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Clinical and Histomorphometric Outcomes Following the Use of Small versus Large Particle Sized Bone Allografts in Ridge Preservation Procedures: A Randomized Clinical Trial

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CLINICAL AND HISTOMORPHOMETRIC OUTCOMES FOLLOWING THE USE OF SMALL VERSUS LARGE PARTICLE SIZED BONE ALLOGRAFTS IN RIDGE PRESERVATION PROCEDURES: A RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Alveolar ridge preservation at the time of tooth extraction is a commonly performed procedure as it significantly minimizes ridge resorption and allows for more optimal implant placement. The amount of new vital bone has been shown to vary with the use of different types of bone replacement grafts, however, the effect of bone graft particle size on the outcomes following ridge preservation has not been elucidated in the scientific literature. The present randomized clinical trial compared clinically, radiographically and histomorphometrically the use of small- (0.25-1.0mm) versus large- (1.0-2.0mm) sized particle mineralized cortico-cancellous bone freeze-dried bone allografts (FDBAs) in ridge preservation procedures.

Nineteen patients (22 sites) were initially enrolled and randomized to receive either small- or large-sized particle FDBA. Flapless extractions were performed, and sockets grafted with the corresponding allograft. A CBCT scan was taken immediately following the ridge preservation procedure. After an average of 16 weeks of healing, a second CBCT scan, as well as bone core biopsy and implant placement were performed. Vertical and horizontal dimensional changes were assessed clinically by direct measurements, a novel CBCT superimposition technique was used for radiographic measurements and bone cores were analyzed by histomorphometric analysis. Two-sample t-tests were conducted to compare the outcomes in the two groups.

The small-sized particle group had an average of 17.3% ± 12.8% new vital bone, 43.0% ± 11.7% residual graft particles and 39.7% ± 12.8% soft tissue. The large-sized particle group had a mean of 25.9% ± 9.5% new vital bone, 37.6% ± 13.8% residual graft particles and 36.5% ± 7.8% soft tissue. The difference between the two groups lacked statistical significance for any of the tissue types. Similarly, no statistically significant variation was found between the two groups in clinical and radiographic dimensional changes. These findings suggest that both types of graft material can be used in clinical setting with similar outcomes.

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A-PRF	advance platelet-rich fibrin
ABBM	anorganic bovine bone matrix
BMP	bone morphogenetic protein
β-TCP	β- tricalcium phosphate
CT	connective tissue
CBCT	cone beam computed tomography
DBM	demineralized bone matrix
DFDBA	demineralized freeze-dried bone allograft
FDBA	freeze-dried bone allograft
HMAC	Histomorphometry and Molecular Analysis Core
IRB	Institutional Review Board
LP	large particle
MM	millimeter
μM	micrometer
MPS	multiple particle sizes
PRP	platelet-rich plasma
rhPDGF-BB	recombinant human platelet derived growth factor
SD	standard deviation
SPS	single particle size

SP	small particle
TGF β-1	transforming growth factor β-1
UAB	University of Alabama at Birmingham
VEGF	vascular endothelial growth factor

INTRODUCTION

Healing of Extraction Sockets

Following tooth extraction, the healing phase and resultant bone and soft tissue volumes can determine the feasibility of tooth replacement therapies at those sites. The healing process following tooth extraction is characterized by multiple events and can be divided into three phases: Inflammatory, proliferative, and modeling/remodeling phases (Aukhil, 2000, Araujo et al., 2015). The inflammatory phase itself is subdivided into the blood clot formation and the inflammatory cell migration (Araujo et al., 2015). Immediately after tooth extraction, hemorrhage occurs, and the socket is filled with blood. A clot forms, consisting of platelets within a network of cross-linked fibrin fibers along with plasma fibronectin, vitronectin, and thrombospondin (Araujo et al., 2015). The growth factors and cytokines present in the fibrin clot provide the start signals for wound repair and within a few days a large number of inflammatory cells, primarily polymorphic neutrophils in the early phase, migrate to the wound in order to sterilize the site (Araujo et al., 2015). Neutrophils release enzymes as well as oxygen products to remove the bacterial debris and along with macrophages they release pro-inflammatory cytokines providing signals that activate adjacent fibroblasts and keratinocytes (Aukhil, 2000). Migration of the epithelial cells requires the creation of a migrating path that is achieved by the dissolution of the fibrin barrier by enzyme plasmin deriving from the activation of plasminogen in the clot. Chemotactic factors, active contact guidance and absence of neighboring

cells drive the epithelial cell migration and allow for re-epithelialization. Granulation tissue which consists of new capillaries, macrophages, fibroblast and loose connective tissue begins to form around day four after the extraction. The proliferative phase is characterized by an intense and rapid tissue formation and alike the inflammatory phase it is divided into two parts: Fibroplasia and the woven bone formation (Araujo et al., 2015). The release of cytokines by macrophages stimulates fibroblasts to synthesize a new, collagen-rich extracellular matrix. Endothelial cells migrate into the provisional matrix and form tubes surrounded by basement membranes. Subsequently, the provisional matrix is penetrated by several bone-forming cells, and finger-like projections of woven bone are laid down around the blood vessels. The projections eventually completely surround the vessels which leads to the formation of primary osteons (Aukhil, 2000). Woven bone can be identified in the healing socket as early as two weeks following tooth extraction and it remains in the wound for several weeks. Woven bone is known as a provisional type of bone without any load-bearing capacity; It will eventually be replaced with mature bone types like lamellar bone and bone marrow. The third step of the socket-healing process is bone modeling and remodeling (Araujo et al., 2015). Bone modeling corresponds to the osteoclastic bone resorption which takes place on the buccal and lingual walls and on the outer and inner portions of the socket, leading to a dimensional alteration of the alveolar ridge. Many factors may influence the ridge resorption pattern, including the number of neighboring teeth to be extracted, socket morphology (i.e. single- vs multirooted- teeth and socket integrity), periodontal biotype (i.e. bony buccal plate and soft tissue thickness), and systemic factors such as uncontrolled diabetes and bone metabolic disorders (Avila-Ortiz et al., 2014). Bone remodeling does not imply any change in the shape and

architecture of the bone and it correlates with the replacement of woven bone with lamellar bone or bone marrow. It is widely accepted that the cells of the periosteum make a major contribution to the bone healing (Aukhil, 2000). In humans, bone remodeling may take multiple months and it exhibits significant variability among individuals.

The bone resorption that occurs secondary to tooth extraction as part of the modeling phase leads to dimensional changes, the majority of which take place within the first three to six months (Cardaropoli et al., 2003, Araujo and Lindhe, 2005, Nevins et al., 2006, Tan et al., 2012, Jambhekar et al., 2015). The buccal wall of the socket tends to be resorbed to a greater degree than the lingual wall because the coronal aspect of the buccal plate is often comprised of only bundle bone, a very thin layer (≤0.5 mm) of lamellar bone composed of circumferential lamellae (Huynh-Ba et al., 2010). Sharpey's fibers are invested in the bundle bone and connect the periodontal ligament to the alveolar bone, making the bundle bone a tooth-dependent structure (Araujo et al., 2015). Additionally, given its inherent fragility, the buccal plate is occasionally lost at the time of extraction by iatrogenic trauma. Cardaropoli et al. reported a meaningful negative correlation between baseline buccal wall thickness and ridge width changes at extraction sites where no ridge preservation procedures are performed (Cardaropoli et al., 2003). Vertical height loss frequently accompanies the horizontal loss and is usually of greater significance when multiple adjacent teeth are extracted. Some have described a loss of up to 50% of the overall ridge width without ridge preservation at the time of tooth extraction (Schropp et al., 2003, Van der Weijden et al., 2009). In a clinical and radiographic 12-month prospective study, Schropp et al. observed a mean reduction in ridge width from 12.0 mm to 5.9 mm (6.1 mm loss) at one year after tooth extraction without grafting and noted that

the two-thirds of the reduction occurred during the first three months post-extraction (Schropp et al., 2003). The percentage of reduction was larger in the molar areas compared to the premolar areas and the resorption was more pronounced in the mandible compared to the maxilla.

The flapless approach for tooth extraction is known to be minimally invasive, simple, and conservative. Improved clinical outcomes have been reported after flapless extraction, including reduced healing times, discomfort, and signs of inflammation. When the tooth is extracted without elevation of a full thickness flap, the periosteum remains undisturbed and thus, the blood supply to the underlying buccal bone is preserved. It is this blood supply that has been associated with the reduced loss of alveolar bone compared to extraction with flap elevation (Jambhekar et al., 2015). A flapless approach is also recognized for preserving keratinized mucosa at the edentulous site more successfully than the flapped technique for teeth extraction (Barone et al., 2015).

Ridge Preservation Procedures

Ridge preservation can be achieved with different graft materials from autogenous, allogenic, xenograft, and alloplast sources. Autografts may be considered the "gold standard" for bone grafting procedure as they consist of bone and marrow cells that have an osteogenic potential (Reynolds et al., 2010). Autogenous bone can be harvested from intra-oral sites, but it typically yields limited graft volume and it increases surgical morbidity and discomfort. Due to their ability to maintain bone volume, rapid bone turnover, biocompatibility, and the lack of need for a secondary surgical harvest site, commercially available allograft materials of various particle sizes have become increasingly popular

for intra-oral grafting applications, including ridge preservation procedures (Beck and Mealey, 2010). In the United States, certified tissue banks use processing and packaging techniques that ensure their safety and clinical availability. Two of the most commonly used allografts in dentistry are freeze-dried bone allograft (FDBA) and demineralized freeze-dried bone allograft (DFDBA). It has been reported that FDBA physically maintains the socket space and acts as a scaffold for host osteoprogenitor cells during the healing phase. On the other hand, exposed bone morphogenetic proteins (BMPs) from DFDBA give the allograft the added benefit of osteoinductivity by stimulating host osteoprogenitor cells to differentiate into osteoblasts and begin new bone formation (Burchardt, 1983). A wide variation in clinical results has been observed when DFDBA is used and it could be the result of differences in either DFDBA processing technique and/or donor characteristics (Eskow and Mealey, 2014). Schwartz et al. assessed this possibility by comparing the osteoinductive potential of DFDBA from donors of different ages and gender from the same bank. Investigators found there was an age-dependent decrease in new bone induction, with DFDBA for donors over the age of 50 showing significantly less osteoinduction (Schwartz et al., 1998). However, differences in donor gender did not result in significant differences in the bone induction ability.

Xenografts from animal sources are commercially marketed as particulate bone replacement graft. For example, anorganic bovine bone matrix (ABBM) bone consists of naturally derived porous and deproteinized bovine bone mineral with comparable microporous structure and mineral bone composition than human bone (Reynolds et al., 2010). It has an osteoconductive potential and a slow resorption rate. Alloplasts are biocompatible, inorganic, synthetic bone grafting materials. The surface topography, the

morphology and the composition of alloplasts may provide an osteoconductive platform that promotes bone formation along the surface of the grafting material (Reynolds et al., 2010). Commercially available alloplasts include bioactive glass particles, ceramic materials, and calcium phosphates.

Implant dentistry has become a successful routine treatment modality for partially or totally edentulous patients (Albrektsson et al., 1986, van Steenberghe, 1989, van Steenberghe et al., 1990, Lindquist et al., 1996, Buser et al., 1997, Lekholm et al., 1999, Weber et al., 2000). Alveolar ridge preservation at the time of tooth extraction, which consists of placement of biomaterials within the socket, minimizes bone volume loss and allows for esthetic and functional placement of a dental implant (Avila-Ortiz et al., 2014, Jambhekar et al., 2015, Van der Weijden et al., 2009, Darby et al., 2009, Willenbacher et al., 2016). Jambhekar et al. published a systematic review on the histologic outcomes and/or changes in the buccolingual dimension and buccal wall height following flapless extractions with socket grafting using diverse materials, for a re-entry point at implant placement at or beyond 12 weeks (Jambhekar et al., 2015). One-hundred-seventeen extraction sockets grafted with allograft in five studies were assessed in this analysis. The mean loss of buccolingual width at the crest level was 1.63 mm and the mean loss of buccal wall height from the ridge crest was 0.58 mm. The mean value of vital bone formed was 29.93% and the remnant allograft material at these sites was 21.75%. Similarly, 427 extraction sockets grafted with xenograft in 20 studies were analyzed. The mean loss of buccolingual width at the crest level was 1.3 mm and the mean loss of buccal wall height from the ridge crest was 0.57 mm in areas with ridge preservation performed with xenografts. The mean value of vital bone formed was 35.72% and the remnant graft material

was 19.30%. This analysis also evaluated 224 (14 studies) extraction sockets where alloplast graft materials were used. The mean loss of the bucco-lingual width at the level of the crest was 2.13 mm and the loss of buccal wall height was 0.77 mm at sites grafted with alloplasts. The mean value of vital bone formed was 45.53% and the remnant graft material was 13.67%. In a systematic review and meta-analysis on the effect of alveolar ridge preservation after tooth extraction, Avila-Ortiz et al. combined the data from six studies for quantitative analysis (Avila-Ortiz et al., 2014). In terms of buccolingual width changes, they reported a strong ridge preservation effect in favor of the experimental group with a mean difference of 1.89 mm, and a magnitude effect of 2.07 mm for mid-buccal height and 1.18 mm for midlingual height. The authors concluded that socket grafting can be an effective therapy to prevent physiologic bone loss after extraction in both the horizontal and the vertical dimension. Willenbacher et al. reported on the effect of alveolar ridge preservation; 64 studies were included in the qualitative synthesis and 16 in the quantitative synthesis of their meta-analysis (Willenbacher et al., 2016). They showed a mean difference between alveolar ridge preservation and unassisted healing groups of 1.31 mm to 1.54 mm in bone width and 0.91 mm to 1.12 mm in bone height, concluding that alveolar ridge preservation procedures do not completely mitigate the resorption of the alveolar ridge following tooth extraction, but can significantly reduce it compared to unassisted healing. As part of the secondary outcomes, they reported that implants could be inserted in the ideal position without further augmentation in 90.1% of the experimental sites, compared to 79.2% of the control sockets. The authors couldn't draw any reliable conclusion on the histologic effects due to limited data, and no recom-

mendation for a specific technique of socket preservation could be made based upon their analysis.

In a six-month randomized controlled blinded clinical study, Iasella et al. reported on ridge preservation with FDBA and a collagen membrane following tooth extraction, looking into whether or not ridge preservation would prevent post extraction resorptive changes (Iasella et al., 2003). The width of the control group (extraction alone) decreased from 9.1 ± 1.0 mm to 6.4 ± 2.2 mm and the width of the study group (extraction with ridge preservation) decreased from 9.2 ± 1.2 mm to 8.0 ± 1.4 mm for a difference of 1.6 mm between groups. The vertical change for the control group was a loss of 0.9 mm ± 1.6 mm versus a gain of 1.3 ± 2.0 mm for a height difference between groups of 2.2 mm. The histologic analysis revealed a slightly greater amount of bone in the preserved sites, although these sites included both vital and non-vital bone. The authors concluded that following tooth extraction the most predictable maintenance of ridge width, height, and position was achieved when a ridge preservation procedure was performed (Iasella et al., 2003).

Geurs et al. evaluated in a randomized controlled clinical trial the healing of grafted and nongrafted sockets and the effect of platelet-rich plasma (PRP) and recombinant human platelet-derived growth factor (rhPDGF-BB) on early remodeling measured at 8 weeks post-extraction (Geurs et al., 2014). PRP contains at least 60 different growth factors that have an angiogenic potential and can stimulate cell proliferation, differentiation, and chemotaxis (Eskan et al., 2014). rhPDGF-BB also has a significant angiogenic ability as well as a chemotactic and mitogenic effect on mesenchymal cells (Darby and Morris, 2013). Forty-one subjects whose treatment plan involved extraction of premolars

or anterior teeth were randomized into four groups. In group 1 (control group), sockets were not grafted following extraction. In group 2, sockets were grafted with a mixture of mineralized freeze-dried bone allograft (FDBA)/β-tricalcium phosphate (β-TCP). In group 3, sockets were grafted with a mixture of FDBA/β-TCP reconstituted with PRP. In group 4, sockets were grafted with a mixture of FDBA/β-TCP reconstituted with rhPDGF. After eight weeks of healing, trephine cores of 2 × 6 mm were harvested, stained with paragon stain and processed for histologic and histomorphometric analysis. Significant differences in tissue distribution were identified between groups as well as between apical, middle, and coronal thirds of the harvested core. More new bone and organic matrix tissue were noted in non-grafted sockets compared with the groups where bone graft material was used. In the grafted groups (groups 2-4), residual particles comprised 16% to 37% of the core. The highest concentrations of residual graft particles were present in group two. In group four, the amount of graft particles was the least compared with all groups that included grafting. Group four also included the highest percentage of artifact/air of all the groups. Based upon their clinical and histologic findings, the authors concluded that 1) inclusion of bone replacement graft suppressed new bone formation in extraction sockets during the first eight weeks of healing and 2) fewer residual bone graft particles were noted in PRP- and rhPDGF-BB–enhanced groups, indicating more rapid turnover of bone graft in human extraction sockets.

In a randomized controlled clinical trial, Clark et al. aimed to evaluate the efficacy of Advance platelet-rich fibrin (A-PRF) alone or with FDBA in improving vital bone formation and alveolar dimensional stability during ridge preservation (Clark et al., 2018). PRF is an autogenous bioscaffold of a dense fibrin matrix with naturally integrated

growth factor as transforming growth factor β-1 (TGF β-1), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), which promote healing of hard and soft tissues. A total of 45 patients were enrolled and randomized into four groups: A-PRF alone, A-PRF + FDBA, FDBA alone and blood clot alone (i.e. unassisted healing). Clinical measurement of the changes in alveolar dimensions and histomorphometric and micro-CT analysis of bone core biopsies harvested at the time of implant placement were performed. The results showed the greatest reduction of the width of the crest at the coronal third in all groups, but no significant differences between the groups. The treatment groups using A-PRF and A-PRF + FDBA demonstrated significantly less ridge height reduction compared to treatment with blood clot alone. The treatment group A-PRF alone demonstrated the highest percentage of vital bone (46% ± 18%) of all groups and was significantly greater than the treatment group using FDBA alone (29% ± 14%). No significant difference in bone density was found between the 3 treatment groups, but unassisted healing showed significantly less bone mineral density.

A randomized controlled trial conducted by Eskow and Mealey histologically evaluated the new bone formation following ridge preservation with cortical FDBA compared with cancellous FDBA (Eskow and Mealey, 2014). The two types of FDBA have shown a different healing pattern, thus the aim of the study. Cortical allograft heals via reverse creeping substitution, in which early differentiation and recruitment of osteoclast take place and bone resorption precedes bone formation (Eskow and Mealey, 2014). By opposition, cancellous allograft heals by creeping substitution meaning that new bone is formed by the osteoblasts before the osteoclastic bone resorption takes place (Eskow and Mealey, 2014). All bone graft materials were obtained from the same donor and a stand-

ard particle size of 250 to 1,000 μm was chosen. Histologic evaluation was performed on 33 core biopsy specimens, 16 cores in the cortical FDBA group, and 17 in the cancellous FDBA group. A greater percentage of residual graft material was found in the cortical group compared with the cancellous group (28.38% vs 19.94%) and as the percentage of new bone formation increased, the percentage of residual graft material decreased. No statistically significant difference was found in the percentage of new bone formation between the two groups when core biopsies were harvested after an average of 18 weeks of healing.

Beck and Mealey studied the amount of new bone formation at three months and six months after extraction and ridge preservation using small particles (250 to 1,000 μm) of non–freeze-dried cancellous mineralized human bone allograft (Puros™, Zimmer Dental, Warsaw, IN)(Beck and Mealey, 2010). There were 22 sites included in the delayed healing group with an average healing time of 27 weeks, and 16 sites included in the early healing group with an average healing time of 14 weeks. At histology, no statistically significant difference was found between the two groups; The early group had a similar percentage of new vital bone, residual graft material, and non-bone connective tissue compared to the late group and overall the percentage of new vital bone was negatively correlated with the percentage of connective tissue and residual graft material. Considering the dimensional changes between the two healing groups, no statistically significant or clinically relevant difference could be found neither.

In another recent randomized clinical study, Wood and Mealey evaluated and compared clinical and histological the healing of non-molar extraction sockets grafted with DFDBA and FDBA (Wood and Mealey, 2012). As previously stated, both DFDBA

and FDBA are osteoconductive, but only DFDBA has shown to be osteoinductive. However, it is thought that FDBA may be more osteoconductive and may provide a better scaffold for space maintenance (Piattelli et al., 1996). In order to eliminate the potential for the presence of interradicular bone in the core biopsy, multirooted teeth were excluded from the study. All bone graft materials were obtained from the same donor and had a standard particle size of 250 to 750 μm. A total of 33 patients completed the study and 32 biopsies were analyzed histologically. There were no statistically significant differences between the changes in ridge dimension after ridge preservation with DFDBA and FDBA. However, histology showed a significantly greater percentage of vital bone in sites grafted with DFDBA (81.26%) versus FDBA (50.63%), and DFDBA sites had significantly fewer residual graft particles (Wood and Mealey, 2012).

Effect of Particle Size on Healing

Several studies have reported on the effect of particle size on various regenerative procedures (Pallesen et al., 2002, Shapoff et al., 1980, Fonseca et al., 1980, Testori et al., 2013, Chackartchi et al., 2011, Hoang and Mealey, 2012). As a general rule, smaller particles may have enhanced osteogenic potential, greater surface area, and more rapid resorption, but larger particles may provide better space maintenance (Hoang and Mealey, 2012).

Pallesen et al. investigated the influence of particle size of autogenous bone grafts in the early stages of bone regeneration in critical-sized cortical skull defects in rabbits (Pallesen et al., 2002). Small (0.5 to 2 mm^3) and large (10 mm^3) autogenous bone particles were used. The results showed that the total volume of newly formed bone in defects

where small particles were used was greater and more mature compared to defects with large particles after two and four weeks. The resorption of the small particles was enhanced which reflects a higher level of bone substitution compared to large particles over the healing time frame (Pallesen et al., 2002).

Shapoff et al. performed a study to determine if particle size was a factor affecting the osteogenic potential of FDBA (Shapoff et al., 1980). Small particles FDBA (100-300 μm) plus autogenous bone marrow and large particles FDBA (1000-2000 μm) plus autogenous bone marrow were placed in plexiglass diffusion chambers secured to the femurs of six Rhesus monkeys. The authors found significantly more new bone formation and more graft resorption associated with small particle FBDA plus autogenous bone marrow, concluding that within parameters small particles FDBA had the potential to enhance osteogenesis.

In an evaluation of onlay particulate autogenous bone graft healing in monkeys, Fonseca et al. evaluated the differences between two sizes of bone chips (2 x 2x 2 mm and 5 x 5 x2mm) in terms of revascularization and graft resorption (Fonseca et al., 1980). The small-particle graft showed quicker revascularization as well as increased osteoclastic activity and therefore was found to resorb more quickly and fully than the large-particle graft did. The large particles of autogenous cortico-cancellous bone graft led to a greater gain in alveolar ridge contour compare to smaller particles.

The use of large-sized particles of anorganic bovine bone matrix (Bio-Oss™; Geistlich Pharma, North American Inc., Princeton, New Jersey) resulted in significantly more vital bone formation than small particle grafts (26.77% ± 9.63% vs 18.77% ± 4.74%, respectively) in a bilateral sinus augmentation model (Testori et al., 2013). While

the histomorphometric results of this study indicated a statistically significant increase in vital bone formation when the larger particle size was used, these findings were not shown in a previous maxillary sinus augmentation study where there was not a statistically significant difference in the percentage of new vital bone formation (Chackartchi et al., 2011). The authors related the difference between studies to the small sample size included in both (<15 patients).

The effect of particle size on the clinical and histological outcomes of ridge preservation has been only scarcely studied or reported in the literature. Hoang and Mealey conducted a randomized clinical trial after molar extractions with the objective to histologically and clinically compare human demineralized bone matrix (DBM) putty with single particle size (SPS, 125 to 710 μm) to human DBM putty with multiple particle sizes of bone (MPS, 125 to 710 μm and 2 to 4mm)(Hoang and Mealey, 2012). Their hypothesis was that extraction sockets may benefit from the addition of larger particles to increase the ability of the graft to hold its shape within the socket. After 20 weeks of healing, core biopsies were obtained at the time of implant placement and analyzed for the percentage area of vital bone, residual graft particles, and non-mineralized structures (connective tissue (CT)/other non-mineralized tissue). The SPS group had a mean of 49% vital bone, 8% residual graft, and 43% CT and, the MPS group had 53%, 5%, and 42%, respectively. The difference in the percentage of new bone between the two groups was not statistically significant. The changes in alveolar ridge dimension were clinically measured and patients in both groups lost a mean of <1 mm alveolar height on the buccal and lingual aspects and <1.5 mm of total ridge width. The authors concluded that the addition of larger bone particles to DBM putty did not offer additional benefit in the preser-

vation of alveolar bone after the extraction of molar teeth. However, this conclusion is based on the effect of a mixture of different particle sizes within an extra particular matrix and a small number of patients. Most commercially available grafts are categorized in either small or large particle sizes.

A higher percentage of new vital bone is typically desired at the time of implant placement and is thought to be beneficial for the wound healing, as well as the long-term stability of implants (Barone et al., 2012, Wood and Mealey, 2012). The amount of new vital bone has been shown to vary with the use of different types of bone replacement grafts. However, the effect of bone graft particle size on the clinical and histological outcomes following site preservation at the time of tooth extraction has not been fully studied, which leaves us with conflicting information and a paucity of the literature on the topic. The objective of this clinical trial was to shed the light on the influence of bone graft particle size on the amount of new bone formation in the most common bone defect in the oral cavity, i.e. the extraction socket, after ridge preservation.

STATEMENT OF PURPOSE

The randomized trial aimed to clinically, radiographically and histologically compare the healing following ridge preservation procedures when using small- (0.25-1.0mm) versus large- (1.0-2.0mm) sized particle mineralized cortico-cancellous bone allografts (Maxxeus™ Dental, Community Tissue Services, Kettering, OH) at time of implant placement, three months following the surgical intervention. The specific objectives were the following:

A. To compare the dimensional changes (buccolingual ridge width) between the two bone allograft particle sizes by direct clinical measurements;
B. To compare the radiographic dimensional changes (buccolingual ridge width and apicocoronal ridge height) between the two groups using a superimposition of post-grafting and pre-implant cone beam computed tomography scans on a virtual implant planning software, coDiagnostiX™ (Dental Wings GmbH, Chemnitz, Germany);
C. To evaluate and contrast via histomorphometric analysis the new bone formation in the two groups by quantifying the distribution (in %) of new vital bone, soft tissue, and residual graft particles at the healed socket site.

The primary outcome is the new bone formation that is defined as the percentage of new vital bone area in the histomorphometric sections. The secondary outcomes are the clinical and radiographic dimensional changes of the extraction sites in millimeters.

MATERIALS AND METHODS

Patient Enrollment

The Institutional Review Board (IRB) of the University of Alabama at Birmingham (UAB) reviewed and approved the protocol for this study (IRB-161123001). A power analysis was performed in order to determine the minimum number of patients needed to detect a clinically significant difference. The amount of vital bone reported in the clinical trial by Testori et al. for both small- and large-sized particles (Testori et al., 2013) was used to run a two-sided two-sample unequal-variance t-test. It was determined that the inclusion of twenty-two patients in each group (total of 44 patients) would reach 0.90 statistical power to reject the null hypothesis of equal means with a significance level (alpha) of 0.05.

Patients presenting to the postdoctoral periodontal clinic at UAB School of Dentistry with a treatment plan for at least one tooth extraction and implant placement were screened according to the criteria listed in Table 1.

Table 1. Inclusion and Exclusion Criteria

Inclusion Criteria	**Exclusion Criteria**
Patient-related criteria	
• English speaking and able to read and understand the informed consent document • At least 18 years old • Planned for implant(s) to replace missing tooth or teeth in at least	• Systemic conditions contraindicating oral surgical procedures or adversely affecting wound healing • Significant medical conditions or habits expected to interfere with

one quadrant of the mouth • Registered patient at UAB School of Dentistry • Willing and able to comply with the preoperative and postoperative diagnostic and clinical evaluations required.	bone healing. • Por compliance risk (i.e., poor oral hygiene, history of alcohol or drug abuse, history of uncontrolled/severe psychological disease, etc.) • Smoking ≥10 cigarettes/day • Presence of active periodontal disease
Site-related criteria	
• Hopeless non-molar tooth or teeth that are planned to be replaced with dental implants after ridge preservation with healthy adjacent teeth not planned for extraction • Socket with residual 4 walls following minimally invasive tooth extraction and no more than one dehiscence ≤ 3 mm	• Complete or >3mm dehiscence of the facial bone plate as observed following extraction • Periapical pathology, and/or gross carious lesions in adjacent dentition

Molar teeth were not included to eliminate the possibility of existing interradicular bone being harvested as part of the core biopsy procedure. Patients' medical history and electronic health records were reviewed, and study examiners conducted clinical and radiographic examinations to determine eligibility. If patients were deemed eligible, study visits (Table 2) and objectives were explained to all participants and IRB approved written informed consent were obtained.

No monetary compensation was offered to patients, but they were not charged the fee normally associated with the performance of a Cone Beam Computed Tomography (CBCT) scan. A total of 19 patients (22 sites) were enrolled in the study. Although smoking < 10 cigarettes daily was not an exclusion criterion, no smokers were recruited.

Table 2. Schedule of Events

Procedures	Screening/ Baseline	**V-1** Extraction and ridge preservation procedure	**V-2** 2-Week Follow up	**V-3** 3 months post-grafting Biopsy and implant placement
IRB informed consent	X			
Medical history review	X	X	X	X
Inclusion/exclusion criteria	X	X		
Randomization to either SP or LP		X		
Tooth extraction + ridge preservation procedure		X		
Bone core biopsy				X
Implant placement				X
Clinical assessment of ridge dimensions		X		X
CBCT		X		X
Standardized photographs	X	X	X	X
Radiographic and clinical assessment of ridge changes				X

Extraction and Ridge Preservation Procedure (Visit 1)

The surgical procedures were performed by residents in periodontics, under the supervision of the study investigators. Each tooth site was randomized on the day of surgery to receive either Small Particle (SP) or Large Particle (LP) mineralized cortico-cancellous bone allograft by permuted block randomization approach to ensure the same number of patients in each group, using computer-generated random number list (provided by the statistician).

A loading dose of prophylactic antibiotics, either amoxicillin 2000 mg or clindamycin 600 mg, was dispensed 30 minutes to one hour prior to surgery. Local anesthesia was obtained via infiltration and/or nerve block with 4% septocaine 1:100 000 epinephrine, 2%lidocaine 1:100 000 epinephrine and/or 0.5% bupivacaine 1:200 000 epinephrine. Intravenous or inhalation sedation was performed when indicated for anxiolysis. The bone graft material (Maxxeus™ Dental, cortico-cancellous FDBA, Community Tissue Services, Kettering, OH) utilized on every subject came from one manufactured lot, which was obtained from a single donor to account for variation in age, race, gender and other healing potential that may be related to donor sources. The extraction was performed without flap elevation and the tooth was gently elevated with periotomes and other delicate instruments using a minimally traumatic technique in an attempt to prevent iatrogenic loss of supporting bone and minimize trauma to the alveolar bone. Following extraction, the socket was curetted, irrigated and thoroughly inspected for wall defects such as a fenestration, dehiscence, and/or other anomalies. A calibrated UNC-15 probe was used to clinically measure the buccolingual width at the crest, at the center of the extraction site. Measurements were made to the nearest half-millimeter by the same two examiners. Intra-examiner calibration was conducted to ensure the reliability of the measuring method. The randomized bone allograft (SP or LP) was hydrated in saline and the socket was filled to or slightly coronal to the level of the crest. The graft material was compacted prior to being covered with a collagen dressing. Vicryl or chromic gut sutures were used to achieve stability of the collagen dressing. A CBCT scan was taken immediately after completion of the ridge preservation procedure in order to evaluate radiograph-

ically the dimensions of the ridge at the extraction site. This was considered the baseline CBCT radiographic evaluation.

Post-surgical instructions were given verbally and written, and analgesics were prescribed and/or dispensed as deemed necessary by the treating surgeon. All subjects were prescribed 0.12% chlorhexidine gluconate mouth rinse to use for 30 seconds twice daily for two weeks and a seven-day course of either 500 mg amoxicillin or 300mg clindamycin to take 3 times daily.

Follow Up (Visit 2)

The sutures were removed after two weeks (± 3 days). Surgical sites were evaluated for healing status and postoperative instructions on resuming oral hygiene measures were provided to patients.

Core Biopsy and Implant Placement (Visit 3)

Patients returned three months post-socket grafting for the intake of a second CBCT scan used to evaluate the dimensional changes of the healed socket and plan the optimal implant position. The CBCT was captured within one hour of the core biopsy/implant placement surgery. Approximately 30 minutes prior to surgery a loading dose of prophylactic antibiotics was dispensed, either amoxicillin 2000 mg or clindamycin 600 mg. Following local anesthesia, a full-thickness mucoperiosteal flap was elevated at the edentulous space. The buccolingual width at the crest at the center of the healed extraction site was measured by one of the main examiners using a calibrated UNC-15 periodontal probe. Prior to implant placement, a bone biopsy was taken by the calibrated ex-

aminer, from the center of the healed site using a 2 mm internal diameter. The biopsy core was stored in 10% neutral buffered formalin and sent to UAB Histomorphometry and Molecular Analysis Core (HMAC) for histomorphometric analysis. The implant was then placed in the ideal restoratively driven position, using a surgical stent prepared by the restoring dentist. Depending on the primary stability of the implant, either a cover screw or a healing abutment was placed, and flap closure was obtained with vicryl or chromic gut sutures. Standardized intra surgical photographs were to be taken during the process. Post-surgical instructions were given verbally and written, and analgesics were prescribed and/or dispensed as deemed necessary by the treating surgeon. All subjects were prescribed 0.12% chlorhexidine gluconate mouth rinse to use for 30 seconds twice daily for two weeks.

Radiographic Measurements

Buccolingual dimensions and ridge height changes (immediately after extraction and after 3 months of healing) were radiographically evaluated with linear measurements using an implant planning software (coDiagnostiX™, Dental Wings GmbH, Chemnitz, Germany). In the initial CBCT scan, a digital implant was placed at the center of the extraction site, at the level of the crest. In the second scan, either the mandible or the maxilla was segmented, and the resulting segmentation was then superimposed over the initial scan (Figure 1). The same digital implant allowed for making measurements at the exact same locations at both superimposed CBCT scans and facilitated the direct calculation of the change in bone width and height between the scans at those locations.

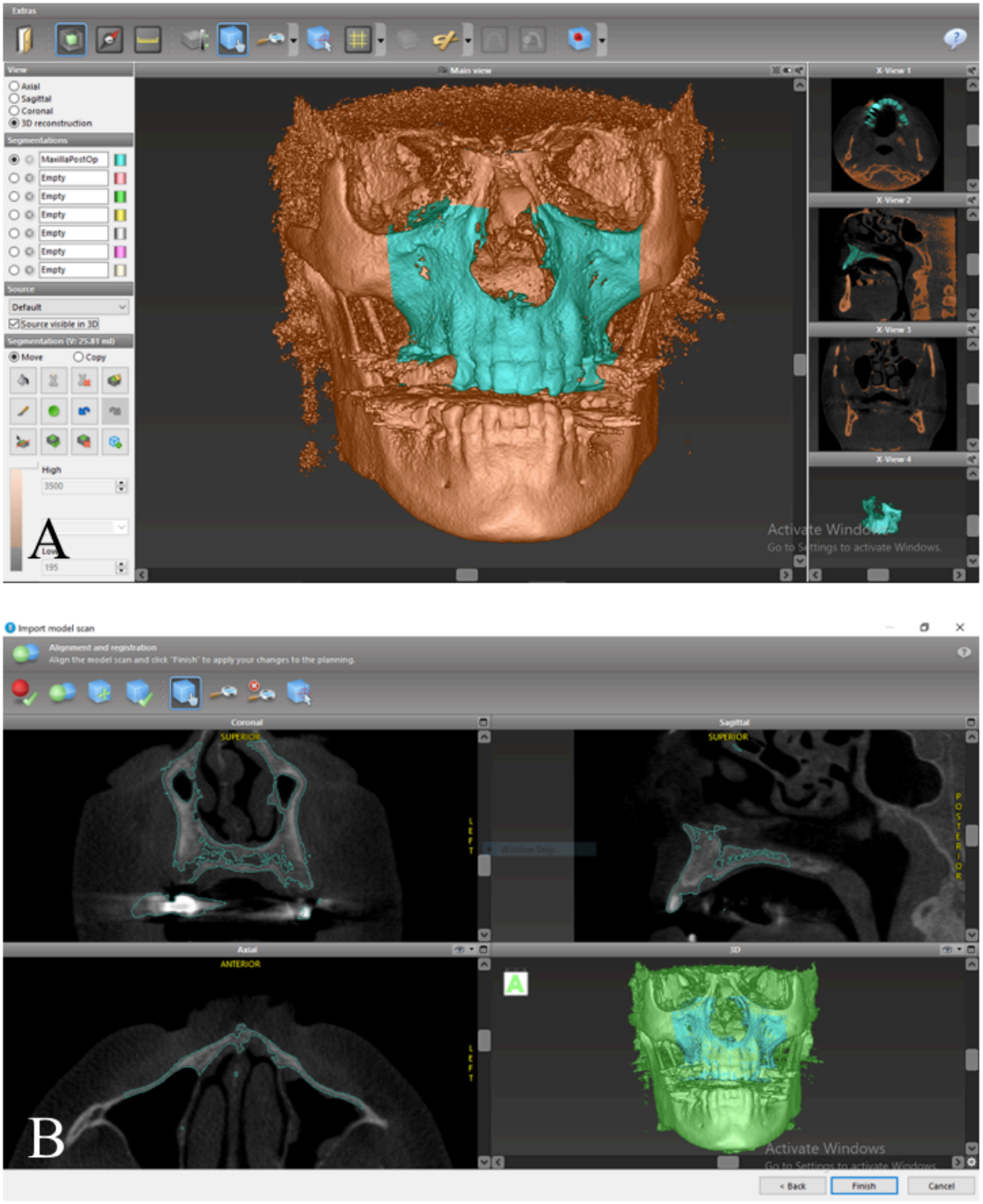


Figure 1. Segmentation process A. Segmentation of the second CBCT scan
B. Verification of the alignment between the baseline CBCT scan and the segmentation

Vertical measurements of the changes in bone height were taken at the facial, the mid-crestal, and the lingual of the crest using the platform of the digitally-placed implant

as a reference point. Horizontal measurements were taken at the crest, as wells as at three, six and nine mm apical to the crest (Figure 2). All measurements were taken by one examiner and were repeated twice in order to verify the reliability of the method and increase precision.

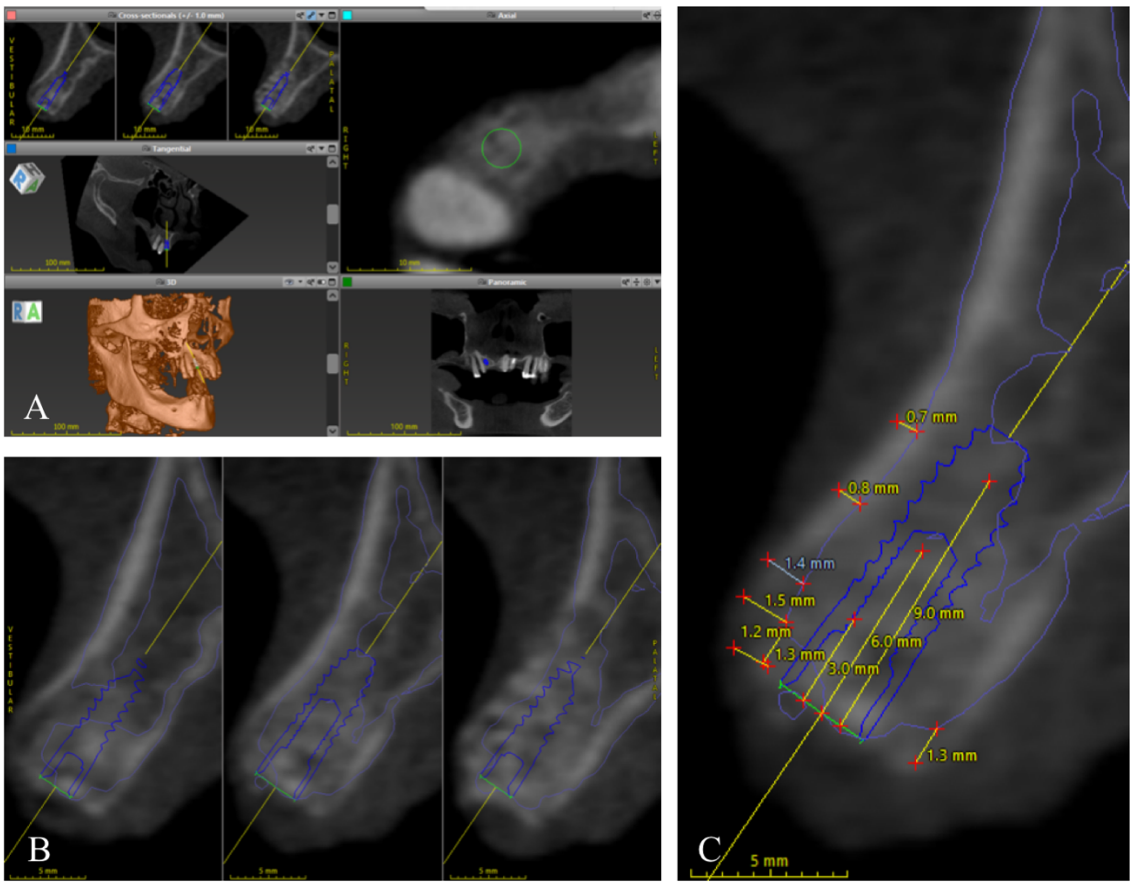


Figure 2. Radiographic measurements A. Digital implant placement in the baseline CBCT scan B. Superimposition of the segmentation over the baseline CBCT scan C. Measurements

Histomorphometric Analysis

Each specimen was fixated with 10% neutral buffered formalin for 48 hours prior to being dehydrated and embedded in methylmethacrylate. It was ground sectioned at the

center of the biopsy in its long axis into 50-70 micron-thick sections (Exakt Technologies, Inc., Oklahoma City, OK), and polished with 4000 grit sandpaper and Novus Polish to create a surface as smooth as possible. All sections were stained with Goldner's Trichrome Bone Stain and imaged for quantification of bone formation. Histomorphometry was done using the Bioquant® Image Analysis Software (R&M Biometrics, Nashville, TN) measuring the total area of new vital bone, residual graft particles, and soft tissue. Corresponding percentages were calculated for each of these tissues and compared between small and large particle grafts. These experiments and measurements were conducted by a single experienced blinded laboratory technician.

Statistical Analysis

All the outcomes were summarized as mean and standard deviation (SD). Comparison of the healing times between the two groups was done using a two-sample t-test (Snedecoor and Cochran, 1989). A two-sample t-test was conducted to compare the difference between the two groups in terms of new bone formation. Both the clinical and the radiographic dimensional changes between the groups were evaluated using two-sample t-tests. A paired t-test was used to compare among all subjects the radiographic vertical loss at the facial, midline, and lingual of the crest. The correlation between the clinical and radiographic changes in width at the crest and the influence of the type of tooth site on the radiographic dimensional changes were evaluated with two-sample t-tests.

RESULTS

Of the 19 subjects initially enrolled, a total of 15 patients (17 sites) completed the study; seven Caucasian males and eight Caucasian females aged between 46 and 86 years old. Among the four patients who were lost, one was disqualified at the time of tooth extraction due to an extensive facial dehiscence, and three were not able to return for the core biopsy and implant placement within the allotted time for the study. Figure 3 presents a flowchart of patients from enrollment to completion of the study including the total number of qualifying sites for clinical, radiographic, and histologic analyses in each group.

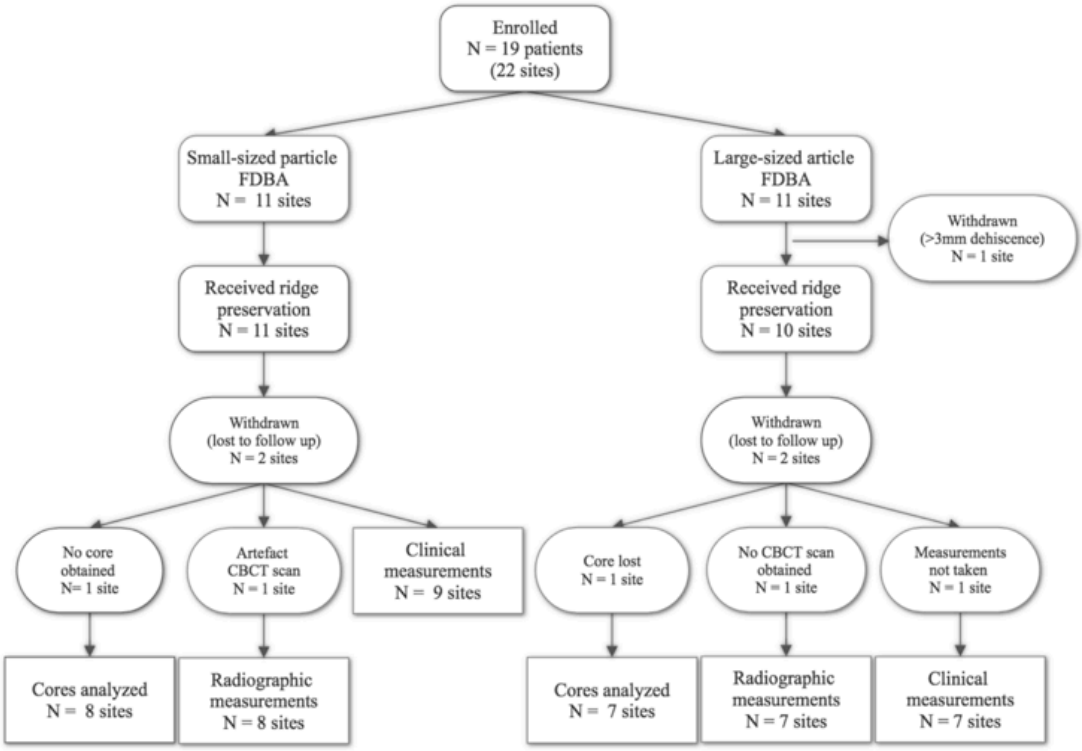


Figure 3. Study flowchart

The distribution of the 17 sites was as follows: two maxillary central incisors, three maxillary canines, five maxillary lateral incisors, five maxillary premolars, and two mandibular premolars.

Histomorphometric Findings

With respect to the primary outcome, data from 13 patients (15 sites) were analyzed, for a total of eight sites in the SP group and seven sites in the LP group. The mean healing time between the ridge preservation procedures and the harvest of the biopsy cores/implant placement was 16.3 ± 4.0 weeks; 14.6 ± 2.9 weeks in the SP group and 18.4 ± 4.0 weeks in the LP group (see Figure 4). The difference between the groups just reached statistical significance (p = 0.049) where biopsies from the SP group sites were harvested 4 weeks earlier (on average) than the LP group sites.

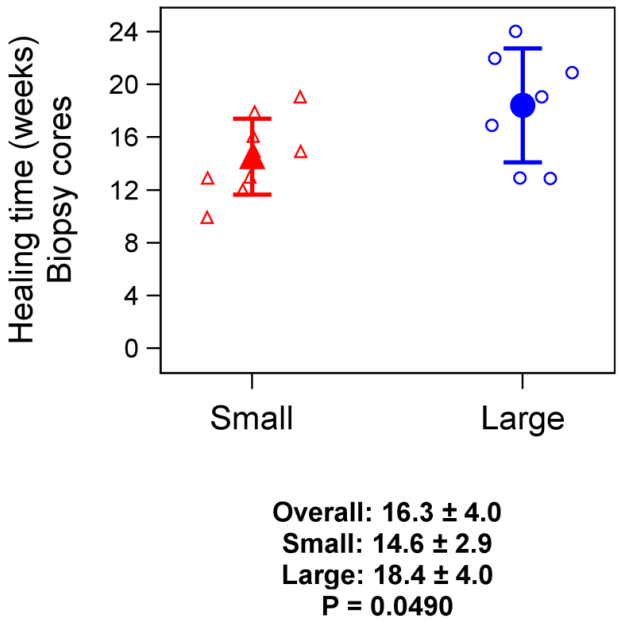


Figure 4. Healing time between the extraction with site preservation procedures and the harvest of the biopsy cores

The SP group had an average of 17.3% ± 12.8% new vital bone, 43.0% ± 11.7% residual graft particles and 39.7% ± 12.8% soft tissue. The LP group had a mean of 25.9% ± 9.5% new vital bone, 37.6% ± 13.8% residual graft particles and 36.5% ± 7.8% soft tissue (Figures 5 and 6). The difference between the two groups was not statistically significant for any of the three types of tissue (p = 0.1689, 0.4233 and 0.5828, respectively) (Table 3).

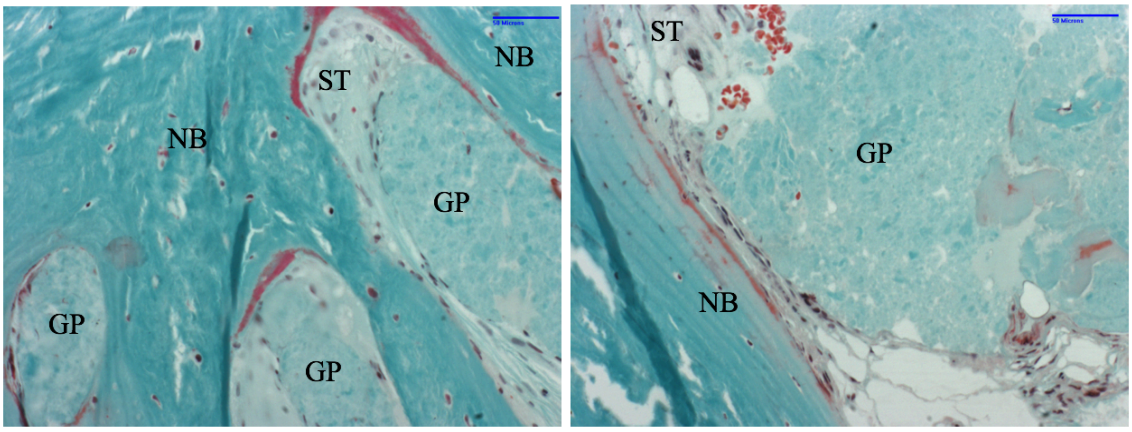


Figure 5. Histology at X20 magnification (NW = new bone, GP = graft particle, and ST = soft tissue)

Table 3. Histomorphometric Analysis

Outcome	N (SP vs LP)	Small	Large	p*
Total % New Bone	8 vs 7	17.3 ± 12.8	25.9 ± 9.5	0.1689
Total % Graft	8 vs 7	43.0 ± 11.7	37.6 ± 13.8	0.4233
Total % Soft Tissue	8 vs 7	39.7 ± 12.8	36.5 ± 7.8	0.5828

Mean ± SD; *t-test

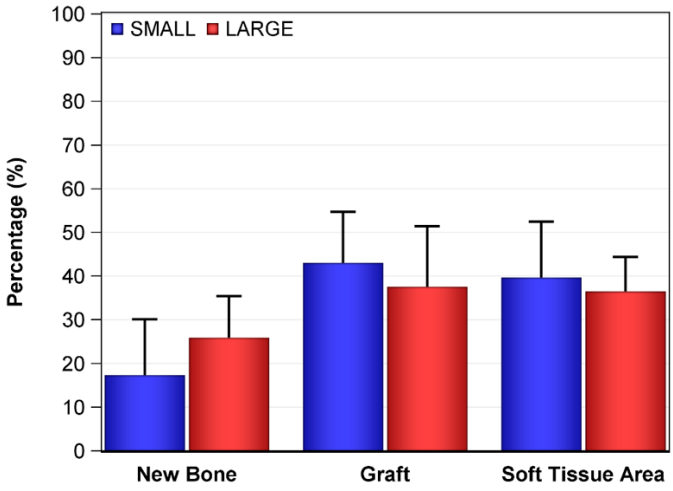


Figure 6. Tissue distribution in core sections per group

When the cores were divided into three zones (coronal, middle and apical), variation in the distribution was noted (Figure 7).

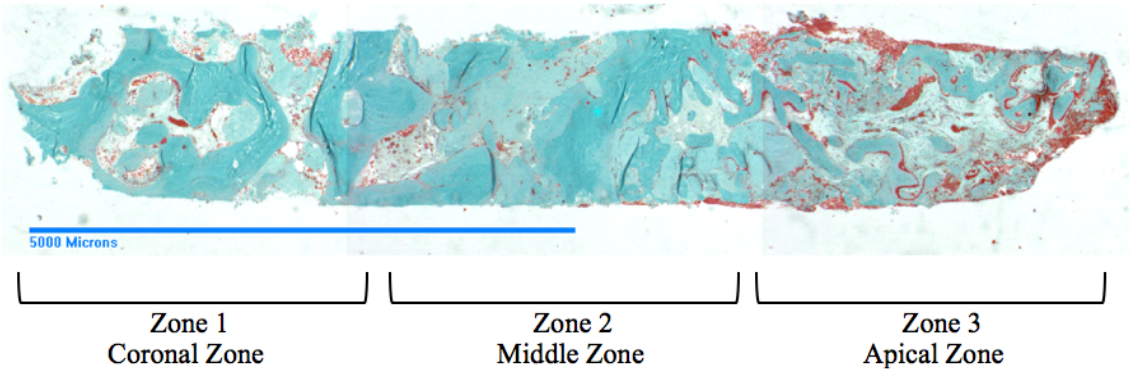


Figure 7. Biopsy core

In the coronal zone, there was 19.4% ± 16.3% new bone in the SP group and 32.5% ± 10.7% in the LP group (p=0.2108); 42.2% ± 20.7% residual graft particles in the SP group and 18.9% ± 6.5% in the LP group (p=0.0696); and 38.4% ± 8.0% soft tissue in

the SP group and 48.7% ± 7.3% in the LP group (p=0.0889) (Figure 8). In the middle zone, there was 25.6% ± 15.8% new bone in the SP group and 24.8% ± 11.2% in the LP group (p=0.9404); 47.2% ± 19.1% residual graft particles in the SP group and 38.5% ± 25.0% in the LP group (p=0.5709); and 27.2% ± 17.4% soft tissue in the SP group and 36.6% ± 14.1% in the LP group (p=0.4103) (Figure 9).

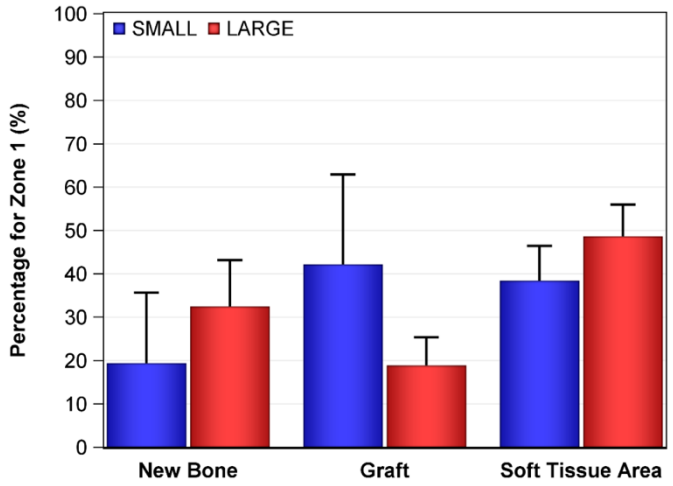


Figure 8. Tissue distribution in the coronal zone

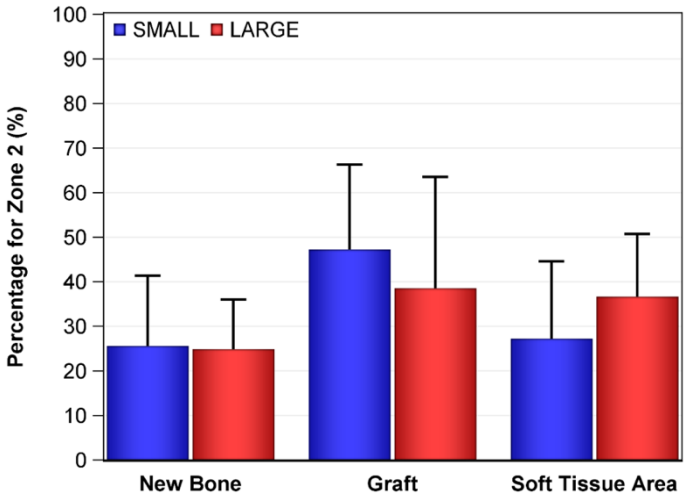


Figure 9. Tissue distribution in the middle zone

The apical zone was present in only two specimens with 48.7% new bone in the SP group and 33.1% in the LP group; 25.1% residual graft particles in the SP group and 31.2% in the LP group; and 26.2% soft tissue in the SP group and 35.7% in the LP group (Figure 10).

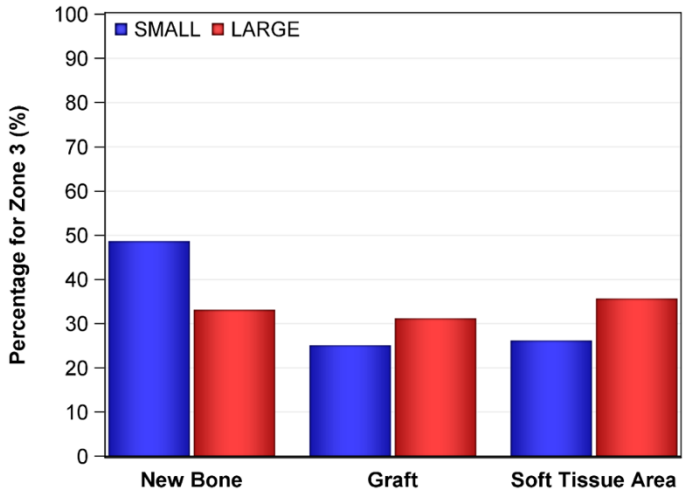


Figure 10. Tissue distribution in the apical zone

Radiographic Findings

The radiographic data from 13 patients/15 sites (eight sites in the SP group and seven sites in the LP group) were available for analysis. The mean healing time between the first and the second CBCT scans was 14.8 ± 4.0 weeks ($p = 0.502$); 14.1 ± 3.8 weeks in the SP group and 15.6 ± 4.3 weeks in the LP group (Figure 11).

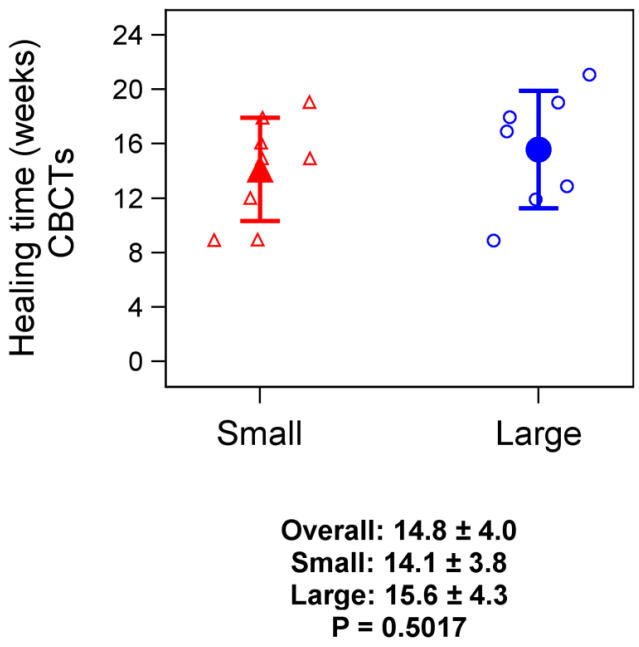


Figure 11. Healing time between CBCT scans

At the crest, the mean loss in ridge width was 1.0 ± 0.6 mm (p = 0.398); 1.1 ± 0.7 mm in the SP group and 0.8 ± 0.5 mm in the LP group (Figure 12). Three millimeters apical to the crest, the mean loss in ridge width was 0.4 ± 0.5 mm (p = 0.432); 0.3 ± 0.4 mm in the SP group and 0.5 ± 0.6 mm in the LP group (see Figure 13). Six millimeters apical to the crest, the mean loss in ridge width was 0.2 ± 0.4 mm (p = 0.558); 0.2 ± 0.3 mm in the SP group and 0.3 ± 0.4 mm in the LP group (Figure 14). Nine millimeters apical to the crest, the mean loss in ridge width was 0.2 ± 0.4 mm (p = 0.232); 0.03 ± 0.1 mm in the SP group and 0.3 ± 0.5 mm in the LP group (see Figure 15). No statistically significant differences could be demonstrated between the two groups at any of the four buccolingual width positions (Table 4). It was noteworthy that the bone width loss at 9mm from the crest in the LP group was almost 7-fold the corresponding loss in the SP group even though it lacked statistical significance.

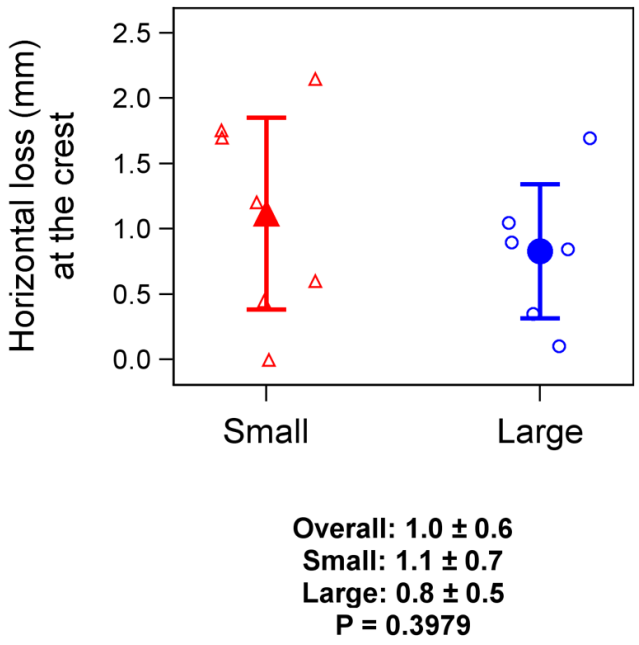


Figure 12. Radiographic change in ridge width at the crest

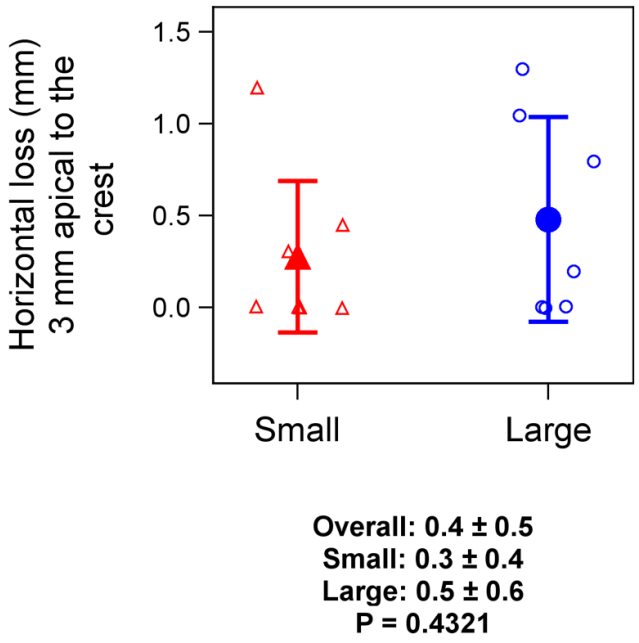


Figure 13. Radiographic change in ridge width at 3 mm apical to the crest

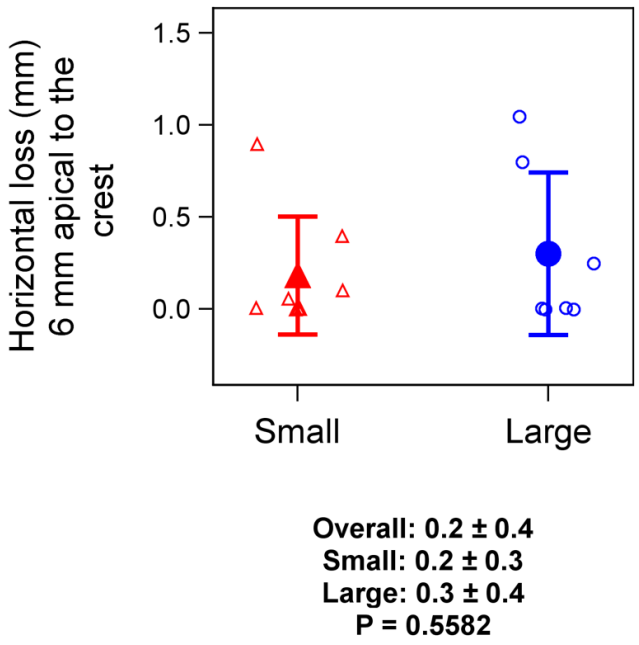


Figure 14. Radiographic change in ridge width at 6 mm apical to the crest

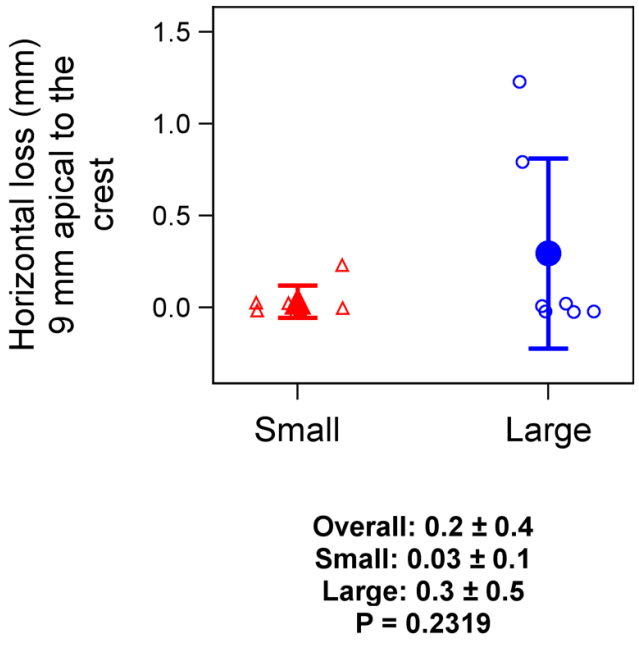


Figure 15. Radiographic change in ridge width at 9 mm apical to the crest

Table 4. Radiographic Outcomes

Outcome (mm)	Small (N=8)	Large (N=7)	P*
Loss of ridge width at the crest	1.1 ± 0.7	0.8 ± 0.5	0.3979
Loss of ridge width 3 mm apical to the crest	0.3 ± 0.4	0.5 ± 0.6	0.4321
Loss of ridge width 6 mm apical to the crest	0.2 ± 0.3	0.3 ± 0.4	0.5582
Loss of ridge width 9 mm apical to the crest	0.03 ± 0.1	0.3 ± 0.5	0.2319
Vertical loss – Facial of the crest	1.3 ± 1.0	1.1 ± 0.8	0.7182
Vertical loss – Center of the crest	0.5 ± 0.4	0.4 ± 0.5	0.6978
Vertical loss – Lingual of the crest	1.2 ± 0.9	0.9 ± 1.0	0.5045

Mean ± SD; * t-test

The mean loss in ridge height at the straight facial of the crest was 1.2 ± 0.9 mm (p = 0.718); 1.3 ± 1.0 mm in the SP group and 1.1 ± 0.8 mm in the LP group (Figure 16). The mean loss in ridge height at the center the crest was 0.4 ± 0.5 mm (p = 0.698); 0.5 ± 0.4 mm in the SP group and 0.4 ± 0.5 mm in the LP group (Figure 17). The mean loss in ridge height at the lingual the crest was 1.0 ± 0.9 mm (p = 0.505); 1.2 ± 0.9 mm in the SP group and 0.9 ± 1.0 mm in the LP group (see Figure 18).

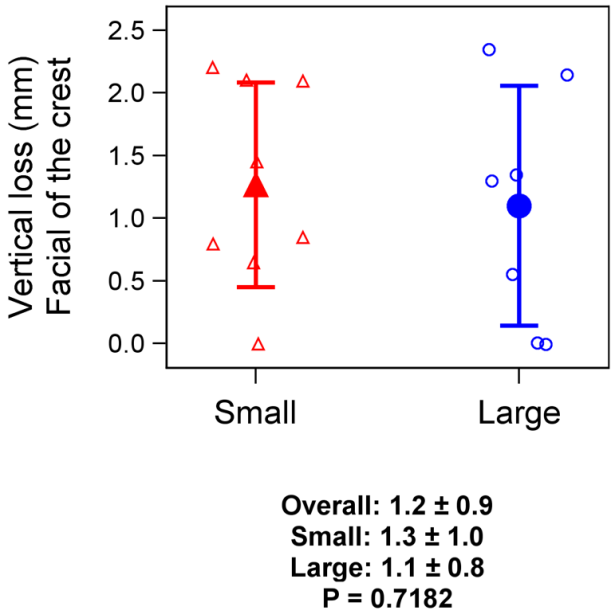


Figure 16. Radiographic change in ridge height at the facial of the crest

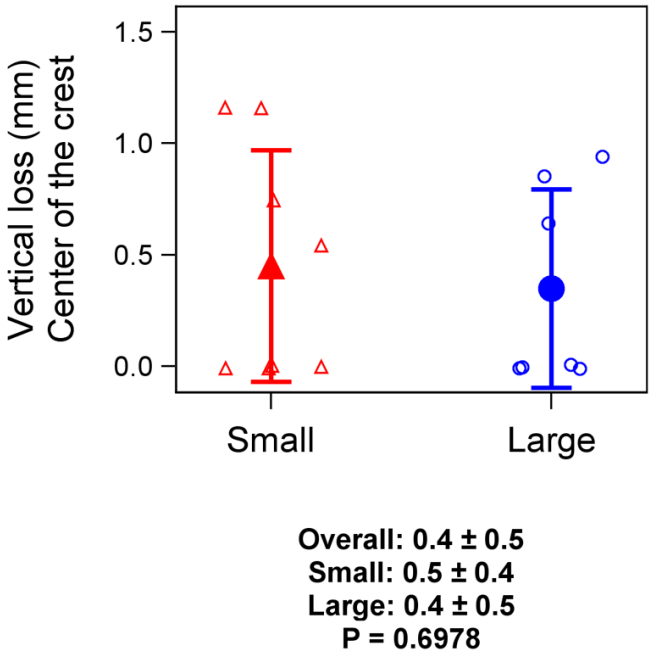


Figure 17. Radiographic change in ridge height at the center of the crest

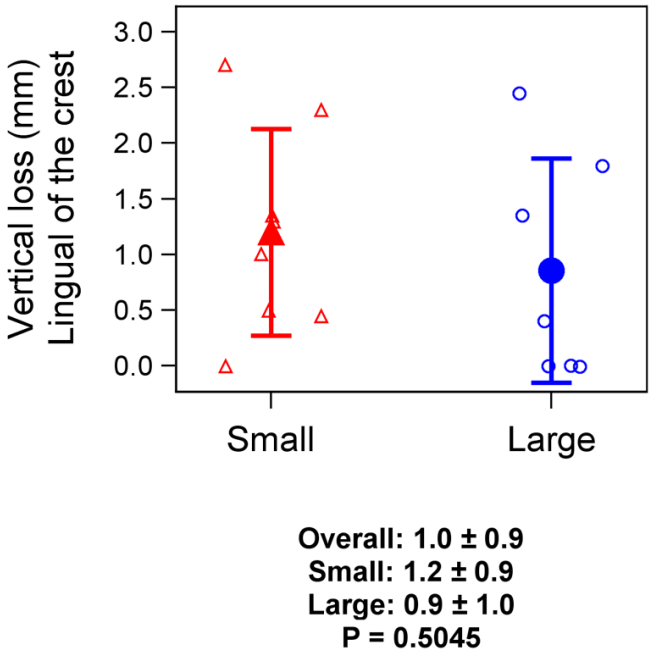
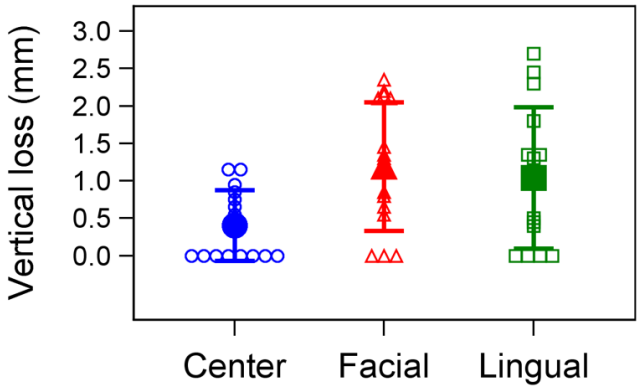


Figure 18. Radiographic change in ridge height at the lingual of the crest

There wasn't any statistically significant difference between the two groups at any of the three positions. However, when both SP and the LP groups were considered together, there was a statistically significant difference between the vertical loss at the facial (1.19 ± 0.86) and the vertical loss at the center (0.4 ± 0.47) (p = 0.002), as well as between the vertical loss at the lingual (1.04 ± 0.95) and the one at the center (p = 0.012) (Figure 19).



Center: 0.40 ± 0.47
Facial: 1.19 ± 0.86
Lingual: 1.04 ± 0.95
Center vs Facial P = 0.0023
Center vs Lingual P = 0.0115
Facial vs Lingual P = 0.5310

Figure 19. Vertical loss comparisons between the facial, center and lingual of the crest

Clinical Findings

The clinical data from 14 patients (nine sites in the SP group and seven sites in the LP group) were analyzed. At the crest the mean loss in ridge width was 1.1 ± 1.3 mm (p = 0.056); 0.6 ± 1.4 mm in the SP group and 1.8 ± 0.6 mm in the LP group (Figure 20). The difference between the two groups was statistically significant, with a greater reduc-

tion in ridge width noted in the LP group. No correlation could be detected between the clinical and the radiographic measurements (Figure 21).

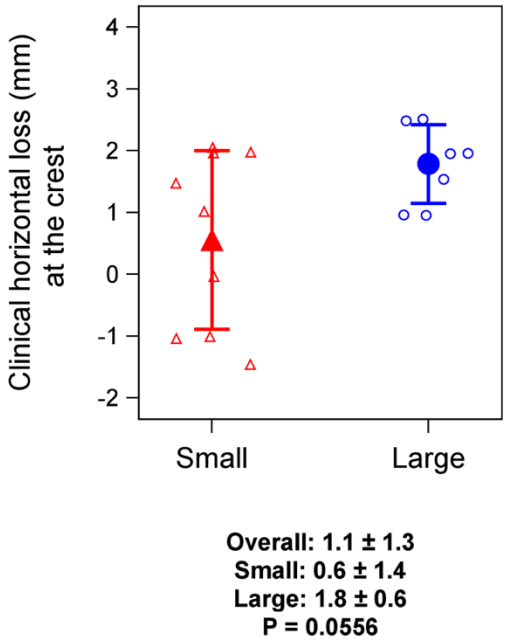


Figure 20. Change in ridge width at the crest measured clinically

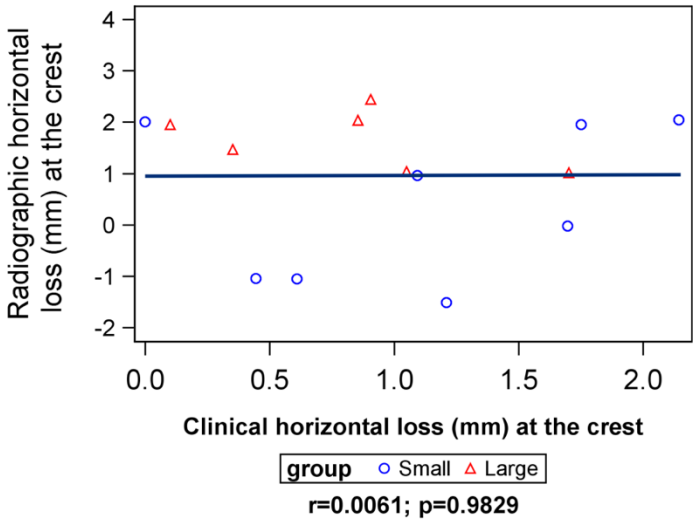


Figure 21. Correlation between radiographic and clinical horizontal ridge changes at the crest

Implant Related Outcomes

Dental implants were placed in all 15 patients (17 implants) without the need for any additional bone grafting procedure. In one patient, the implant was placed mostly in native bone just mesial to the site where grafting had been performed. Therefore, a biopsy core was not harvested from the preserved site to avoid jeopardizing the ideal implant position. In addition, bone density was evaluated by the study investigator performing the bone core biopsy as a tactile perception and categorized according to the Misch classification of bone density. One site was categorized as D1, seven sites as D2, seven sites as D3, and one site as D4.

DISCUSSION

Minimizing loss of alveolar ridge height and width by grafting the socket following tooth extraction (ridge preservation) can provide a better site for implant placement, optimizing the functional and esthetic outcomes of implant therapy. Currently, multiple materials are used for ridge preservation with various degrees of success, as measured by dimensional changes and new vital bone formation. The purpose of this randomized clinical trial was to evaluate and compare the outcomes of ridge preservation using small-sized versus large-sized particles of cortico-cancellous freeze-dried bone allograft.

All grafted sites exhibited new bone formation. Even though biopsy cores from the SP group showed a lower proportion of new vital bone compared to the LP group (17.3% ± 12.8% vs 25.9 ± 9.5%), the difference was not statistically significant. Previous studies have published histomorphometric data on the healing of sockets defects grafted with small particle-sized FDBA. In their attempt to compare the osteoconductive potential of cancellous and cortical FDBA, a clinical trial reported medians of 16.08% new bone in the cortical group and 12.98% in the cancellous group after an average of 18.2 weeks of healing ($p = 0.857$) (Eskow and Mealey, 2014). The graft material used in this study was a mixture of cortical and cancellous bone and after a mean of 14.6 weeks of healing, the small-sized particles led to results comparable to the findings from Eskow and Mealey.

The higher proportion of new bone formation in the LP group in this study may be explained by the larger inter-particular spaces that allowed for more new bone in-growth areas during the healing phase. Another explanation may relate to the longer healing time allowed in the LP group in comparison to the SP group (about four weeks of difference). Nonetheless, it was previously demonstrated in a ridge preservation study that healing time did not affect the proportion of new bone formation between three and six months, nor did it impact the average change in ridge dimension (Beck and Mealey, 2010).

Site preservation with small-sized particle FDBA and a collagen membrane led to 31% ± 9% new bone after four months of healing in a randomized clinical trial comparing the outcomes of extraction with ridge preservation and extraction alone (Iasella et al., 2003). This reported percentage of new bone is greater than the findings of this study (both groups) that included a comparable average healing time. In addition, a collagen dressing was used in the present study to cover the bone graft as opposed to the use of a collagen membrane in the above-mentioned study. It may be theorized that covering the graft with a collagen membrane (more slowly resorbing than collagen dressing) may reduce the soft tissue encapsulation of the coronal particles and therefore may increase the overall quantity of new bone.

Another study published on the outcome of human DBM putty with small particle size (SPS group) compared to human DBM putty with multiple particle sizes (MPS group, particles of 125 to 710 μm and 2 to 4 mm) (Hoang and Mealey, 2012). The average percentage of new bone in the MPS group after 20 weeks of healing was significantly higher than the one found in the LP group from this study after 18.4 weeks of healing

(52.7% ± 13.1% compared to 25.9 ± 9.5%). It is noteworthy that the MPS group included a mixture of larger particles and small-sized particles. In addition, the bovine collagen and sodium alginate carrier may have impacted the healing leading to the formation of a greater amount of bone. Therefore, a direct comparison with the findings of the current study is not possible.

In both the SP and LP groups, this research showed the least amount of new bone in the coronal aspect of the socket, compared to the middle and apical zones. This finding concurs with data previously published showing a higher amount of new bone in the apical zone for four different socket grafting material selections, after eight weeks of healing (Geurs et al., 2014). The natural healing of the socket defect, starting from the apical third with the coronal third being the last to revascularize, may explain the new bone distribution findings in both studies.

Bone loss occurred at all sites, which is consistent with previous reports (Avila-Ortiz et al., 2014, Jambhekar et al., 2015, Willenbacher et al., 2016). No statistically significant difference was found between the two groups in terms of dimensional changes measured clinically and radiographically. The mean loss in ridge width at the crest measured clinically (1.1 ± 1.3 mm) was in accordance with previously published findings (Beck and Mealey, 2010, Cardaropoli et al., 2012, Clark et al., 2018, Hoang and Mealey, 2012, Iasella et al., 2003, Whetman and Mealey, 2016). In this study, the changes in ridge width were measured radiographically at four different levels, and it was demonstrated that the majority of dimensional loss occurs at the most coronal aspect of the extraction socket three months post-extraction. The results were comparable to the findings from another clinical trial in which the ridge width was measured radiographically at three, six

and nine millimeters below the crest 10 to 12 weeks following extraction and site preservation with a composite graft product that combines demineralized bone matrix with cancellous bone chips in a proprietary, reverse phase medium (DynaBlast™). The authors reported a mean loss of 1.6 ± 0.8 mm, 0.8 ± 0.4 mm, and 0.6 ± 0.9 mm, respectively (Brownfield and Weltman, 2012). The dimensional changes in width also concurred with data demonstrating that extraction sites grafted with FDBA lost a mean of 2.5 ± 0.1 mm in the coronal third of the ridge, and a mean of 1.2 ± 1.3 in the apical third when measured clinically with surgical calipers after an average of 15 weeks healing (Clark et al., 2018). While the trend of bone loss is similar in decreasing from the coronal to the apical aspect, the amount of reported ridge width loss is significantly less in the present study when compared to the results by Clark et al. Differences may be explained by the type of FDBA used, graft compaction into the sockets, and the measurement methodology (clinical versus radiographic).

Previous research has shown a significant correlation between the loss of vertical ridge height and buccal plate thickness (Brownfield and Weltman, 2012). In that investigation, thick sites (mean buccal plate thickness of 1.3 mm) lost a mean of 0.2 mm vertical ridge height after extraction and grafting, while thin sites (mean buccal plate thickness of 0.9 mm) lost a mean of 1.9 mm. Interestingly, in the current study, a significant difference was found between the loss of ridge height at the facial and lingual aspects of the crest versus the vertical loss of bone at the center. The facial and lingual mean vertical loss was 1.19 ± 0.86 mm and 1.04 mm ± 0.95, respectively. The mean vertical loss at the midline of the crest, however, was only 0.4 ± 0.47 mm. That finding is clinically relevant and provides information on the pattern of resorption of the ridge following site preserva-

tion and may allow for a more accurate prediction of alveolar ridge height changes at sites where implants are planned after post-extraction remodeling.

To our knowledge, this is the first study that evaluates differences in ridge height changes at the aforementioned locations (facial, mid-crest, lingual) using a CBCT superimposition technique. Historically, ridge height changes are clinically reported at the facial and lingual (Eskow and Mealey, 2014, Hoang and Mealey, 2012, Whetman and Mealey, 2016) or coronally (Brownfield and Weltman, 2012) but do not include mid-crestal changes. The current study demonstrates a statistically significant and clinically relevant distinction between the middle of the ridge and its facial and lingual boundaries. The healed ridge appears to preserve its pre-extraction architecture where the mid-interproximal bone tends to be more coronal than the facial and lingual bone levels. The current findings suggest that ridge preservation procedures help maintain the overall shape of the alveolar bone during the remodeling process, in part due to the greater vascularity and thickness of the interproximal bone when compared to the much thinner and less vascularized facial and lingual bone where more bone loss occurs.

Most data available on the dimensional changes of the ridge following tooth extraction and ridge preservation procedures come from clinical measurements (Beck and Mealey, 2010, Cardaropoli et al., 2012, Clark et al., 2018, Eskow and Mealey, 2014, Hoang and Mealey, 2012, Iasella et al., 2003, Whetman and Mealey, 2016). However, few have published on the radiographic assessment of the reduction in ridge height and width resulting from tooth loss (Brownfield and Weltman, 2012, Schropp et al., 2003). This clinical trial has used CBCT scans to evaluate radiographically the changes in dimension following tooth extraction and ridge preservation.

Multiple published studies on the outcomes of tooth extraction and ridge preservation procedures have used a physical intraoral stent to standardize their clinical, cast-based, or radiographic measurements of the alveolar ridge (Brownfield and Weltman, 2012, Cardaropoli et al., 2012, Clark et al., 2018, Eskow and Mealey, 2014, Iasella et al., 2003, Whetman and Mealey, 2016). One specific study used a radiographic stent with three radiopaque markers serving as reference points on the CBCT scans; one at the facial, one at the coronal and one at the lingual aspect of each treated site (Brownfield and Weltman, 2012). While the current investigation did not use a physical stent, radiographic measurements were standardized via segmentation and superimposition of the two CBCT scans using coDiagnostiX™ and the placement of a digital implant in the initial scan at the level of the crest, in the center of each grafted extraction socket. This workflow mitigated the use of a physical stent with radiopaque markers by making linear measurements at the exact locations in width and height on the superimposed scans simultaneously. It may be argued that the methodology was validated by the concordance of the present results with the reported literature on dimensional changes of sockets defects.

Statistical analysis showed no correlation between the clinical and the radiographic measurements of the change in ridge width at the crest. The clinical measurements may have lacked precision since they were taken by different examiners using a UNC-15 probe without any site-related standardization technique. As part of their study protocol, Clark et al. created a measurement stent made of light-cured resin for each of the patients. The alveolar ridge width was measured with calipers at the coronal, middle and apical third of the ridge at the extraction site. In order to allow for reproducible measurements,

demarcations were made on the stent flanges indicating where the initial measurements were taken (Clark et al., 2018).

Due to time restriction, difficulty recruiting qualifying participants, as well as strict inclusion and exclusion criteria, the sample size of this clinical trial was small resulting in a limited statistical power. A total of 19 subjects were enrolled, among which 15 completed the study for a total of 17 sites (nine sites in the SP group, eight sites in the LP group). Another limitation is that most of the treated sites were located at the maxilla, therefore the conclusions may not apply to the mandible.

Variability in bone grafting material was well controlled in this study as the graft material used for all subjects came from one single donor and was processed as one lot. Only the particle size varied between the LP and SP groups. This allowed for standardization of graft-related healing potential that has been purported to relate to the age, race, or gender of the donor (Schwartz et al., 1998). It is interesting to note that the range of particle size (0.25-1.0 mm for the SP group and 1.0-2.0 mm for the LP group) practically allowed for some particles of similar size (close to 1.0mm) to be included in either group. The cortical chips were polyhedral in shape and consistent in size. However, the cancellous chips were frangible and of smaller size. In particular, the use of the cancellous particles in the LP group likely resulted in the inclusion of particles of ≤1mm in the sites grafted with large particles after compaction in the socket defects, thus potentially affecting the overall histologic outcomes.

Bone biopsy cores were significantly more difficult to harvest in the LP group compared to SP, which resulted in smaller and more crumbled specimens. Certain samples, because of their limited size, were not divided into three zones, namely the coronal,

middle and apical zones. Another challenge at histomorphometry was the difficulty in distinguishing the coronal portion of the core from the apex.

In a patient-level analysis of radiographic horizontal bone loss measured nine mm apical to the crest, the data from two patients in the LP group were found to be outliers. On both of the initial scans, a lack of continuity in the apical portion of the buccal plate of bone was identified, which may represent buccal fenestrations that were not diagnosed clinically. It has been previously shown that a significant reduction in the ridge dimension can be expected in the presence of a fenestration defect (Chen and Darby, 2017). Excluding these samples from the analysis considerably reduces the mean change in ridge width at nine mm apical to the crest for the LP group. Furthermore, in one subject in the LP group, the second scan was taken after replacement of the CBCT scan equipment with a newer model. This may have had an impact on the radiographic measurements recorded for that specific patient and the ability to compare baseline and post-healing radiographic images.

The long-term stability of dental implants is thought to depend, among other things, on the quality and the quantity of the available alveolar bone volume at the implant placement site. It is notable that this has not been fully examined in the current scientific literature (Whetman and Mealey, 2016). A previous study evaluated the need for additional augmentation procedures at the time implant insertion, as well as the success rate and the marginal bone loss for implants placed in sites where ridge preservation was performed at the time of tooth extraction versus those placed in naturally healed sites. At the end of the three-year examination, the cumulative implant survival rate was 95% with no significant difference between the two groups in terms of implant failure rate and

mean marginal bone loss (Barone et al., 2012). Similarly, a systematic review found no difference was in term of dental implant survival rates between implants placed in sites where ridge augmentation or preservation techniques had previously been used compared to implants placed in native bone (Fiorellini and Nevins, 2003). Furthermore, two systematic reviews compared various grafting technique in regard to their ability to support implant placement and survival, and no conclusions could be drawn about the effectiveness those graft materials in a ridge preservation model due to the small sample size and the lack of homogeneity within and across studies (Aghaloo and Moy, 2007, Darby et al., 2009). Two studies, however, found a cumulative implant survival rate of 90.3% at three years for implants placed at sites where ridge preservation had been performed (Norton and Wilson, 2002, Sandor et al., 2003). It is notable that alloplast materials were used in both of these clinical trials and a relatively long healing time was required to achieve a small amount of new vital bone incorporation into the graft (Norton and Wilson, 2002). The outcome of implant therapy at sites with previous alveolar ridge preservation was not evaluated in the current clinical trial and it remains unknown if the comparatively higher proportion of new vital bone observed in this study when compared with previous investigations may have a positive impact on the implants' survival and success rates.

CONCLUSION

The present randomized clinical trial aimed to compare the outcomes of extraction with site preservation performed with small-sized particles versus large-sized particles FDBA. The reported horizontal and vertical bone loss in ridge dimensions (in mm) is in accordance with the findings of previously published studies. The clinical and radiographic data indicate that there is no statistically significant variation in terms of dimensional changes between the two groups. Overall, the histologic outcomes also mirror those of similarly designed studies. The histomorphometric analysis showed no statistically significant difference in the amount of new bone, residual graft particles and soft tissue present among the SP and LP groups after an average of 16 weeks of healing, even though a greater percentage of new bone was achieved in the LP group. The clinical significance of this finding cannot be demonstrated within the study limitations. In conclusion, both small-sized and large-sized FDBA particles are effective in ridge preservation following tooth extraction with comparable clinical and histologic results.

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APPENDIX A

IRB APPROVAL

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Form 4: IRB Approval Form
Identification and Certification of Research
Projects Involving Human Subjects

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federalwide Assurance with the Office for Human Research Protections (OHRP). The Assurance number is FWA00005960 and it expires on November 8, 2021. The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56.

Principal Investigator:	ABOU-ARRAJ, RAMZI V
Co-Investigator(s):	AKERS, SHEILA D. GEISINGER, MARIA GEURS, NICOLAAS KAUR, MANINDER REDDY, MICHAEL S
Protocol Number:	**F161123001**
Protocol Title:	*A Randomized Trial to Evaluate the Effect of Allograft Bone Particle Size on Histomorphometric and Clinical Outcomes Following Ridge Preservation/Augmentation Procedures*

The IRB reviewed and approved the above named project on 2/15/2017. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received FULL COMMITTEE review.

IRB Approval Date: 2/15/2017

Date IRB Approval Issued: 3/1/17

IRB Approval No Longer Valid On: 2/15/18

Identification Number: IRB00000196

Ferdinand Urthaler MD/aw

Ferdinand Urthaler, M.D.
Chairman of the Institutional Review
Board for Human Use (IRB)

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.

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THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

February 1, 2018

MEMORANDUM

TO: Whom It May Concern

FROM: Margie Lawson, BS, CIP
Assistant Director, OIRB

RE: Protocol Number: IRB-161123001

Protocol Title: A Randomized Trial to Evaluate the Effect of Allograft Bone Particle Size on Histomorphometric and Clinical Outcomes Following Ridge Preservation/Augmentation Procedures

The above noted protocol was approved on March 1, 2017 with Drs. Hussein Basma and Gentiane Valiquette listed as investigators. This protocol is being conducted by Dr. Ramzi V Abou-Arraj.

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APPENDIX B

PROCEDURE CHECKLISTS

Randomized Clinical Trial: Outcomes Following the Use of Small versus Large Particle Sized Bone Allografts in Ridge Preservation Procedures

Please check yes or no as procedures are completed

Patient ID _____ Visit Date ____/ ____ /___

____ ___ ___

Mo Day Year

Visit-1 - Extractions and Grafting

1: Extracted Teeth # Site 1_#_____ ☐ Site 2_ #_____ ☐ Site 3_ #_____ ☐ Site 4_#_____ ☐

2: Amount used: _____ Bone Allograft ☐ LP ☐ SP

3: Socket Integrity (*measurements taken with a UNC 15mm periodontal probe - rounded up to the nearest millimeter)*

Sites	Socket Width (mm)		# Walls 1, 2, 3 or 4	Dehiscence		Fenestration		Wound Width (mm)	
	Mesio/ Distal	Bucco/ Lingual		Yes	No	Yes	No	Mesio/ Distal	Bucco/ Lingual
1									
2									
3									
4									
GM to Crest/Soft Tissue Measurements:			D:	M:		P:		F:	

4: Surgical Comments *(if any)*:

_____

_____

_

Please attach label from each graft vial used

Clinician's Signature _____ *Date:* _____

Randomized Clinical Trial: Outcomes Following the Use of Small versus Large Particle Sized Bone Allografts in Ridge Preservation Procedures

Patient ID _____ Visit Date ____/ ____ /___

___ ___ ___

Mo Day Year

Visit-1 - Procedures Checklist

Extractions and Grafting

PROCEDURE	YES	NO
Randomization assignment obtained		
Payment for Procedure (*copy of receipt*)		
Medical and Medication Review/Update *(if changed complete Medical and Meds log)*		
Pre-treatment antibiotics dispensed		
Vital signs measured		
Anesthesia accomplished		
Teeth Extracted		
Socket with residual 4 walls following minimally invasive tooth extraction (a dehiscence ≤ 3 mm may be included)		
Socket dimensions measured		
Standardized clinical photographs taken		
Post-extraction procedures completed in accordance with randomized group therapy		
Post-operative prescriptions written		
Post-operative CT Scan obtained (CBCT-1)		
Written post-operative instructions given		
Print-out of the EDR procedural note		

Clinician's Signature _____ *Date:* _____

Patient ID _____ Visit Date ____/ ____ /___

____ ___ ___

Mo Day Year

Visit- 3 - Procedure Checklist
Bone Biopsy and Implant Placement
(12 weeks +/- 3 days)

PROCEDURE	YES	NO
Medical and Medication Review/Update *If changes, complete Medical & Meds log*		
Pre-implant CBCT scan taken (CBCT-2)		
Pre-treatment antibiotics dispensed		
Vital signs measured		
Anesthesia accomplished		
Ridge dimensions measured at crest and 4 mm apical to crest		
Biopsy completed		
Implants Inserted		
Subjective Bone Quality Assessment		
Standardized clinical photographs taken		
Post-insertion Periapical Radiographs obtained		
Post-operative prescriptions written		

Clinician's Signature _____ *Date:* _____

Written post-operative instructions given		

*Please check **yes** or **no** as procedures are completed*

Patient ID _____ Visit Date ____/ ____ /___

____ ___ ___

Mo Day Year

Visit- 3- Bone Biopsy and Implant Placement

1: Procedures: Specify sites using relative tooth numbers (1-32)

Procedures	Site 1	Site 2	Site 3	Site 4	Indices
Bone biopsy (2 mm trephine) from one randomized site)					
Implant System					
Diameter and Length of Implants (mm)					
Subjective Bone Density *(D-1 -D-4)*					*D-1 Homogeneous dense compact bone D-2 Thick cortical compact bone surrounding a core of coarse dense trabecular bone D-3 Thin cortical compact bone surrounding a core of coarse dense trabecular bone D-4 Thin cortical compact bone surrounding a core of fine low-density trabecular bone*
Ridge B-L dimensions at crest (mm)					
Ridge B-L dimensions at 4mm apical to crest (mm)					
Estimated Torque					
Osstell	B P D M	B P D M	B P D M	B P D M	

Clinician's Signature _____ *Date:* _____