Early Implant Placement In Ridge Preserved Extraction Sockets: A pre-clinical in vivo study

Running title: Early implant placement in ridge preserved extraction sockets

Authors: Gabriel Rodriguez-Ortiz[1], Stephen Chen[1], Helen Davies[2], Wayne Fitzgerald[2], Ivan Darby[1*]

[1]Periodontics, Melbourne Dental School, University of Melbourne, Parkville, Victoria, Australia

[2]Faculty of Veterinary Science, University of Melbourne, Parkville, Victoria, Australia

*** Corresponding author:**

Ivan Darby, Head of Periodontics

Melbourne Dental School, University of Melbourne, 720 Swanston Street, Carlton, Victoria 3053, Australia

Tel.: +61 3 9341 1471

Fax: +61 3 9341 1599

E-mail: idarby@unimelb.edu.au

Acknowledgements: Implants were kindly provided by Straumann Dental Implant System (Straumann, Basel, Switzerland) and the bone graft materials were kindly provided by Geistlich Pharma AG (Wolhusen, Switzerland). The authors would like to thank staff at the Melbourne University Veterinary School for their assistance and exceptional care of the animals. The authors would also like to thank Annette Lindner at Cell Tissue Analysis, University of Freiburg for her expertise in preparation and processing of the histological specimens.

Author contributions: ID and SC conceived the design of the study. ID, SC and GR undertook the treatment procedures, HD and WF were responsible for animal care, GR, ID and SC undertook the histological analysis and data analysis, preparation and writing of the manuscript.

Conflicts of interests: The authors report no conflicts of interest.

Abstract

Objectives: The aim was to analyze the outcomes of early implant placement after 6 and 12 weeks of healing in ridge reserved sites in a canine model.

Materials and methods: Implants were placed in second maxillary incisors sites in 9 dogs 6 weeks after grafting of the sockets with 90% deproteinized bovine bone mineral in 10% collagen matrix (DBBMC) and closure with resorbable type I/III porcine collagen matrix (PCM). The implants were randomly assigned to 6 (T6) and 12 (T12) weeks of healing.

Results: The percentage of bone to implant contact (%BIC), old bone, new bone and residual DBBMC was similar between T6 and T12. In relation to the implant shoulder (IS), the original bone crest (IS-ROB) was more apical on the buccal than the palatal side. The regenerated bone crest (IS-C) and IS-ROB were similar between groups. However, the distance from IS to first bone to implant contact (IS-fBIC) was significantly less in T12 compared to T6 ($p=0.022$; Wilcoxon signed rank test). The bucco-palatal ridge dimensions between T6 and T12 were similar.

Conclusions:

This study confirms that implants can successfully be placed early in ridge preserved maxillary second incisor sites and are osseointegrated by 6 weeks. There was significantly lower IS-fBIC values at 12 weeks than at 6 weeks on the buccal aspect. The original buccal bone crest underwent greater corono-apical resorption than the palatal crest. The %BIC, relative proportions of mineralized tissues and dimensions of the alveolar ridge demonstrated stability between 6 and 12 weeks of healing.

Key words: Ridge preservation, dental implant, extraction, early implant placement, type 2 placement, buccal bone

Abstract word count 249

Introduction

After tooth extraction, healing of the socket takes place with gradual regeneration of bone within the socket. Concurrently there is loss of apico-coronal height and oro-buccal width of the alveolar ridge due to physiological resorption of the external socket walls, predominantly on the buccal side of the socket (M. G. Araujo & Lindhe, 2005; Cardaropoli, Araujo, & Lindhe, 2003). Bone resorption leads to a reduction in the volume of the ridge (Cardaropoli, Lekholm, & Wennstrom, 2006; Schropp, Wenzel, Kostopolous, & Karring, 2003). When dental implant therapy is proposed for the replacement of extracted teeth, the post-extraction dimensional changes can hinder the ideal position of the implant for the subsequent implant prosthesis (Farmer & Darby, 2014). As a consequence, there has been a growing interest in alveolar ridge preservation (ARP) techniques to minimize these dimensional changes. ARP is defined as a grafting procedure that takes place at the time of extraction, or soon thereafter, with the aim of minimizing resorption of the alveolar ridge and maximizing bone formation within the socket (Darby, Chen, & De Poi, 2008). Dental implants have been placed into ridge preserved sites with successful short-term (Apostolopoulos & Darby, 2017; Darby, Chen, & Buser, 2009; Patel, Mardas, & Donos, 2013; Vignoletti et al., 2012) and long-term survival rates (Roccuzzo, Gaudioso, Bunino, & Dalmasso, 2014). ARP is successful at maintaining ridge dimensions for subsequent implant placement, but the technique does not prevent some loss of bucco-lingual ridge width (Bassir, Alhareky, Wangsrimongkol, Jia, & Karimbux, 2018; Darby et al., 2009; Vignoletti et al., 2012).

The majority of clinical reports of ARP have recommended post-grafting healing

periods of 3 to 6 months (Mardas, Trullenque-Eriksson, MacBeth, Petrie, & Donos, 2015). However, earlier placement of implants within ridge preserved sockets has previously been described. A clinical study in which biopsies were obtained at the time of implant placement revealed marked de novo bone formation after a healing period of 6 weeks following tooth extraction and ARP with deproteinised bovine bone mineral and 10% collagen (DBBMC) (Heberer, Al-Chawaf, Hildebrand, Nelson, & Nelson, 2008). A mean overall new bone formation of 28% (range 9–57%) was recorded. In a recent case series study of 10 consecutively enrolled patients, implants were placed in single tooth maxillary central incisor sites 8 weeks after extraction and grafting of the sockets with DBBMC (Chen & Darby, 2020). The authors reported a 100% survival rate after 1 year.

Pre-clinical evidence to support early implant placement after ARP is limited. In a recent study of hemisected mandibular premolar sockets in a canine model, implants were placed 4 weeks after ARP with DBBMC (Thoma et al., 2017). Histomorphometric analysis was performed after healing periods of 4 and 12 weeks. The authors found that implants placed at this early time frame successfully osseointegrated, with higher bone to implant contact at 12 weeks than 4 weeks. Furthermore, the osseointegration was not affected by the presence of the DBBMC biomaterial. Recently, a maxillary second incisor model in the canine has been used for ARP and guided bone regeneration studies (De Santis et al., 2011; Janner et al., 2017; Mellati, Chen, Davies, Fitzgerald, & Darby, 2015). This model has several advantages, including the provision of a single root socket similar in dimension to a human maxillary lateral incisor, and avoidance of additional endodontic/hemisection procedures (De Santis et al., 2011). In addition, maxillary and mandibular bone have different tissue composition and embryonic origin (Lindhe et al., 2013). The maxillary second incisor model may therefore have translational advantages when applying preclinical data to peri-implant bone healing in the anterior maxilla in a clinical setting. To date, there is lack of data on bone healing and osteointegration of implants placed in ridge preserved maxillary second incisor sockets in a canine model.

The aim of this study was therefore to histomorphometrically evaluate bone regeneration and healing outcomes 6 and 12 weeks following early implant placement

in ridge preserved maxillary second incisor sites.

Material and methods

The study protocol was approved by the Animal Ethics Committee of the University of Melbourne (Ethics ID no. 1513668.1) and the study report has been written according to the ARRIVE guidelines (Kilkenny et al. 2010). The study was undertaken between February 2016 and September 2017. Nine female greyhound dogs were used, each weighing approximately 30 kg and aged 1–2 years. All procedures were performed under general anaesthesia combined with bilateral infra-orbital nerve block anaesthesia.

At baseline, one maxillary second incisor was randomly selected by coin toss. This tooth was extracted and grafted with DBBMC. Six weeks later, the grafted socket had an implant placed. At the same time, the contralateral maxillary second incisor was extracted and the socket grafted. A further 6 weeks later an implant was placed into the second grafted site. All implants were therefore placed 6 weeks after ARP with DBBMC. At 18 weeks from baseline, the animals were sacrificed. This study design provided 2 groups with post-implant placement healing periods of 6 and 12 weeks.

The surgical procedures are described as follows. At the time of extraction, an intrasulcular incision was performed around the maxillary second incisor. Without flap elevation, extraction was performed carefully using luxators and fine tipped extraction forceps. Care was taken not to damage the socket walls, particularly the buccal wall. The extraction socket was irrigated with saline to remove all debris and soft tissue remnants. The integrity of the socket was checked and the presence of dehiscence and/or fenestration defects were recorded. The apico-coronal, mesio-distal and bucco-palatal dimensions of the socket were measured using a Michigan O probe with Williams markings (Hu-Friedy, Chicago, IL, USA) and recorded to the nearest half millimeter.

A commercial preparation of 90% deproteinized bovine bone mineral in a 10% collagen matrix (DBBMC) (100 mg Bio-Oss Collagen®, Geistlich Pharma, Wolhusen, Switzerland) was hydrated in saline solution and placed into each extraction socket up to the mid-buccal bone crest. The graft was covered by a resorbable type I/III porcine collagen matrix (CM) (8mm Mucograft Seal®, Geistlich Pharma, Wolhusen,

Switzerland) which was secured using 4-0 synthetic bio-resorbable sutures (Monosyn®; B. Braun, Melsungen, Germany).

At the scheduled time, buccal and palatal mucoperiosteal flaps were reflected without vertical releasing incisions to expose the alveolar ridge. Implant placement was completed in accordance with the manufacturer's recommendations (Straumann Dental Implant System®, Institut Straumann, Basel, Switzerland). In each site, an 8mm long implant with a 3.3 mm neck diameter and apical taper (Straumann Roxolid® narrow CrossFit® NC bone level tapered implant with an SLA® surface) was installed with the shoulder of the implant placed level with the buccal bone crest. The implant stability quotient (ISQ) was recorded for each implant after placement with a resonance frequency device (Osstell Mentor®; Integration Diagnostics AB, Göteborg, Sweden).

A closure screw was connected to the implant and primary closure of the site was achieved using 4-0 synthetic bio-resorbable sutures (Monosyn®, B. Braun, Germany) (figure 1).

Post-surgery wounds were inspected daily over the first two weeks and a plaque control regimen including once daily application of chlorhexidine formulation (Hexarinse®, Virbac, Regents Park, NSW, Australia) was used. Immediately following the surgical procedures, the dogs were individually housed at the veterinary clinic with close monitoring by a registered vet. Thereafter, they were returned to their shared purpose-designed enclosures with associated outdoor runs. After the initial post-operative healing phase (14 days), mechanical plaque removal was commenced and performed every day for the remainder of the experimental period. The teeth were cleaned for two minutes using an individual soft toothbrush soaked in chlorhexidine and primed with chicken flavoured toothpaste (Dentipet, Ceva Animal Health Care Australia) at each brushing session. Soft food was prescribed in the first week followed by a normal diet thereafter.

At the end of the study (18 weeks), the dogs were sacrificed using an intravenous injection of pentobarbitone (Lethabarb®, Virbac, Regents Park, NSW, Australia) into the cephalic vein. Block specimens were prepared for histologic analyses of the tissues.

Histological preparation

The blocks were immediately placed in 10% buffered formalin (Orion, Balcatta, WA, Australia). The fixed specimens were dehydrated in a series of graded ethanol solutions with increasing concentration (70%, 80%, 90% and 100% ethanol), for a day at each concentration. Defatting was carried out by soaking the specimens in xylene (Merck, Darmstadt, Germany) for 24 hours. Specimens were infiltrated and embedded in resin (Technovit 9100; Heraeus Kulzer, Wehrheim, Germany) and polymerized according to the manufacturer's instructions. After polymerization, radiographs were taken to determine the position of each implant to ensure sectioning was parallel to the long axis of the implant. Samples were then cut in a para-sagittal plane coincident with the long axis of the implant using a low-speed rotary diamond saw (Microslice®; Metals Research, Cambridge, UK). Two central sections of 500 µm thickness were obtained from each implant. Sections were mounted, ground and polished to a final thickness of approximately 60 µm. Subsequently, sections were stained with azure II and pararosaniline (Merck, Darmstadt, Germany).

Histomorphometric measurements

Sections were viewed with an Axio Imager M1 microscope equipped with a digital Axio-Cam HRc camera (Carl Zeiss, Göttingen, Germany). The following landmarks were identified on each slide (Fig. 2a-d):

- IS: implant shoulder
- C: crest of the regenerated buccal bone
- fBIC: the first bone-to-implant contact
- ROB: the most coronal remnant of the original bone crest

Subsequently, the following measurements were performed:

IS-fBIC: distance from the implant shoulder to the most coronal bone-to-implant contact

IS-C : the apico-coronal distance from the implant shoulder to the regenerated crestal bone

IS-ROB: the apico-coronal distance from the implant shoulder to the most coronal remnant of the original bone crest

To evaluate the percentage of bone-to-implant contact (%BIC), four areas of interest were determined around each implant; corono-buccal, apico-buccal, corono-palatal and apico-palatal. %BIC at each area of interest was the linear percentage of bone in direct contact with surface of the implant. The amount of residual graft material and new bone formation was determined for each area of interest using the analysis FIVE® software (Soft Imaging System, Münster, Germany). The bucco-palatal thickness of the alveolar ridge was measured 0 to 4 mm from the implant shoulder at 1 mm increments. Measurements were taken twice and averaged. A sample of measurements were repeated a week later with an intra-examiner correlation of 0.99 (SE 10.3 µm and SD of 38.7 µm for the mean differences).

Data Analysis

Based on %BIC results in a similar model (Mellati et al. 2015) and assuming alpha value of 0.05 and a power of 0.80, a sample size calculation showed that a minimum of 8 animals were required. Therefore 9 animals were included in case of implant failure or histological processing errors. As there were two sections per implant, the average value of the two measurements for each parameter was calculated to represent the implant as the experimental unit. The Wilcoxon signed rank test was used to analyze differences between the treatment groups for each parameter (Minitab 16; Minitab Inc., State College, PA, USA) and the level of significance was set at 0.05.

Results

Following extractions, all sockets were intact without fenestration or dehiscence defects present. There were no statistically significant differences in the apico-coronal, bucco-palatal and mesio-distal dimensions of the sockets at the time of extraction at either group (mean dimensions for the groups combined: apicocoronal 11.3 ± 0.8 mm; bucco-palatal 5.7 ± 0.8 mm; mesio-distal 4.9 ± 0.5 mm). Following grafting, all sites healed uneventfully with complete mucosal coverage of the sockets.

At the time of implant placement, the median ISQ values for the T6 and T12 groups were 65 and 70 respectively, with no significant difference between groups (median 65 and mean 65.6 ± 6.2 for both groups combined; range 55 – 77).

After 6 and 12 weeks of healing, all implant sites had healed without complications. There was complete mucosal coverage at all sites. Histologically, both implants (T6 and T12) in one animal and one implant (T6) in a second were encapsulated with fibrous connective tissue indicating a failure of osseointegration. The remaining paired specimens from seven animals were therefore included for further analysis.

Histological outcomes

The morphological and histological appearance of the alveolar bone was similar in specimens in the T6 and T12 groups. All implants were located completely within the alveolar bone and were relatively centrally positioned. Cortical and cancellous compartments could be identified. The palatal and buccal cortices consisted of mature lamellar bone with scattered Haversian systems throughout. The original buccal plate was reduced in corono-apical height relative to the palatal bone crest and was replaced by DBBMC surrounded by new bone. The cancellous compartment was present between the cortices and the implants, and consisted of mature lamellar bone with some areas undergoing bone turnover. DBBMC particles were found predominantly on the buccal aspect of the implants and were contained within the cancellous compartment. The DBBMC particles were either completely surrounded by lamellar bone or a combination of lamellar bone and connective tissue. In the coronal buccal region, DBBMC particles tended to be surrounded entirely by connective tissue. In some specimens, DBBMC particles were observed within the buccal connective tissue at the buccal crestal region. DBMMC particles within bone were free of osteoclastic activity. However, DBBMC particles present in the buccal connective tissue showed evidence of osteoclastic activity and resorption. The connective tissue in contact with graft particles was free of inflammation. There was clear evidence of osseointegration with bone in direct contact with the buccal and palatal surfaces of the implants. A few DBBMC particles were also observed to be in direct contact with the implant surface.

Histomorphometric results

The percentage by area of new bone, old bone and DBBMC particles, as well as % BIC in the 4 regions of interest are tabulated in table 1. For %BIC, greater variability was observed at the apico-buccal, corono-buccal and corono-palatal regions in the T6 group compared to the T12 group (figure 3). There was a trend for greater %BIC at the apico-buccal region in T12 compared to T6; however, the differences were not statistically significant. Similarly, there were no statistically significant differences between groups in the other 3 regions of interest.

For percentage new bone, there was a trend for greater percentage new bone at the apico-buccal, apico-palatal and corono-palatal regions in the T12 group compared to the T6 group (figure 4). However, the differences between groups were not statistically significant in the 4 regions of interest. There were also no differences between groups for percentage old bone, although a trend for more old bone was observed for the apico-palatal and corono-palatal regions (figure 5). A clear trend for greater %DBBMC was observed in the corono-buccal region compared to the other 3 regions of interest (figure 6). There were no significant between-group differences in the 4 regions of interest.

The results for IS-fBIC, IS-C and IS-ROB at the buccal and palatal aspects of both groups are provided in table 2. For IS-fBIC, the distance was less in the T12 group compared to the T6 group on the buccal aspect (figure 7). This difference was statistically significant ($p=0.022$; Wilcoxon signed rank test), suggesting that the reduction in IS-fBIC may be time dependent. On the palatal aspect, there was a larger variation in fBIC in T12 compared to T6. The median was less in the T12 group than in the T6 group; however, the difference between groups was not statistically significant.

Box plots of IS-C and IS-ROB at the buccal and palatal aspects of implants in each group are shown in figure 8. Overall, the results were variable. The median position of the buccal bone crest was coronal to IS, whereas the median position of the palatal bone crest was slightly apical to IS. There were no significant differences between the T6 and T12 groups at the buccal and palatal aspects. Within group comparison of IS-C indicated that the difference between the buccal and palatal at T6 was borderline significant ($p=0.052$; Wilcoxon rank sum test). No significant differences were noted between IS-C at the buccal and palatal aspects in the T12 group. There was a trend

for greater IS-ROB at the buccal compared to the palatal side, indicating more corono-apical resorption of the original buccal bone than the original palatal bone. There were no significant between-group differences for IS-ROB at the buccal and palatal aspects. However, there was a significant within-group difference between the buccal and palatal IS-ROB in the T6 group ($p = 0.022$; Wilcoxon signed rank test).

At 1 mm increments from 0 to 4 mm apical of IS, the bucco-palatal ridge width tended to increase (figure 9). There were no statistically significant differences in bucco-palatal ridge width between T6 and T12 at all increments.

Discussion

The results of this experimental study in a canine model indicated that implants placed early in ridge preserved maxillary second incisor sites (i) successfully integrated in 15 of 18 implants placed, (ii) had lower IS-fBIC values at 12 weeks than at 6 weeks, (iii) maintained overall %BIC and relative proportions of mineralised tissues between 6 and 12 weeks, and (iv) were associated with dimensional stability of the alveolar ridge between 6 and 12 weeks of healing.

These findings are largely in agreement with Thoma and co-workers who used a mandibular premolar canine model (Thoma et al., 2017). The authors reported lower median buccal fBIC at 12 weeks than at 4 weeks, which is consistent with the findings of the present study. In contrast to Thoma et al. in which the implants were placed relatively centrally in the grafted mandibular socket, implants in the present study were placed more palatally in the maxillary sites, a position consistent with the correct 3-dimensional positioning of implants in the anterior maxilla. This resulted in proportionally more DBBMC graft material being present in the corono-buccal region of the implants. It is interesting to speculate on the influence of the graft material on healing. In a preclinical study that analysed DBBMC graft into sockets of hemisected premolars in a canine model, the marginal and central regions of the socket contained graft particles surrounded by non-mineralized connective tissue with limited woven bone formation after 2 weeks of healing (M. Araujo, Linder, & Lindhe, 2009). Bone formation was observed to be delayed in comparison to non-grafted sockets that were

allowed to heal spontaneously. A follow-up study by the same research group concluded that the delay in bone healing was apparent even after 3 months of healing (M. Araujo, Linder, Wennstrom, & Lindhe, 2008). This delay in bone healing in the presence of DBBMC may explain the observation in the present study that a further 6 weeks of healing between T6 and T12 resulted in a smaller IS-fBIC. It would seem, therefore, that IS-fBIC may be time dependent and directional (apico-coronal). It should be noted, however, that although a statistically significant difference in IS-fBIC between the T6 and T12 groups was observed, the median change of 300 μm was relatively small in relation to the dimensions of the implant.

Thoma et al. noted greater %BIC on the lingual compared to the buccal side at 4 weeks, and greater buccal %BIC at 12 weeks than at 4 weeks (Thoma et al., 2017). These findings are at odds with the present study, where there were no between-group and within-group differences in %BIC at the four regions of interest. This may be explained by the longer healing period of 6 weeks to the first observation point in the present study compared to 4 weeks of Thoma et al. The lack of difference between the T6 and T12 groups suggests that osteointegration as measured by %BIC was established by 6 weeks. Furthermore, the presence of DBBMC material did not impede the process of osseointegration, although interestingly, some DBBMC particles were observed to be in direct contact with the implant surface. The overall histological appearance of the bone surrounding the implants was similar for specimens in the T6 and T12 groups, with similar proportions of new and old bone undergoing normal turnover. In light of these observations, it may be speculated that early loading of implants following early placement into ridge preserved sites may theoretically be feasible. Further studies would be required to test this hypothesis.

The principal indication for ARP is to minimize the dimensional change that follows tooth extraction. In the present study, it was noted that the buccal bone crest had resorbed corono-apically, and was replaced with a regenerated bone wall comprised of DBBMC and new bone. This is consistent with previous preclinical studies of ARP and immediate implant placement using the maxillary second incisor model (De Santis et al., 2011; Ellis et al., 2020; Raveendiran, Chen, Davies, Fitzgerald, & Darby, 2019) and mandibular hemisected premolar model (M. G. Araujo & Lindhe, 2009; Fickl et al., 2008). The findings are also consistent with clinical studies of ARP which confirmed

that the procedure is effective at reducing but not preventing resorption of the ridge in the corono-buccal region (Botilde et al., 2020; Fickl et al., 2017; Jung et al., 2018; Mardas, Chadha, & Donos, 2010). A recent case series study reported on early implant placement in ridge preserved sockets grafted with DBBMC. 10 consecutively included patients had implants placed 8 weeks after grafting of maxillary central incisor sockets with DBBMC. At the time of implant placement, all 10 sites required additional grafting with DBBM due to partial resorption in the corono-buccal region (Chen & Darby, 2020). In the present study, the bucco-lingual ridge dimensions between the T6 and T12 groups were similar, indicating that once initial resorption of the buccal bone had occurred, the alveolar ridge dimensions were stable thereafter. A recent prospective clinical study of ARP reported dimensional stability over a 5 to 7 year observation period (Botilde et al., 2020).

The study was limited by exclusion of 2 out of the 9 animals due to fibrous encapsulation and non-integration of 3 implants. The reasons for this are not clear, but a similar finding was reported in a previous study of immediate implants in the same second incisor model (Ellis et al., 2020). Care should be therefore taken in interpreting the results of the study.

Conclusions

This study confirms that implants can successfully be placed early in ridge preserved maxillary second incisor sites and are osseointegrated by 6 weeks. There was significantly lower IS-fBIC values at 12 weeks than at 6 weeks on the buccal aspect, suggesting that IS-fBIC may be time dependent. The %BIC, relative proportions of mineralized tissues and dimensions of the alveolar ridge demonstrated stability between 6 and 12 weeks of healing.

References

Apostolopoulos, P., & Darby, I. (2017). Retrospective success and survival rates of dental implants placed after a ridge preservation procedure. *Clin Oral Implants Res, 28*(4), 461-468. doi:10.1111/clr.12820

Araujo, M., Linder, E., & Lindhe, J. (2009). Effect of a xenograft on early bone formation in extraction sockets: an experimental study in dog. *Clin Oral Implants Res, 20*(1), 1-6.

Araujo, M., Linder, E., Wennstrom, J., & Lindhe, J. (2008). The influence of Bio-Oss Collagen on healing of an extraction socket: an experimental study in the dog. *International Journal of Periodontics & Restorative Dentistry, 28*(2), 123-135.

Araujo, M. G., & Lindhe, J. (2005). Dimensional ridge alterations following tooth extraction. An experimental study in the dog. *J Clin Periodontol, 32*(2), 212-218.

Araujo, M. G., & Lindhe, J. (2009). Ridge preservation with the use of Bio-Oss collagen: A 6-month study in the dog. *Clin Oral Implants Res, 20*(5), 433-440. doi:10.1111/j.1600-0501.2009.01705.x

Bassir, S. H., Alhareky, M., Wangsrimongkol, B., Jia, Y., & Karimbux, N. (2018). Systematic Review and Meta-Analysis of Hard Tissue Outcomes of Alveolar Ridge Preservation. *Int J Oral Maxillofac Implants, 33*(5), 979-994.

Botilde, G., Colin, P. E., Gonzalez-Martin, O., Lecloux, G., Rompen, E., & Lambert, F. (2020). Hard and soft tissue analysis of alveolar ridge preservation in esthetic zone using deproteinized bovine bone mineral and a saddle connective tissue graft: A long-term prospective case series. *Clin Implant Dent Relat Res, 22*(3), 387-396. doi:10.1111/cid.12899

Cardaropoli, G., Araujo, M., & Lindhe, J. (2003). Dynamics of bone tissue formation in tooth extraction sites. An experimental study in dogs. *J Clin Periodontol, 30*(9), 809-818.

Cardaropoli, G., Lekholm, U., & Wennstrom, J. L. (2006). Tissue alterations at implant-supported single-tooth replacements: a 1-year prospective clinical study. *Clin Oral Implants Res, 17*(2), 165-171.

Chen, S. T., & Darby, I. (2020). Alveolar ridge preservation and early implant placement at maxillary central incisor sites: a prospective case series study. *Clin Oral Implants Res, 31*(9), 803-813. doi:10.1111/clr.13619

Darby, I., Chen, S., & De Poi, R. (2008). Ridge preservation: what is it and when should it be considered. *Aust Dent J, 53*(1), 11-21. doi:ADJ008 [pii] 10.1111/j.1834-7819.2007.00008.x

Darby, I., Chen, S. T., & Buser, D. (2009). Ridge preservation techniques for implant therapy. *Int J Oral Maxillofac Implants, 24 Suppl*, 260-271.

De Santis, E., Botticelli, D., Pantani, F., Pereira, F. P., Beolchini, M., & Lang, N. P. (2011). Bone regeneration at implants placed into extraction sockets of maxillary incisors in dogs. *Clin Oral Implants Res, 22*(4), 430-437. doi:10.1111/j.1600-0501.2010.02122.x

Ellis, R., Chen, S., Davies, H., Fitzgerald, W., Xu, J., & Darby, I. (2020). Primary stability and healing outcomes of apically tapered and straight implants placed into fresh extraction sockets. A pre-clinical in vivo study. *Clin Oral Implants Res, 31*(8), 705-714. doi:10.1111/clr.13618

Farmer, M., & Darby, I. (2014). Ridge dimensional changes following single-tooth extraction in the aesthetic zone. *Clin Oral Implants Res, 25*(2), 272-277. doi:10.1111/clr.12108

Fickl, S., Fischer, K., Petersen, N., Happe, A., Schlee, M., Schlagenhauf, U., & Kebschull, M. (2017). Dimensional Evaluation of Different Ridge Preservation Techniques: A Randomized Clinical Study. *International Journal of Periodontics & Restorative Dentistry, 37*(3), 403-410.

Fickl, S., Zuhr, O., Wachtel, H., Stappert, C. F., Stein, J. M., & Hurzeler, M. B. (2008). Dimensional changes of the alveolar ridge contour after different socket preservation techniques. *J Clin Periodontol, 35*(10), 906-913. doi:10.1111/j.1600-051X.2008.01305.x

Heberer, S., Al-Chawaf, B., Hildebrand, D., Nelson, J. J., & Nelson, K. (2008). Histomorphometric analysis of extraction sockets augmented with Bio-Oss Collagen after a 6-week healing period: a prospective study. *Clin Oral Implants Res, 19*(12), 1219-1225. doi:10.1111/j.1600-0501.2008.01617.x

Janner, S. F. M., Bosshardt, D. D., Cochran, D. L., Chappuis, V., Huynh-Ba, G., Jones, A. A., & Buser, D. (2017). The influence of collagen membrane and autogenous bone chips on bone augmentation in the anterior maxilla: a preclinical study. *Clin Oral Implants Res, 28*(11), 1368-1380. doi:10.1111/clr.12996

Jung, R. E., Sapata, V. M., Hammerle, C. H. F., Wu, H., Hu, X. L., & Lin, Y. (2018). Combined use of xenogeneic bone substitute material covered with a native bilayer collagen membrane for alveolar ridge preservation: A randomized controlled clinical trial. *Clin Oral Implants Res, 29*(5), 522-529. doi:10.1111/clr.13149

Lindhe, J., Bressan, E., Cecchinato, D., Corra, E., Toia, M., & Liljenberg, B. (2013). Bone tissue in different parts of the edentulous maxilla and mandible. *Clin Oral Implants Res, 24*(4), 372-377. doi:10.1111/clr.12064

Mardas, N., Chadha, V., & Donos, N. (2010). Alveolar ridge preservation with guided bone regeneration and a synthetic bone substitute or a bovine-derived xenograft: a randomized, controlled clinical trial. *Clin Oral Implants Res, 21*(7), 688-698. doi:CLR1918 [pii]
10.1111/j.1600-0501.2010.01918.x

Mardas, N., Trullenque-Eriksson, A., MacBeth, N., Petrie, A., & Donos, N. (2015). Does ridge preservation following tooth extraction improve implant treatment outcomes: a systematic review: Group 4: Therapeutic concepts & methods. *Clin Oral Implants Res, 26 Suppl 11*, 180-201. doi:10.1111/clr.12639

Mellati, E., Chen, S., Davies, H., Fitzgerald, W., & Darby, I. (2015). Healing of Bio-Oss((R)) grafted marginal gaps at implants placed into fresh extraction sockets of incisor teeth in dogs: a study on the effect of submerged vs. non-submerged healing. *Clin Oral Implants Res, 26*(5), 553-562. doi:10.1111/clr.12442

Patel, K., Mardas, N., & Donos, N. (2013). Radiographic and clinical outcomes of implants placed in ridge preserved sites: a 12-month post-loading follow-up. *Clin Oral Implants Res, 24*(6), 599-605. doi:10.1111/j.1600-0501.2012.02500.x

Raveendiran, N., Chen, S., Davies, H., Fitzgerald, W., & Darby, I. (2019). The influence of deproteinised bovine bone mineral on dimensional changes in the

maxillary second incisor socket. *Clin Oral Implants Res, 30*(7), 670-681. doi:10.1111/clr.13452

Roccuzzo, M., Gaudioso, L., Bunino, M., & Dalmasso, P. (2014). Long-term stability of soft tissues following alveolar ridge preservation: 10-year results of a prospective study around nonsubmerged implants. *International Journal of Periodontics & Restorative Dentistry, 34*(6), 795-804. doi:10.11607/prd.2133

Schropp, L., Wenzel, A., Kostopolous, L., & Karring, T. (2003). Bone healing and soft tissue contour changes following single-tooth extraction: A clinical and radiographic 12-month prospective study. *International Journal of Periodontics & Restorative Dentistry, 23*, 313-323.

Thoma, D. S., Naenni, N., Benic, G. I., Munoz, F., Hammerle, C. H. F., & Jung, R. E. (2017). Effect of ridge preservation for early implant placement - is there a need to remove the biomaterial? *J Clin Periodontol, 44*(5), 556-565. doi:10.1111/jcpe.12709

Vignoletti, F., Matesanz, P., Rodrigo, D., Figuero, E., Martin, C., & Sanz, M. (2012). Surgical protocols for ridge preservation after tooth extraction. A systematic review. *Clin Oral Implants Res, 23 Suppl 5*, 22-38. doi:10.1111/j.1600-0501.2011.02331.x

Table 1 The percentage of bone to implant contact and percentages by area of new bone, old bone and DBBMC particles in the 4 regions of interest.

	Apico-buccal		Corono-buccal		Apico-palatal		Corono-palatal	
	T6	T12	T6	T12	T6	T12	T6	T12
%BIC								
Median (25^{th}/75^{th} percentiles) (%)	38.3 (26.8/61.5)	62.0 (50.6/69.0)	48.7 (32.3/64.7)	46.0 (39.1/55.8)	52.9 (45.4/60.1)	57.9 (51.4/68.4)	50.7 (30.7/58.5)	47.2 (44.8/52.3)
Range (%)	26.0 – 78.8	34.4 – 73.2	11.3 – 68.0	25.6 – 63.4	39.1 – 65.2	45.3 – 84.8	21.5 – 73.0	22.7 – 68.8
p-value	0.205		0.933		0.205		0.800	
% New bone								
Median (25^{th}/75^{th} percentiles) (%)	32.0 (30.9/40.4)	35.5 (31.7/37.4)	46.3 (38.9/49.3)	43.1 (40.2/46.4)	25.7 (20.4/27.6)	30.7 (25.2/38.6)	31.6 (27.2/41.6)	35.7 (30.4/39.2)
Range (%)	29.2 – 46.0	28.9 – 37.5	37.1 – 51.2	37.4 – 48.3	19.4 – 35.9	21.9 – 40.1	25.3 – 45.7	29.0 – 41.0
p-value	0.544		0.933		0.151		0.800	
% Old bone								

Median (25^{th}/75^{th} percentiles) (%)	14.5 (6.8/18.8)	9.4 (2.3/16.6)	5.0 (1.6/8.2)	2.4 (0.1/8.7)	7.9 (4.2/23.4)	15.8 (13.4/27.8)	20.3 (14.8/31.1)	28.2 (25.2/33.7)
Range (%)	1.6 – 21.5	1.9 – 31.5	0.1 – 20.6	0.1 – 11.9	2.8 – 47.2	11.3 – 28.9	11.4 – 58.9	24.9 – 38.1
p-value	0.544		0.800		0.272		0.353	
% DBBMC								
Median (25^{th}/75^{th} percentiles) (%)	0.4 (0.3/3.3)	4.2 (2.5/5.5)	10.0 (9.0/13.9)	10.1 (5.2/17.7)	1.6 (0/2.7)	0.2 (0/1.4)	0.4 (0/1.4)	2.1 (0.5/4.2)
Range (%)	0 – 11.7	1.9 – 5.5	4.9 – 16.5	5.2 – 23.4	0 – 4.0	0 – 7.3	0 – 7.1	0.4 – 6.9
p-value	0.272		0.447		1.000		0.205	

Table 2 IS-fBIC, IS-C and IS-ROB measurements at the buccal and palatal aspects at both timepoints.

	IS-fBIC				IS-C				IS-ROB			
	Buccal		Palatal		Buccal		Palatal		Buccal		Palatal	
	T6	T12	T6	T12	T6	T12	T6	T12	T6	T12	T6	T12
Median (25^{th}/75^{th} percentiles) (µm)	585 (345/730)	175 (0 – 225)	585 (455/850)	380 (70/1020)	-802 (-1205/-28)	-518 (-873/407)	79 (-989/290)	219 (88/538)	2418 (1233/2478)	678 (480/2027)	554 (-335/684)	653 (379/1389)
Range (µm)	260 – 1015	0 – 490	405 – 1350	0 – 2075	-1516 – -4	-1117 – -699	-1015 – 544	88 – 1353	688 – 3275	387 – 3806	-345 – 1101	-539 – 1625
p-value	0.022 *		0.933		0.108		0.353		0.353		0.447	

A negative value indicates a location coronal to the implant shoulder (IS)

* - significant ($p < 0.05$) Wilcoxon signed rank test

Figure legends:

Figure 1. Surgical procedures. (a) Flapless extraction of a right second incisor followed by (b) grafting of the socket with 90% deproteinized bovine bone mineral in 10% collagen matrix (DBBMC). (c) The socket was sealed with a resorbable type I/III porcine collagen matrix (PCM) which was sutured in place. (d) Situation 6 weeks after healing, and (e) following flap reflection. (f) An implant has been inserted into the site.

Figure 2. Histological sections from the same specimen showing (a) the maxillary right second incisor implant (T6 group) and (b) histomorphometric colour labelling of the same implant; and (c) the contralateral maxillary left second incisor implant (T12 group) and (d) histomorphometric colour labelling of the implant. Regions of interest – 1: apico-buccal, 2: corono-buccal, 3: apico-palatal, and 4: corono-palatal. IS, implant shoulder; C, bone crest; ROB, remnants of the original buccal bone. Note – images (c) and (d) are mirror images to facilitate comparisons between T6 and T12.

Figure 3. Box plot of percentage bone to implant contact (%BIC) at T6 and T12 by regions of interest.

Figure 4. Box plots of percentage new bone at the 4 regions of interest, by groups (T6 and T12).

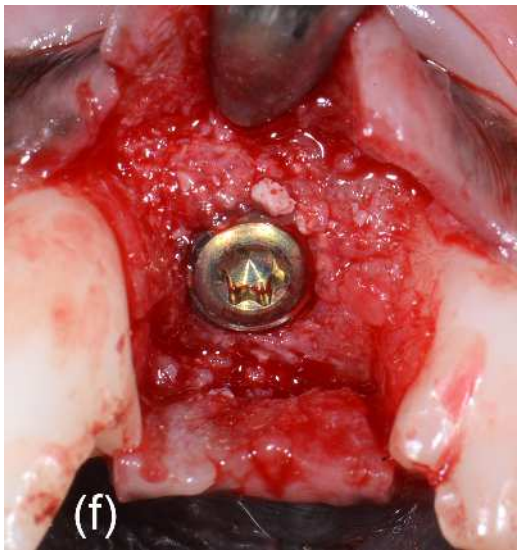
Figure 5. Box plots of percentage old bone at the 4 regions of interest, by groups (T6 and T12).

Figure 6. Box plots of percentage DBBMC at the 4 regions of interest, by groups (T6 and T12).

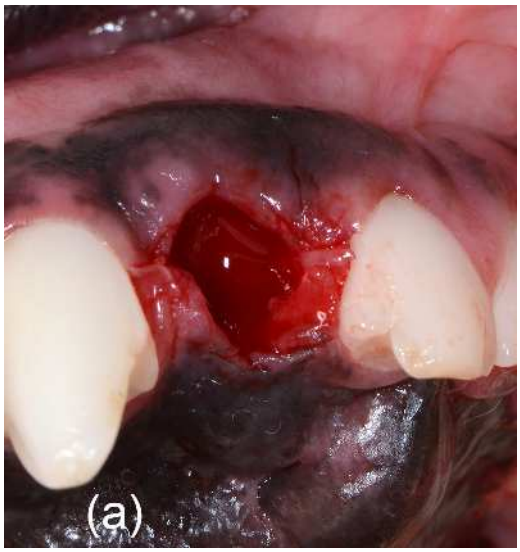
Figure 7. Boxplot of distance from the implant shoulder to first bone to implant contact (IS-fBIC) at T6 and T12 on the buccal and palatal aspects of implants.

Figure 8. Box plots of distance from implant shoulder to bone crest (IS-C) and implant shoulder to remnants of the original bone crest (IS-ROB) at the buccal and palatal aspects of implants by groups (T6 and T12).

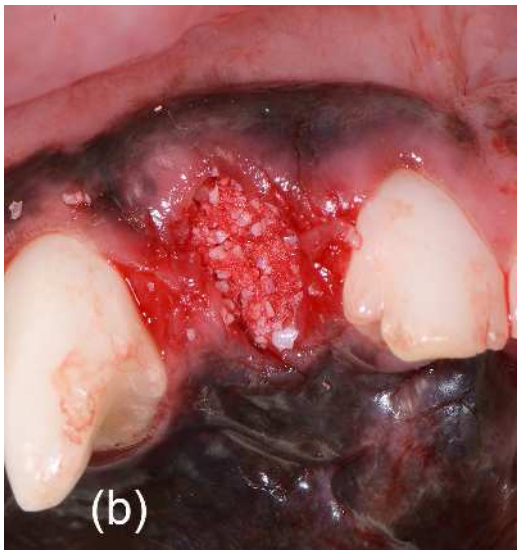
Figure 9. Bucco-palatal ridge with at 1 mm increments apical to the implants shoulder (IS) by groups (T6 and T12).



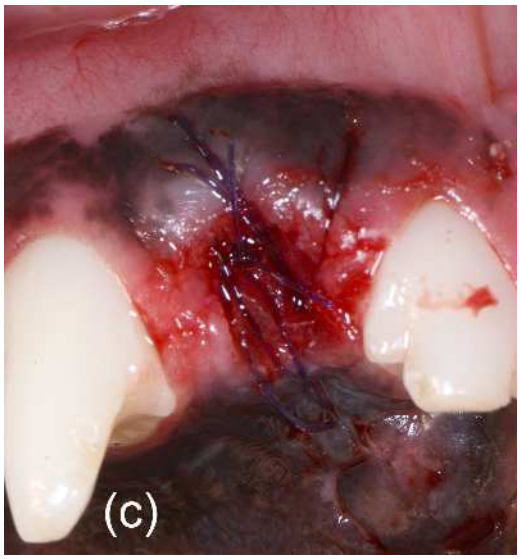
clr_13744_f1.tif



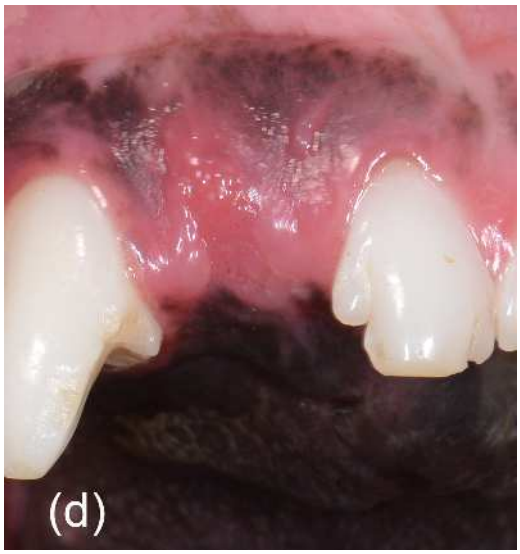
clr_13744_f1a.tif



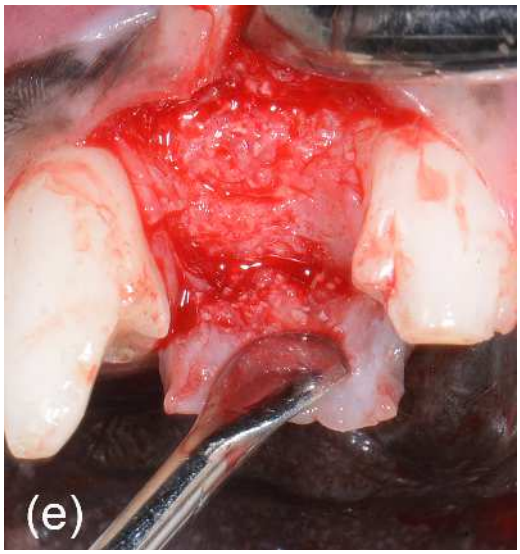
clr_13744_f1b.tif



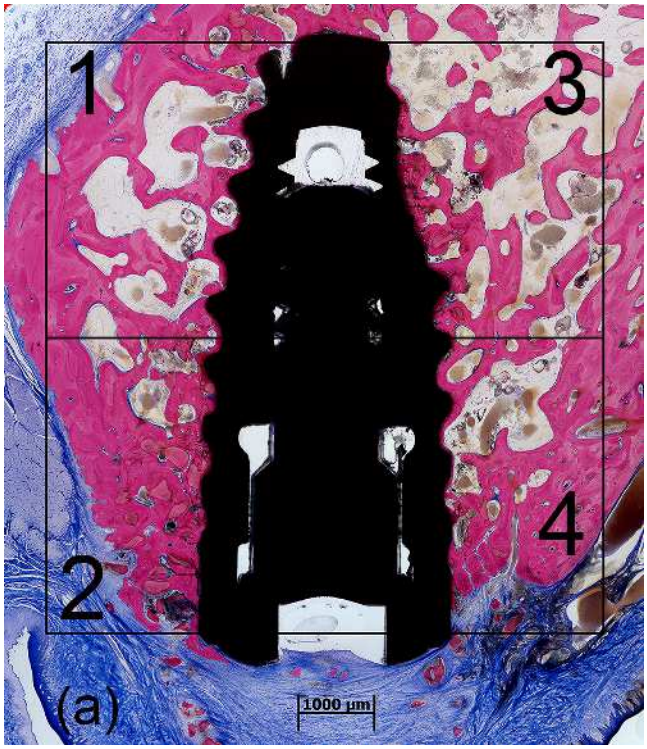
clr_13744_f1c.tif



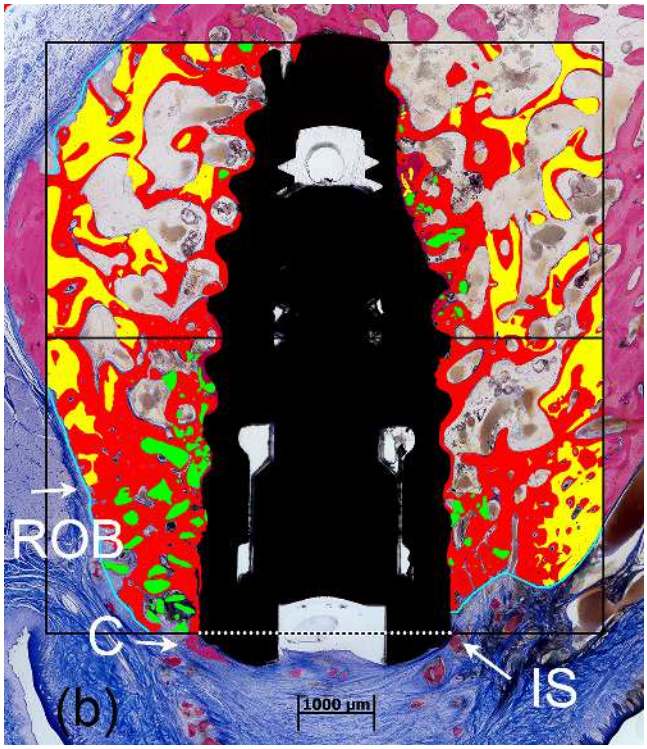
clr_13744_f1d.tif



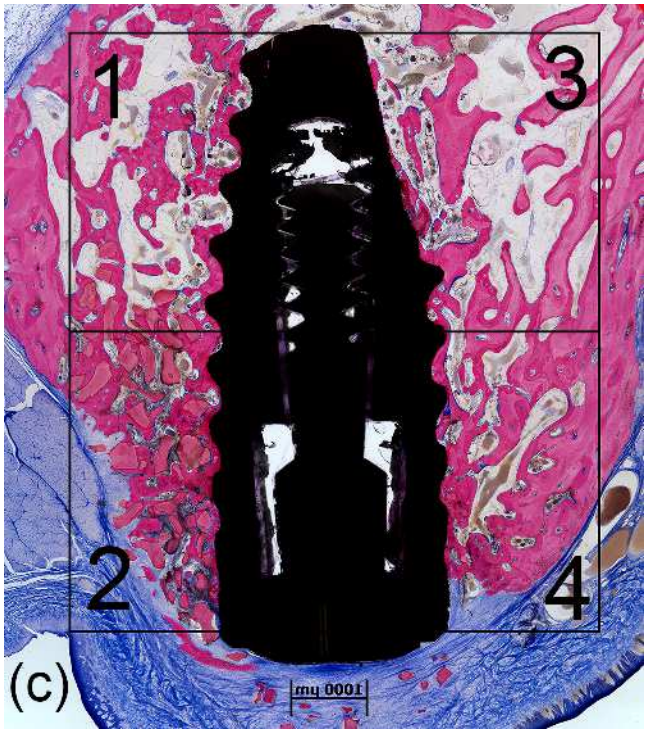
clr_13744_f1e.tif



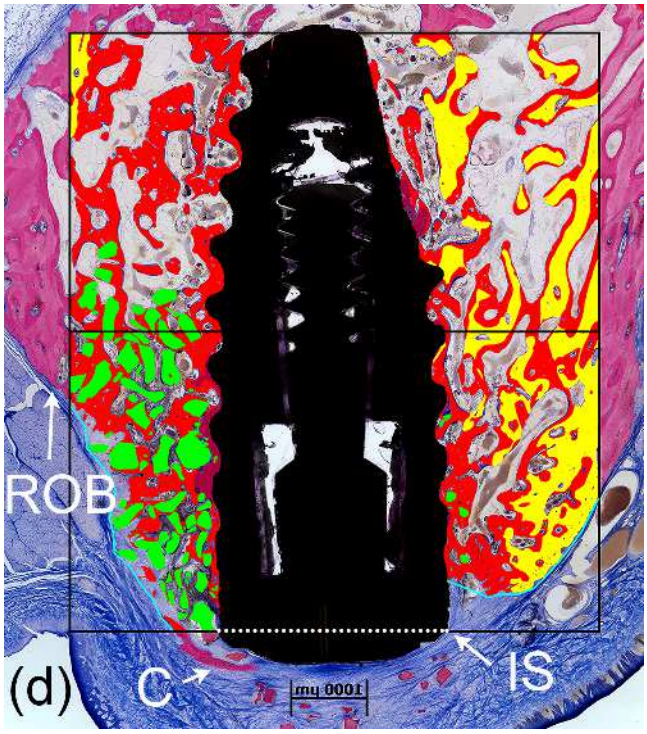
clr_13744_f2a.tif



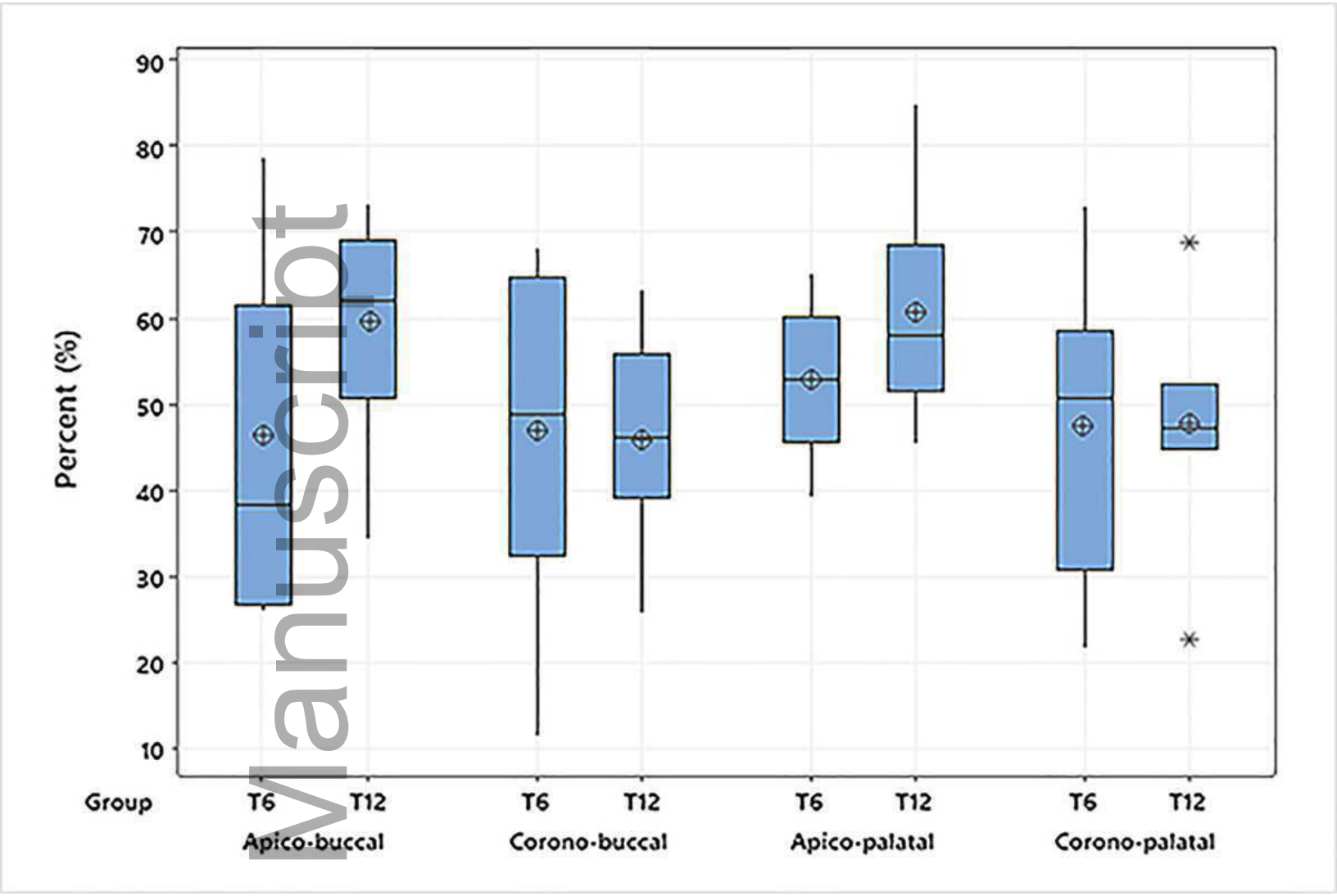
clr_13744_f2b.tif



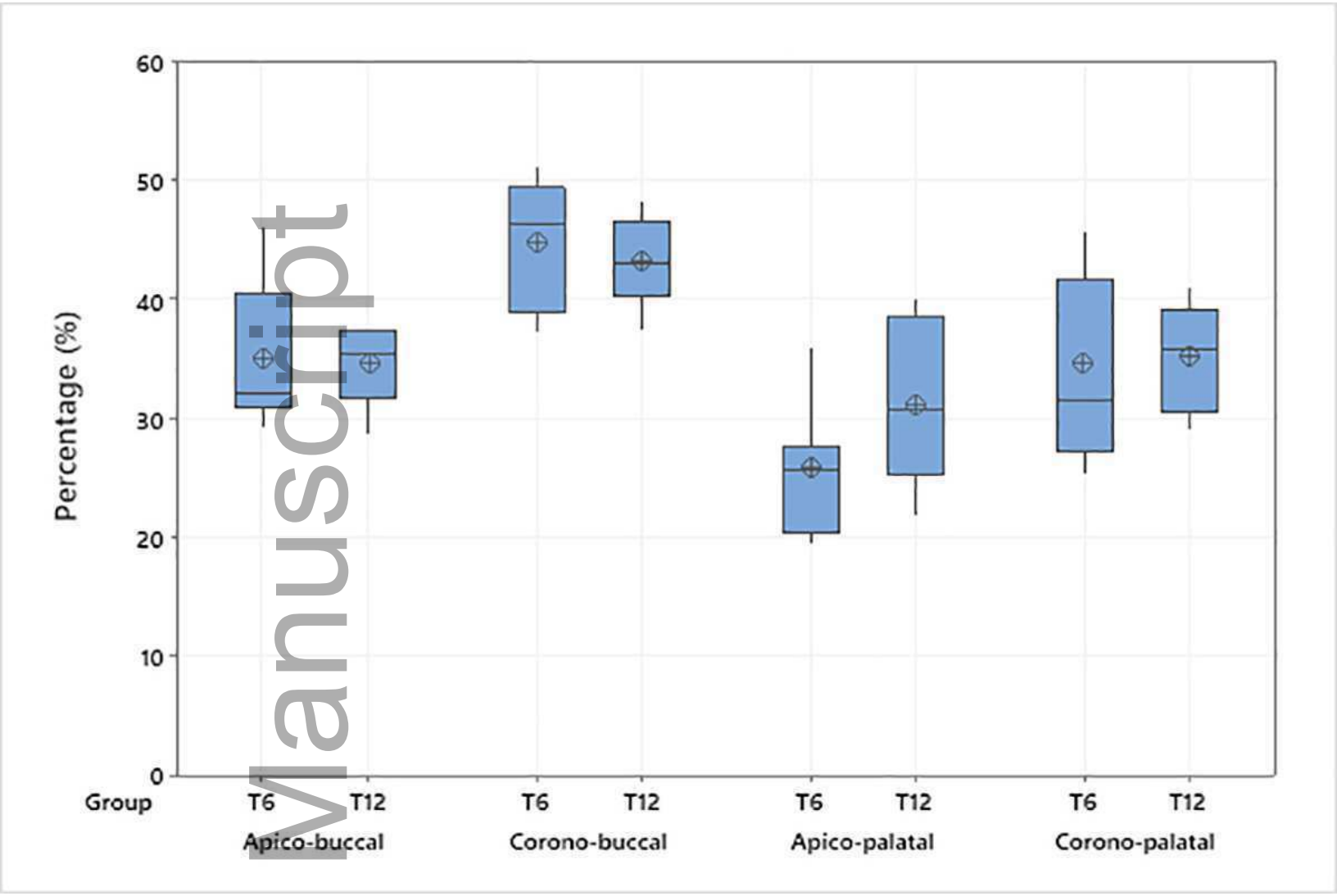
clr_13744_f2c.tif



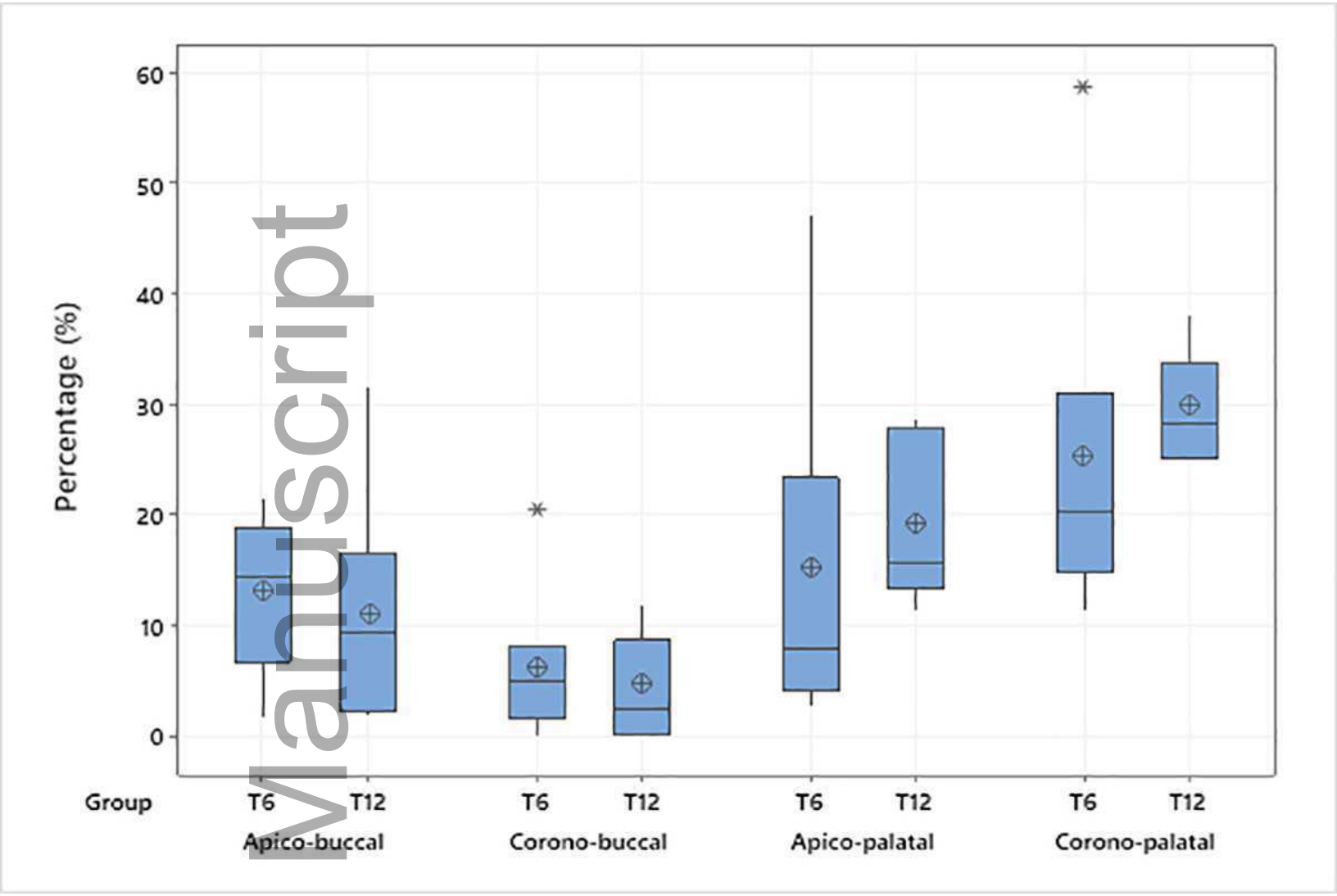
clr_13744_f2d.tif



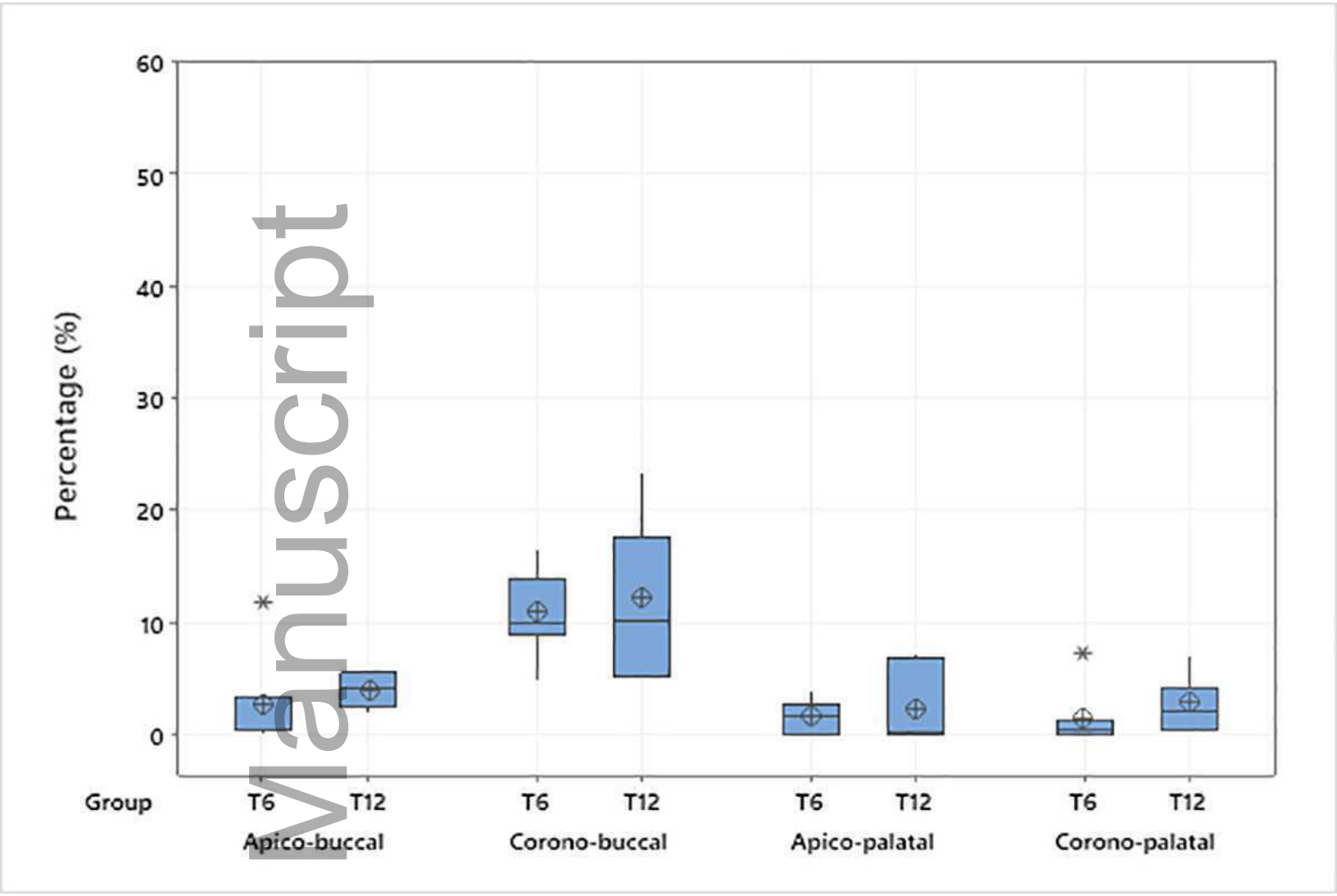
clr_13744_f3.eps



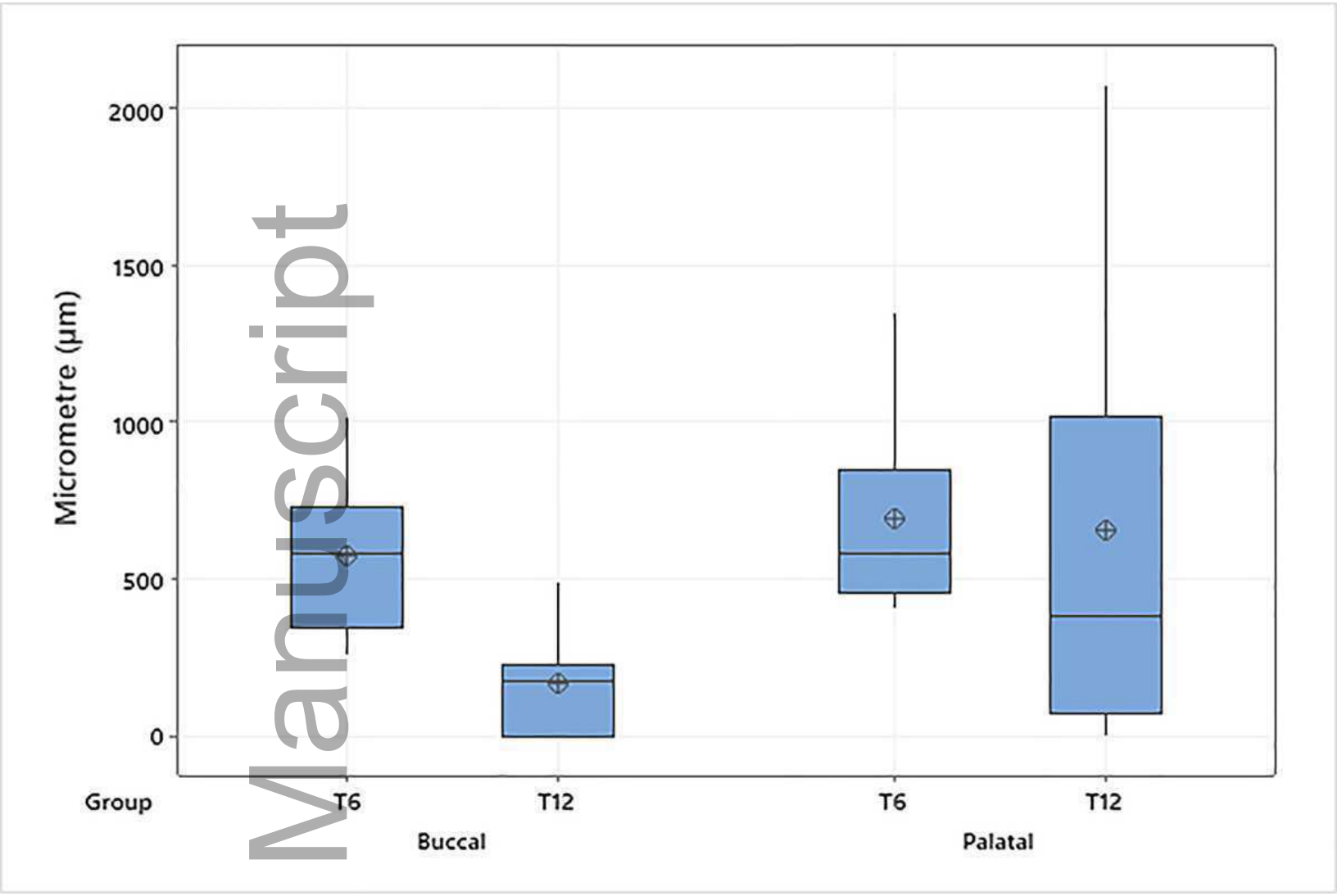
clr_13744_f4.eps



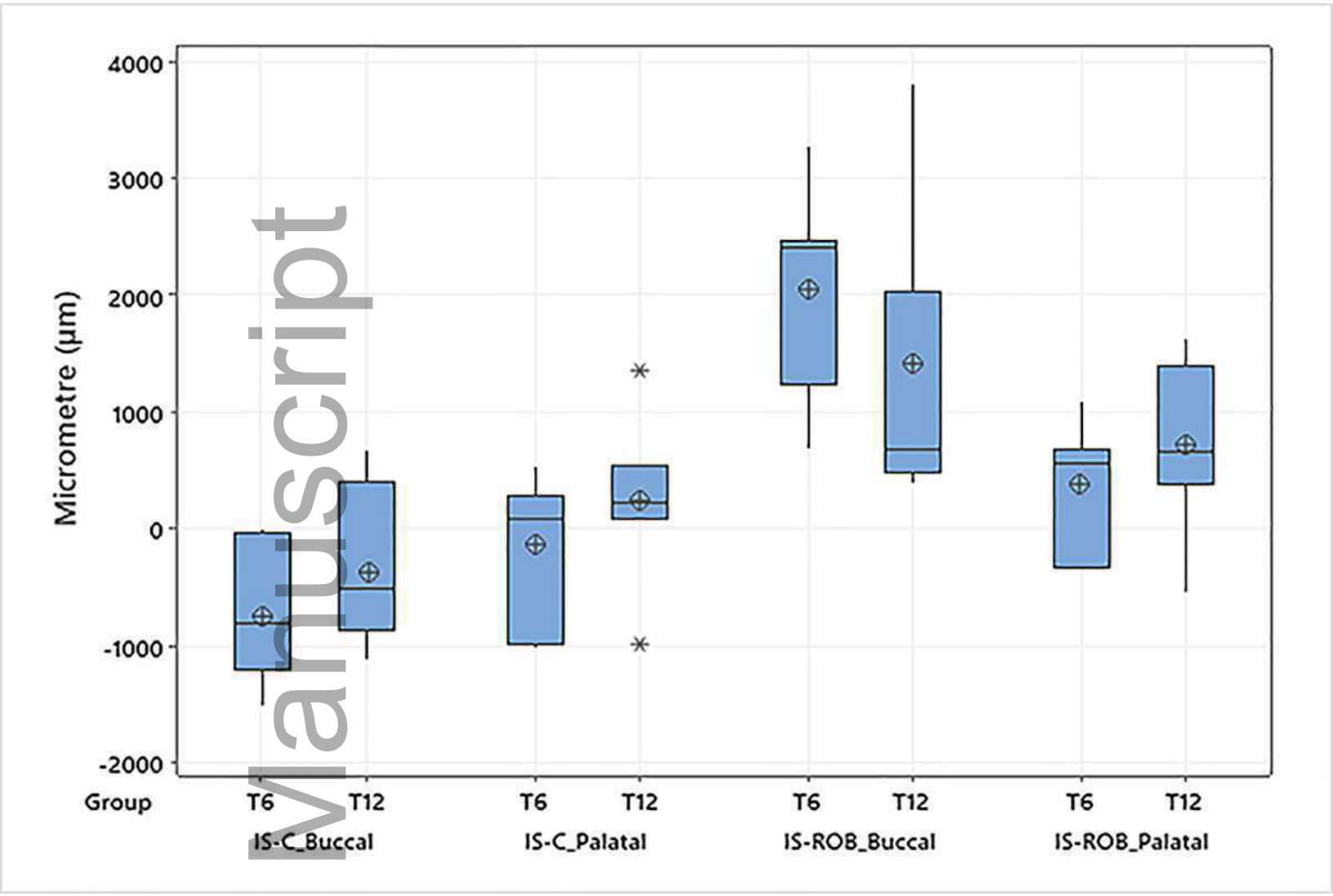
clr_13744_f5.eps



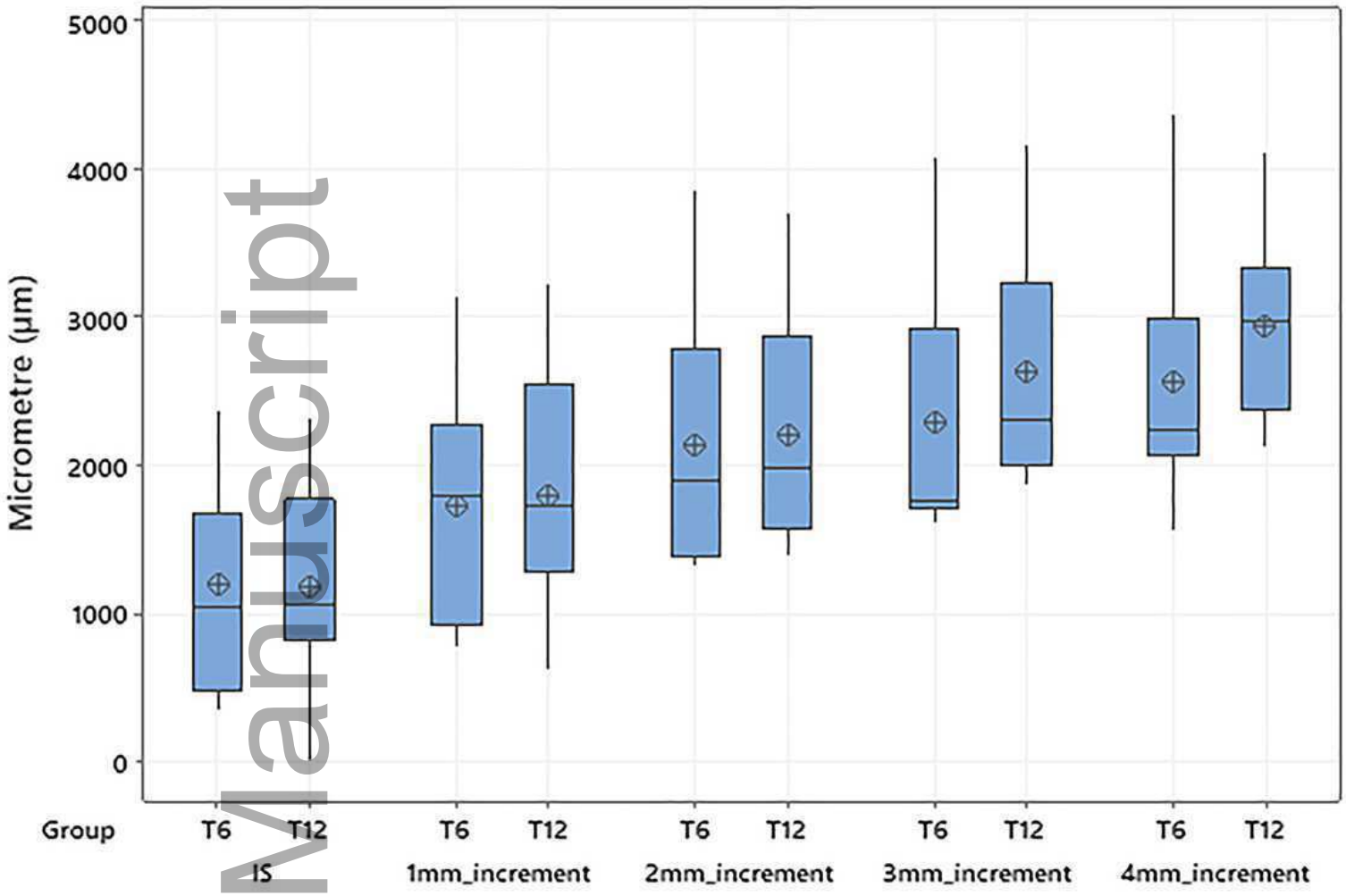
clr_13744_f6.eps



clr_13744_f7.eps



clr_13744_f8.eps



clr_13744_f9.eps