bone, mag. x 200. E: seams of osteoblasts (OB) forming osteoid (O) which is mineralized into new bone (NB), with osteocytes (OC) embedded within. Cement line (CE) separates new bone from original alveolar bone (AB). mag.x 630. F. Dense trabecular network of newly formed bone, mag. x 50.

Figure 7: Detailed Histology of sockets grafted with BioOss® Collagen. A: epithelium and uninflamed connective tissue, embedded BioOss® particle (BO). Mag. X 50. B: Bio-Oss® particles embedded in a dense trabecular network of newly formed bone (NB), OT: Osteon. Mag. x 50. C: seams of osteoblasts (OB) add osteoid (O) which mineralizes into new bone (NB) adjacent to the Bio-Oss® particles (BO). Mag. x 400. D: mature lamellar bone (LB) with numerous osteons (OT) adjacent to newly deposited immature woven bone (WB). Image shows a rare instance of the formation of osteons inside a Bio-Oss granule (BO). Mag. x 200.

Figure 8: CBCT images of both Dog 7. A: image of non-grafted socket, B: image of grafted socket

Figure 9: Volumetric analysis of grafted and non-grafted sockets via surface scan subtraction. Below: original surface scans, pre- (A) and post- (B) extraction. (C): Output after subtraction.

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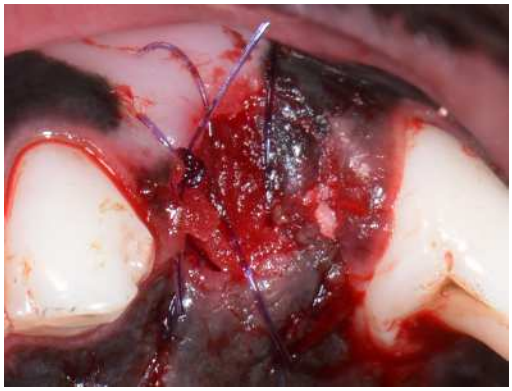

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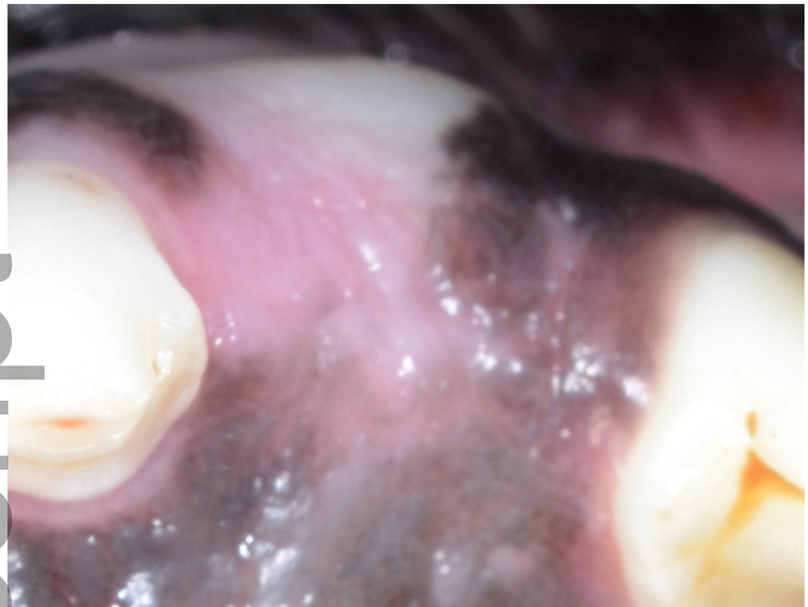

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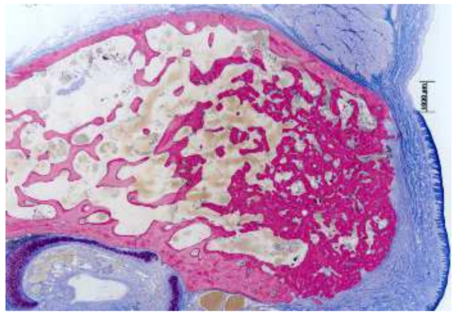

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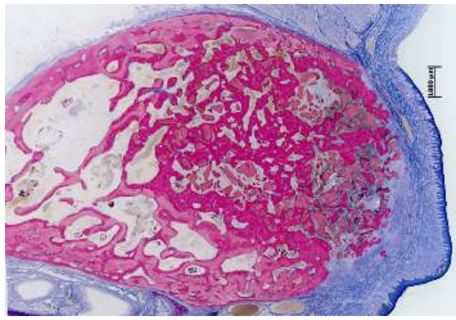

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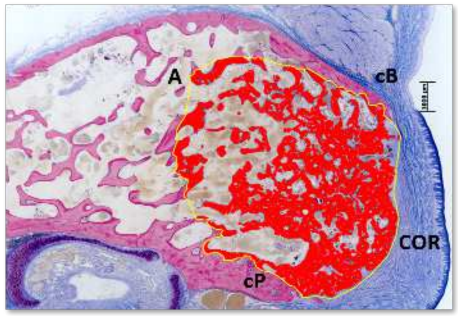

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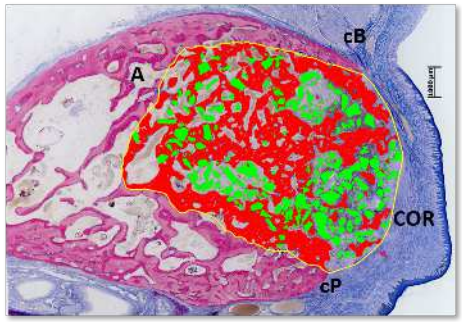

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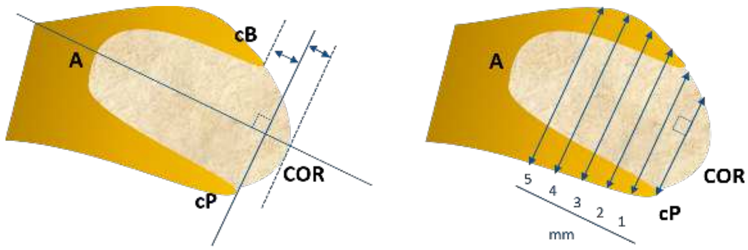

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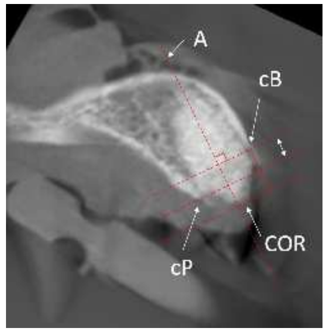

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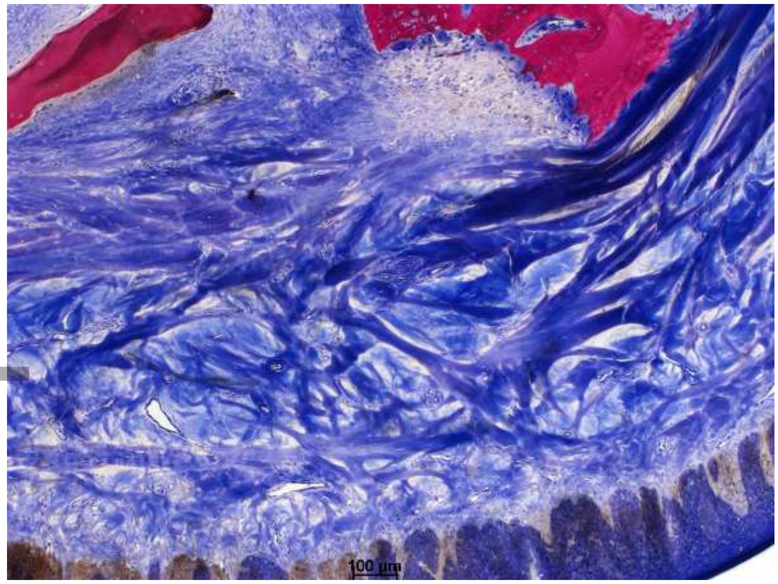

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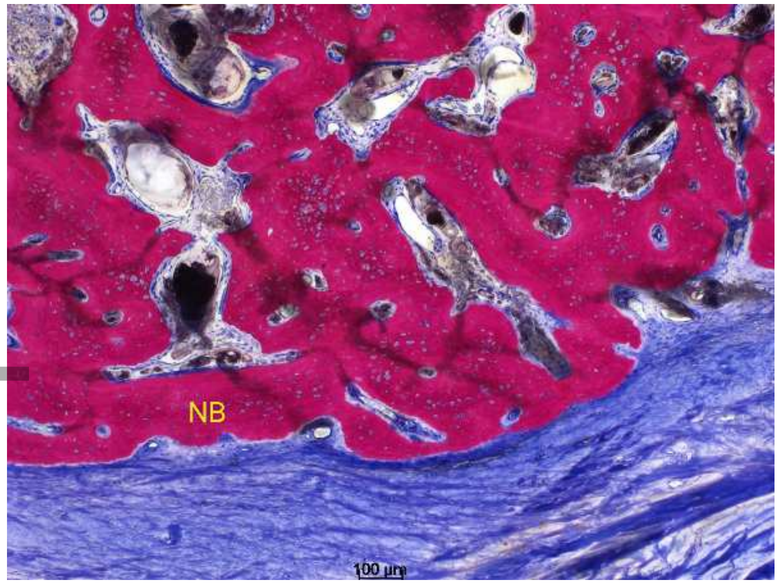

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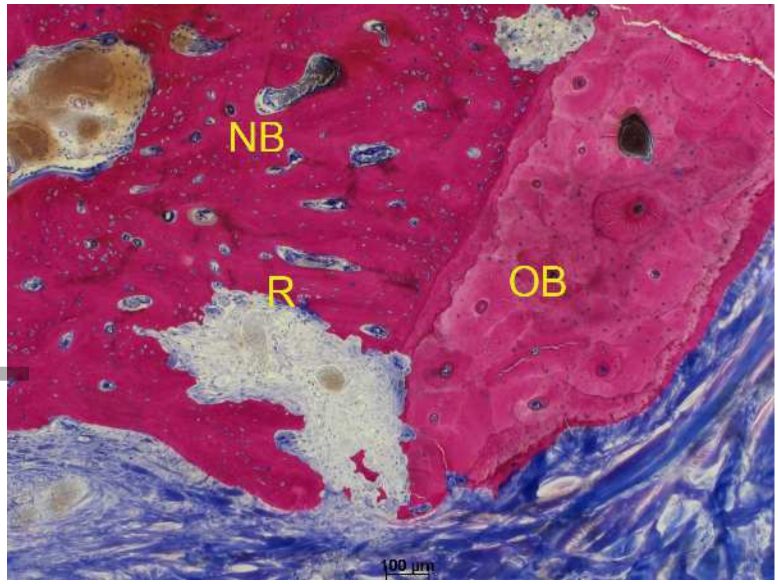

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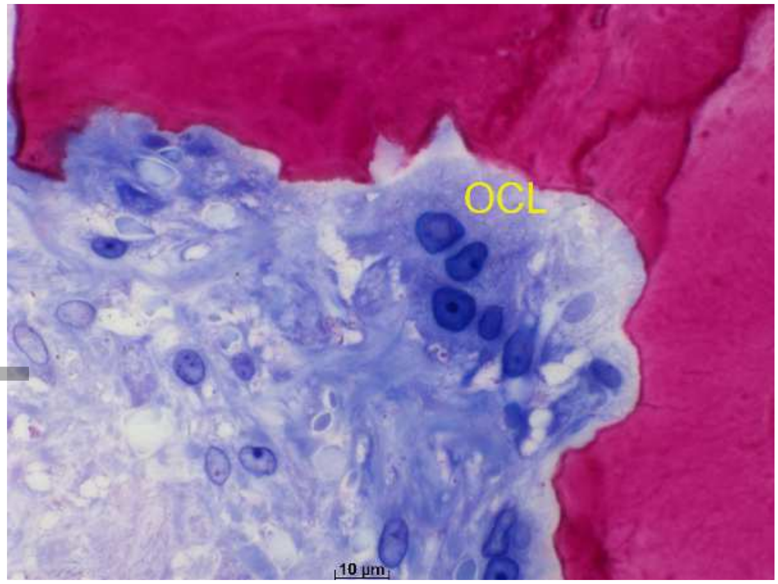

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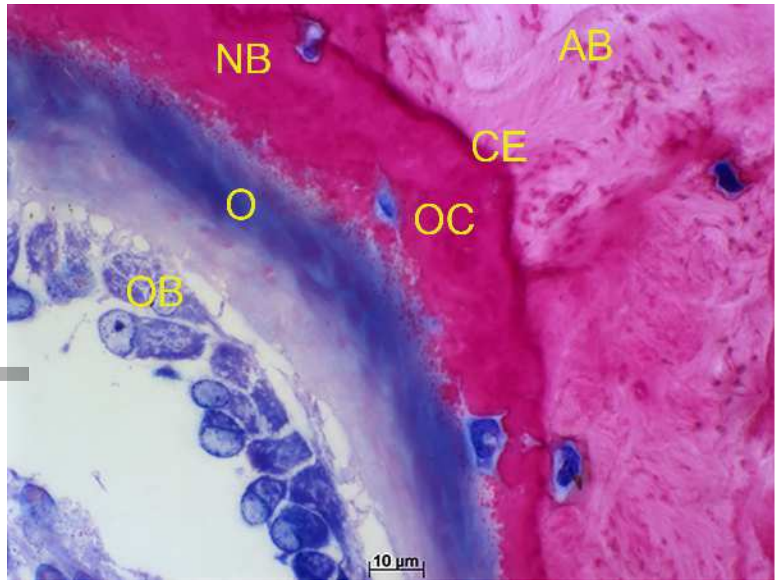

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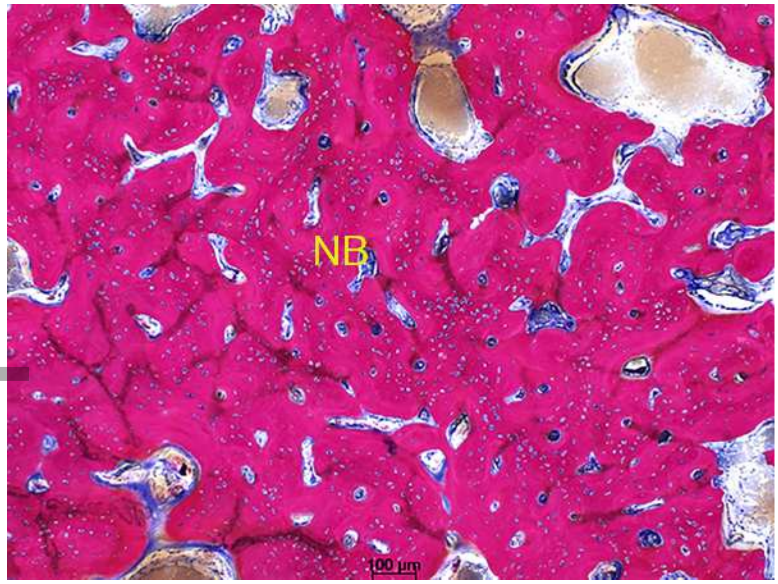

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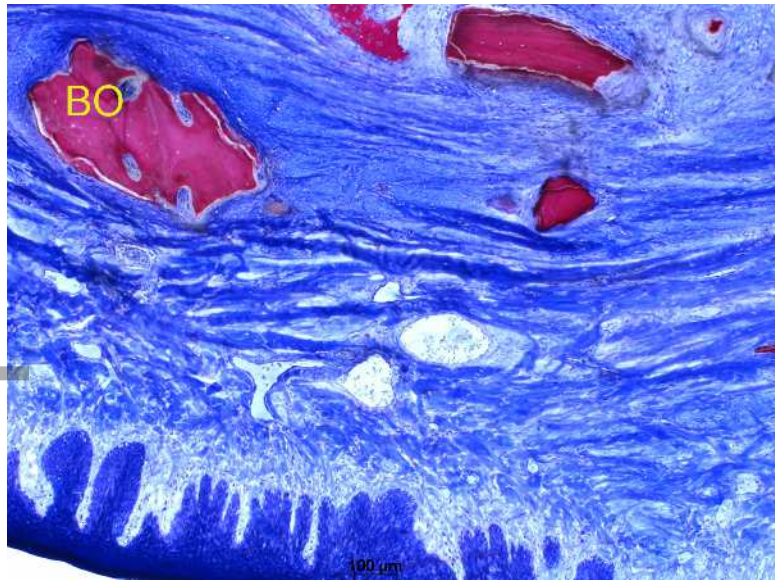

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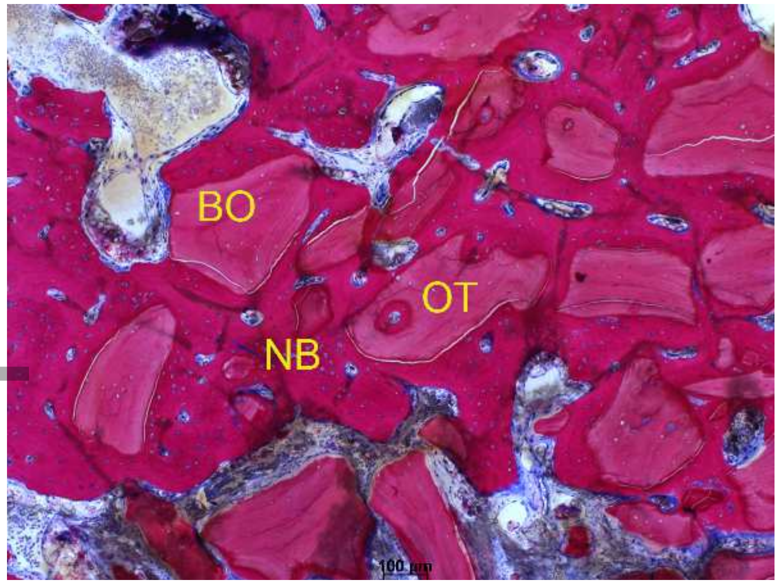

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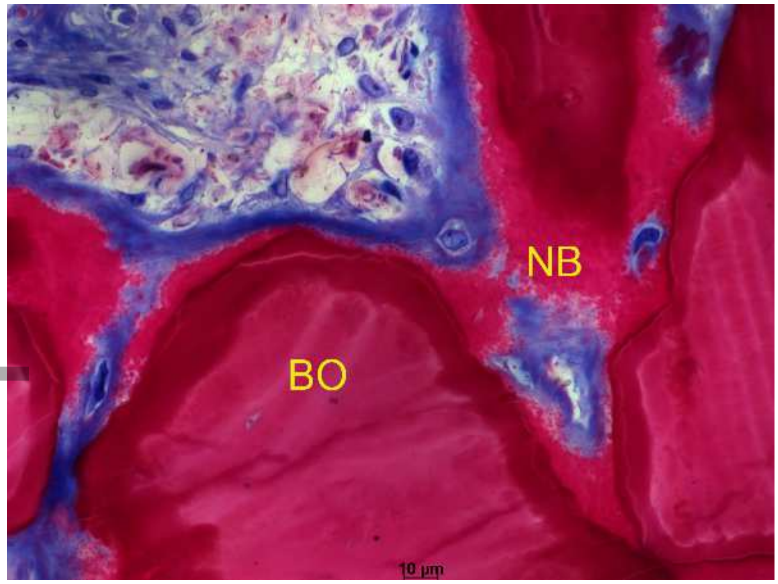

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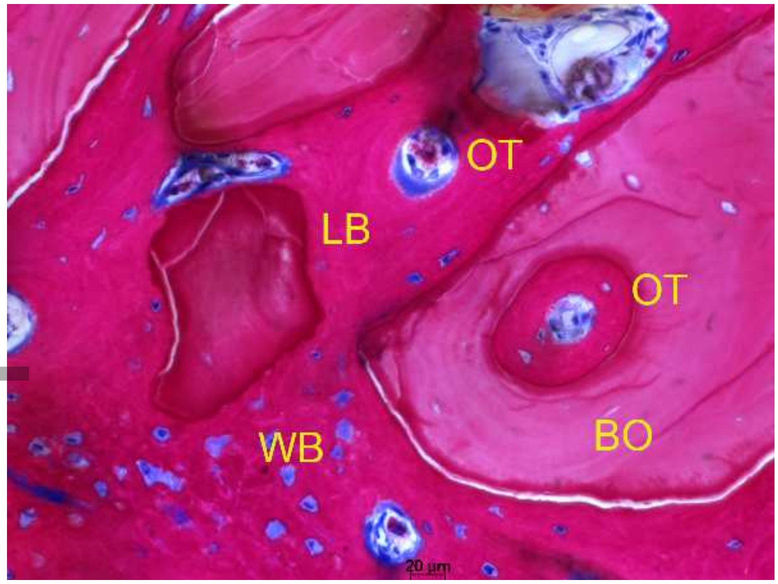

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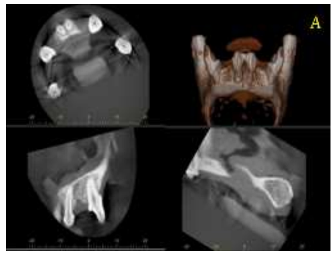

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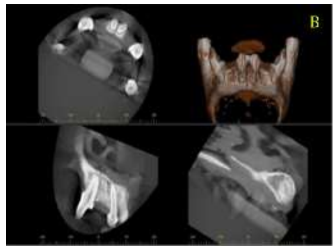

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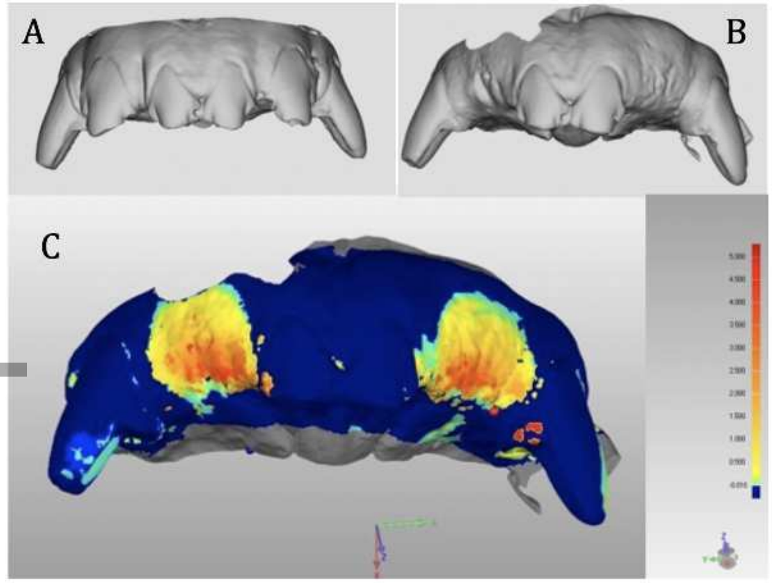

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