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A randomized trial to evaluate the effect of allograft bone particle size on histomorphometric and clinical outcomes following ridge augmentation procedures

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A RANDOMIZED TRIAL TO EVALUATE THE EFFECT OF ALLOGRAFT BONE PARTICLE SIZE ON HISTOMORPHOMETRIC AND CLINICAL OUTCOMES FOLLOWING RIDGE AUGMENTATION PROCEDURES

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A RANDOMIZED TRIAL TO EVALUATE THE EFFECT OF ALLOGRAFT BONE PARTICLE SIZE ON HISTOMORPHOMETRIC AND CLINICAL OUTCOMES FOLLOWING RIDGE AUGMENTATION PROCEDURES

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ABSTRACT

Ridge deficiency is an unfortunate obstacle that obviates the placement of dental implants or results in placing them at an angle that compromises the prosthetic restoration. An ideal volume is essential for implant placement in an optimal three-dimensional position. Several methods for augmenting the alveolar ridge in preparation for implant placement have been described.

Autogenous bone grafts, considered to be the “gold standard”, are associated with significant morbidity and require a second surgical site. Guided bone regeneration (GBR), is an alternative technique that use bone-substitute materials as adjuncts to or replacements for autografts in bone augmentation procedures to overcome the limitations related to the use of autografts. Freeze-dried bone allograft (FDBA), of various particle sizes, is commonly used today and has shown success in augmenting deficient ridges.

A graft material that promotes a high percentage of new vital bone is beneficial for implant placement and stability. The effect of particle size on the clinical and histological outcomes of lateral ridge augmentation (insufficient edentulous ridge width) has been scarcely studied or reported in the literature.

This randomized clinical trial aims to clinically and histologically compare the amount and quality of the bone gained after lateral ridge augmentation procedures performed using small (250-1000μm) versus large (1000-2000μm) particle size cortico-cancellous bone allografts at 6 months following surgical intervention.

Twenty-two patients, each presenting with ridge width less than 5mm received a lateral ridge augmentation. The patients were randomly allocated to small and large particle size graft. Trephine bone cores were taken from the 19 augmented sites out of 17 patients who completed the study, 6 months after augmentation for clinical, histologic and histomorphometric analysis. The gain in ridge width at the level of the crest and 4mm apical to the crest, was assessed before grafting and at time of implant placement, using a calibrated surgical caliper and coDiagnostiX software.

Large particle size graft (large, 5.1 ± 1.7; small, 3.7 ± 1.3 mm graft size) resulted in greater ridge width gain at the level of the crest and also 4mm apical to the crest (large, 5.9 ± 2.2 small, 5.1 ± 1.8 mm graft size) compared with the small particle size graft. No statistical significance for both outcomes (p=0.0642), (p=0.4480) respectively.

Bone samples from both the large and small particle size groups showed evidence of vital bone formation similar to that seen in previous studies, confirming the osteoconductivity of FDBA. Vital bone formation was more extensive in the small particle grafts compared with the large particle grafts (41.0 ± 10.1 % vs 31.4 ± 14.8 %, respectively. The most apical zone of the biopsy sample showed the highest percentage of vital bone in both groups.

The clinical and radiographic results showed that large particles result in more gain ridge width than small particles FDBA. However no statistical significance found .

The histologic results reaffirm the osteoconductive ability of FDBA when used as the sole grafting material in GBR procedures. The histomorphometric results at 6 months revealed an increase in vital bone formation when the small particle size was used. Additional studies should be performed to confirm these results.

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ABBM	anorganic bovine bone matrix
BMP	bone morphogenetic protein
CBCT	cone beam computed tomography
DFDBA	demineralized freeze-dried bone allograft
e-PTFE	Expanded polytetrafluorethylene
FDBA	freeze-dried bone allograft
GBR	Guided bone regeneration
IRB	Institutional Review Board
LP	large particle
MM	millimeter
SD	standard deviation
SOD	school of dentistry
SP	small particle
UAB	University of Alabama at Birmingham

INTRODUCTION

Dental rehabilitation of partially or totally edentulous patients with endosseous implants has become a routine treatment modality in the last decades, with reliable long-term result (Albrektsson, Zarb et al. 1986, van Steenberghe 1989, Lindquist, Carlsson et al. 1996, Buser, Mericske-Stern et al. 1997, Lekholm, Gunne et al. 1999, Weber, Crohin et al. 2000) However, early loss of teeth due to trauma, caries or periodontitis often leads to deformities in these resulting edentulous ridges. To treat this dimensional loss of bone volume, ridge preservation techniques have been used to maintain the alveolar ridge secondary to tooth extraction. In fact, without further treatment, crestal bone resorption is common and can't be avoided, which can lead to significant ridge dimensional changes. Bone resorption secondary to tooth extraction tends to occur over a 12-month period, mostly in the first 4 months following extraction **(Fig. 1)** and, depending upon location, may range up to 5–7 mm buccolingually.

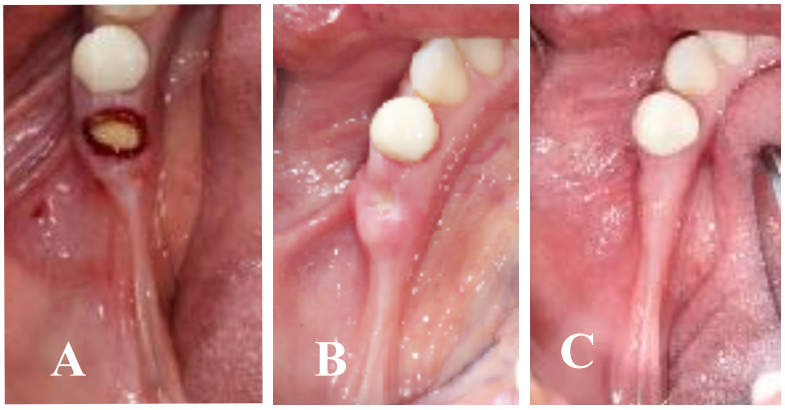


Fig. 1. Ridge resorption following tooth extraction. (A) Non Restorable tooth planned for extraction. (B) 1 week after tooth extraction (C) 12 weeks after extraction

In addition, 2–4 mm of vertical height loss frequently occurs along with the horizontal loss and usually is more seen when multiple adjacent extraction sites are combined (Cardaropoli, Araujo et al. 2003, Araujo and Lindhe 2005, Nevins, Camelo et al. 2006). Cardaropoli et al (Cardaropoli, Araujo et al. 2003) reported a negative correlation between initial buccal plate thickness and ridge width change at sites receiving tooth extraction without ridge preservation procedures. Furthermore, the presence of bone dehiscences or fenestrations during extraction may worsen post-extraction alveolar remodeling, leading to a severe buccal concavity after healing (Carlsson, Bergman et al. 1967).

As a result, insufficient bone volume or unfavorable vertical, horizontal, and sagittal inter-maxillary relationships may render implant placement, impossible or incorrect from functional and esthetic viewpoints or allow for inaccurate implant angulation (Khraisat, Abu-Hammad et al. 2004). In many cases, hard tissue augmentation is required prior to implant placement. Several methods for augmenting the alveolar ridge in preparation for implant placement have been described, including but not limited to: guided bone regeneration, block grafting techniques, ridge split, distraction osteogenesis, dental nerve repositioning, onlay bone grafting and also the use of narrow diameter implants.

Guided Bone Regeneration

Among these techniques, guided bone regeneration (GBR) has probably generated the most interest (Buser, Dula et al. 1996, Fiorellini and Nevins 2003). A main limitation

for successful bone healing and for formation of new bone is the rapid formation of the soft connective tissue that may disturb or totally prevent osteogenesis in a wound area. The influence of soft connective tissue on osteogenesis mechanisms is not yet fully understood. In vitro experiments showed that bone cell differentiation and osteogenesis are inhibited by one or more soluble factors that are secreted by fibroblasts (Wilson and Buser 1994). Another explanation suggested by Schmitz et al (Schmitz, Schwartz et al. 1990) is that a failure in bony-union development may be because of the failure of the cells that aid in calcifying the matrix, probably caused by the absence of suitable bone derived growth and differentiation factors in large bony defects. GBR **(Fig. 2)** works on the principle of compartmentalization, that is based upon the usage of a barrier membrane to prevent rapidly growing soft tissue cells from a bony defect allowing osteoblasts to populate the wound site before epithelial and connective tissue cells and, more importantly, to maintain a space for the slower process of bone formation. In a clinical situation, it is hard to predict the efficacy of ridge augmentation. To ensure successful GBR, four principles need to be met: space maintenance, exclusion of epithelium and connective tissue, stability of the fibrin clot, and passive wound closure.

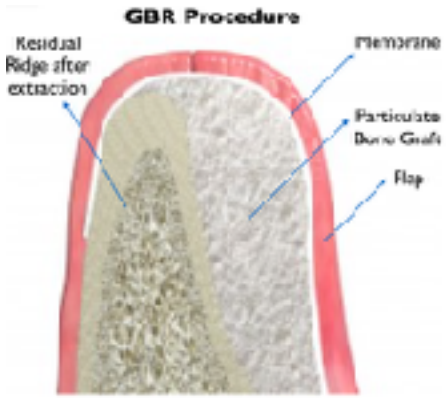


Fig. 2. GBR concept

Bone Substitutes

Bone grafts or bone substitutes **(Table 1)** are commonly used in GBR procedures to provide support for the barrier membrane, for additional space maintenance, and/or other properties. The four desired properties of bone graft materials are osteogenesis, osteoinduction, osteoconduction, and osteointegration. Osteogenesis is the formation and development of bone that occurs from osteoprogenitor cells that are present in the graft, survive the transplant, and proliferate and differentiate to osteoblasts, even in the absence of local undifferentiated mesenchymal stem cells. Osteoinduction entails the stimulation and recruitment of nearby undifferentiated mesenchymal stem cells to the graft site with in turn transform into osteoblasts or chondroblasts through growth factors that exist only in living bone. Osteoconduction is the process that provides a bio-inert scaffold, or physical matrix, suitable for the deposition of new bone from the surrounding bone or encourage differentiated mesenchymal cells to grow along the graft surface. This is an ordered process that results in the formation of new Haversian systems in a predictable pattern along the host-graft interface, which subsequently infuse into the graft material (Misch and Dietsh 1993) . Osteointegration is described as connection between the host and the graft material. This phenomenon is vital to graft survival. For the graft to be functional, a suitable amount of new bone must exist in the graft and unite with the host bone(Khan, Cammisa et al. 2005).

Graft Material	Source	Characteristic	Example
Autogenous Graft	taken from the host himself	osteogenesis, osteoinduction, osteoconduction	Intraoral: Ramus , Chin(Symphysis) Extraoral: Tibia
Allograft	taken from a member of the same species as the host but is genetically dissimilar	osteoinduction, osteoconduction	Cadaver cortical/ cancellous bone, FDBA, DFDBA
Xenograft	Grafts derived from a genetically different species than the host	osteoconduction	Deproteinized Bovine Bone Mineral(DBBM)
Alloplast	Fabricated graft materials	osteoconduction	Calcium sulfate, bioactive glass, Hydroxyapatite

Table 1: Bone graft and bone substitute synopsis

The different types of bone graft materials are autogenous, allografts, xenografts and alloplasts. All these types have one or more of the mechanisms of action mentioned earlier. The mechanisms by which the grafts act are normally determined by their origin and composition. Autogenous bone harvested from the host himself forms new bone by osteogenesis, osteoinduction, and osteoconduction. Allografts harvested from a member of the same species as the host, have osteoconductive and possibly osteoinductive properties, but they are not osteogenic. Xenografts (Fig. 3) are derived from a genetically different species than the host typically only osteoconductive. Alloplasts are fabricated and synthetic bone material that are also osteoconductive.

Fig. 3: Xenograft/Alloplast

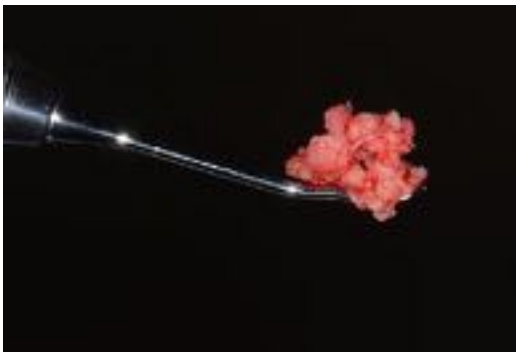


Fig. 4: Autogenous Bone graft harvested from the ramus of the same patient

Fig. 5: Allograft

So, the only graft material that contains all four qualities is autogenous bone. Autogenous bone grafts (Fig.4) are still considered the gold standard in bone regeneration procedures (Buser, Dula et al. 1996). However, donor site morbidity, unpredictable resorption, limited quantities available, and the need to include additional surgical sites are drawbacks related to autografts that have intensified the search for suitable alternatives (Buser, Bragger et al. 1990, Hjorting-Hansen 2002) Bone-substitute materials such as allografts, xenografts and alloplasts have increased in popularity as adjuncts to or

replacements for autografts in bone augmentation procedures to overcome the limitations related to the use of autografts

Allografts (**Fig.5**) consist of tissue transferred from one individual to another genetically dissimilar individual of the same species. Allografts are non-osteogenic and formation of bone usually takes longer and results in less regeneration than autogenous grafts. With allografts, there were concerns regarding the possibility of disease transmission through grafting; however, with careful donor screening and specimen processing, the risk is extremely low (Quattlebaum, Mellonig et al. 1988). The goal of these steps is to remove antigenic components and reduce host immune response while retaining the biologic characteristic of the graft. The grafts are prepared as fresh, frozen, freeze-dried, mineralized, and demineralized, and each preparation may be purchased as cortical chips, cortical granules, cortical wedges, or cancellous powder. Fresh or frozen allografts possess the highest osteoinductive and osteoconductive potential, but they are rarely used because of increased risk of host immune response and disease transmission. Compared with freeze-dried allografts, fresh or frozen allografts induce much stronger immune response, which is the primary reason why processed grafts are favored (Strong, Friedlaender et al. 1996). Freeze-dried, or lyophilized, grafts are the least immunogenic, but they possess inferior osteoinductive properties, mechanical properties, and strength compared with fresh or frozen (Gazdag, Lane et al. 1995). Host immune response and infection are reduced by eliminating the cellular phase of the allograft. Although freeze drying kills all cells, the chemical integrity of the graft remains intact (Mellonig 1992). Freeze-drying process is one of the used sample processing methods that can further help

prevent the risk of disease transmission (Mellonig, Prewett et al. 1992). Freeze-dried bone can be used in two forms, demineralized freeze-dried bone allograft (DFDBA) or mineralized freeze-dried bone allograft (FDBA).

FDBA has slower resorption than DFDBA because it is mineralized. FDBA provides an osteoconductive scaffold when placed in mesenchymal tissues. Regarding DFDBA, the demineralization process removes the mineral phase of the graft which can expose the underlying bone collagen and possibly leads to the release of bone growth factors like BMPs (Urist 1965, Mellonig, Bowers et al. 1981). Hence, DFDBA has more osteoinductive properties than FDBA. Most commercial bone banks do not confirm the presence or activity of BMPs in DFDBA nor the ability of DFDBA to form new bone. Schwartz *et al.* (Schwartz, Mellonig et al. 1996) found that DFDBA from different tissue banks had a variety of shapes and sizes and variable osteoinductive potential that are age-dependent, with stronger potential from younger donors (<50 years). Even from the same tissue bank, different batches may have different clinical results. This may partially explain why Rummelhart found similar clinical results between DFDBA and FDBA for periodontal osseous regeneration (Rummelhart, Mellonig et al. 1989). A study by Piattelli and colleagues (Piattelli, Scarano et al. 1996) found that FDBA particles farthest away from the host-graft interface were embedded in new bone, whereas DFDBA particles farthest away from the host-graft interface were surrounded with connective tissue.

Due to the success in space maintenance, rapid bone turnover, biocompatibility, and the lack of need to harvest from another site, allograft materials have become increasingly popular. Evidence-based treatment results indicate that guided bone

regeneration (GBR) for localized alveolar ridge deformities can effectively augment the ridge with new bone in the range of 1.5 to 5.5 mm (Buser, Bragger et al. 1990, Mellonig and Nevins 1995, Buser, Dula et al. 1996). Freeze-dried bone allograft (FDBA) is commonly used today and has shown success, both clinically and histologically in augmenting deficient ridges. Since particulate FDBA is clinically perceived to produce denser bone, its often preferred to DFDBA for lateral ridge augmentation prior to implant placement (Nevins and Mellonig 1992, Shanaman 1994). However, there is a paucity of documentation to verify this clinical impression. A graft material that promotes a high percentage of new vital bone is thought to be beneficial for implant placement and stability.

Barrier Membranes

Guided tissue regeneration is a barrier technique **(Fig.6)** that is used for the treatment of periodontal bone defects. Studies by Cosci *et al.* showed that if a barrier membrane was placed in direct contact with the surrounding bone surface and a space was created, only cells from the neighboring bone or bone marrow can migrate into this bone defect, without in-growth of competing soft tissue cells from the overlying mucosa (Cosci and Cosci 1994). There may be additional benefits to the use of a membrane, such as protection of the wound from mechanical disruption and salivary contamination. The criteria required to select appropriate barrier membranes for guided bone regeneration encompass biocompatibility, integration by the host tissue, cell occlusiveness, space-making ability and adequate clinical manageability. Currently, barrier membranes are of

two types, non-resorbable and resorbable. Expanded polytetrafluorethylene (e-PTFE), an ideal membrane for guided bone regeneration (GBR), is a fluorocarbon polymer that can be reinforced with titanium for increased strength, rigidity, and space maintenance. However, a second surgery is required for its removal, which increases site morbidity, patient discomfort, cost, and time. The main disadvantage to using e-PTFE is a high incidence of membrane exposure leading to bacterial colonization, bone loss, and failure of regeneration(Machtei 2001). Absorbable membranes are mainly collagen (porcine or bovine type I or III collagen) and synthetic membranes.

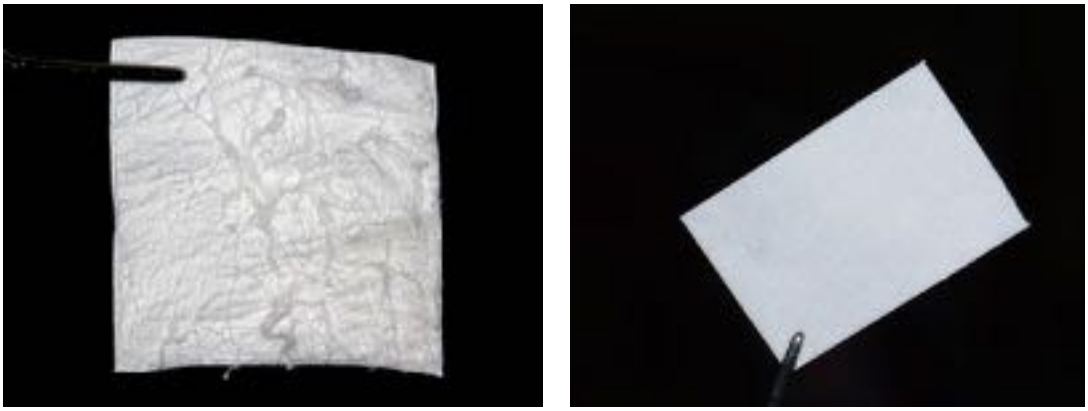


Fig. 6. Collagen Membrane Samples

The advantages of using collagen membranes include early wound stabilization through faster clot formation, increased migration of fibroblasts to the wound site, increased transfer of nutrients, and ease of handling (Bunyaratavej and Wang 2001). However, these membranes typically lack the rigidity of e-PTFE membranes, resulting in

membrane collapse, hence limiting regeneration. Therefore, they are frequently used together with bone grafts, which will support the membrane and maintain the space for regeneration.

Healing of Guided Bone Regeneration

Healing after a GBR Procedure follows a specific sequence of events. Within the first 24 hours after a bone graft, the graft material/barrier created space is filled with the blood clot that releases growth factors (e.g., platelet derived growth factor) and cytokines (e.g., IL-8) to attract neutrophils and macrophages. The clot is absorbed and replaced with granulation tissue which is highly vascularized and rich in blood vessels. Through these blood vessels, nutrients and mesenchymal stem cells capable of osteogenic differentiation can be transported and contribute to osteoid formation. Mineralization of osteoid forms woven bone (Schenk, Buser et al. 1994), which later serves as a template for the apposition of lamellar bone (Javed, Chen et al. 2010). This transformation of primary sponge work would eventually constitute both compact and reticular bone with mature bone marrow. These events occur 3 to 4 months post-surgery.

Effect of Particle Size on GBR

The effect of particle size **(Fig. 7**) on the clinical and histological outcomes of lateral ridge augmentation (insufficient edentulous ridge width) has been scarcely studied or reported in the literature. The published data have shown that a controversy in the effects of bone particle size on bone augmentation outcomes. In a study in Rhesus monkeys to

determine if particle size should be considered as a factor for evaluation of osteogenic activity of FDBA, there was significantly more new bone formation associated with small particle FDBA (100-300 μm) when mixed with autogenous marrow than that of the large particles (1000-2000 μm) (Shapoff, Bowers et al. 1980). Also, there was a marked resorption of small graft particles in the new bone formed. It was concluded that small particles FDBA enhance osteogenesis when mixed with autogenous marrow by increasing the number of pores.

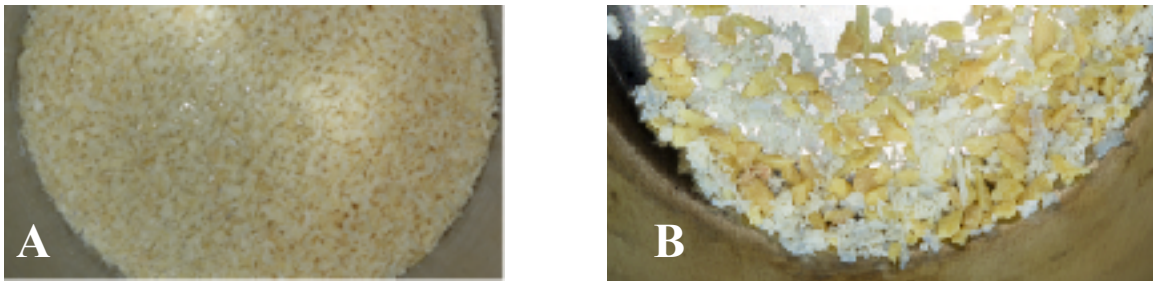


Fig 7. Allograft: (A)Small particles (250-1000μm),(B)Large particles (1000-2000μm).

A study done by Kon et al (Kon, Shiota et al. 2009) to investigate the impact of autogenous bone particle size in vertical augmentation using either small (150 to 400 mm) or large particles (1 to 2 mm). Results showed that large particle bone grafts have better outcomes in terms of bone volume, bone height and resorption rate when compared to small particles graft. In addition, a mixture of different sized autogenous bone particles was not found to be effective in maintaining the augmented bone volume.

Contradictory to these results, Zhou et al (Zhou, Zhang et al. 2011) and Pallesen et al (Pallesen, Schou et al. 2002) demonstrated that the use of small particle size bone grafts

can be more effective in bone augmentation procedures. Zhou et al. studied the effect of small and large Xenograft bone particles (300–500 and 850–1000 m) on the formation of new bone in GBR procedures in a rabbit cranial vertical augmentation model. They found a higher density of newly formed bone in the small-particle group at 4 and 10 weeks after implantation. Also, they concluded that the space between the bone particles is an important factor regarding osteoconduction, the contact length between newly formed bone and particles were significantly higher in the small-particle group at both time points. Pallesen et al. studied the influence of autogenous graft particle size on the early stages of bone regeneration in a rabbit Calvarium. In their study, small particles autogenous graft resulted in higher volume of newly formed bone after 2 and 4 weeks. Furthermore, this study stated that the resorption of small particles is faster and at 4 weeks there was higher level of bone substitution compared to large particles. With their experiment on the healing of onlay particulate autogenous bone grafts in monkeys, Fonseca et al (Fonseca, Clark et al. 1980) evaluated the differences between two sizes of bone chips (2x2x2 mm and 5x5x2mm) in terms of revascularization and graft resorption. The small-particle graft showed quicker revascularization as well as increased osteoclastic activity and therefore resorbed faster than the large-particle graft did. The large particles of autogenous cortico-cancellous bone graft led to greater gain in alveolar ridge contour compare to smaller particles

In a prospective randomized controlled clinical trial, Testori et al. (Testori, Wallace et al. 2013) compared the histologic and histomorphometric vital bone formation and residual graft volume in human bilateral sinus augmentations performed with either

large (1.0 to 2.0 mm) or small (0.25 to 1 mm) particle size anorganic bovine bone matrix (Bio-Oss) in 13 patients. For each patient, one compartment was grafted with 100% large particle Bio-Oss and the contralateral compartment was grafted with small particle Bio-Oss. At stage-one implant placement surgery 24 to 32 weeks later, a trephine core sample was taken (10 × 3 mm) from the former lateral window site as identified by measurements taken at the time of sinus elevation. Blinded paired histomorphometric and histologic analysis was subsequently performed on 11 bilateral cases. Vital bone formation was 26.77% ± 9.63% vs 18.77% ± 4.74% for the large particle and small particle grafts, respectively. Residual xenograft was 20.01% ± 8.97% vs. 21.66% ± 10.47% for the large and small particle grafts, respectively. At the 24- to 32-week time interval, the new bone appeared as woven bone with several large rounded osteocyte lacunae. Close contact between graft granules embedded in the mineralized bone and bone matrix was observed. Hence, the histologic results of this study indicate a statistically significant increase in vital bone formation when the larger particle size is 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, Iezzi et al. 2011). The authors related the difference between studies to the small sample size included in both (Testori, Wallace et al. 2013).

Thus, there was no final conclusion in the published data on the particle size that should be used to achieve the optimal ridge width and higher percentage of newly formed bone for placement of dental implants in the staged ridge. A higher percentage of new

vital bone is typically desired at time of implant placement and is thought to be beneficial for implant wound healing. 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 paucity of the literature on the topic. The objective of this clinical trial was to investigate the influence of bone graft particle size on the amount of new bone formation in ridge augmentation procedures.

OBJECTIVES / SPECIFIC AIMS

The randomized trial aimed to clinically, radiographically and histologically compare the quantity and quality of bone gained following ridge augmentation procedures when using small- (0.25-1.0mm) versus large- (1.0-2.0mm) sized particle mineralized cortico-cancellous bone allografts. Specific objectives of this clinical study (Table 2) were to histomorphometrically quantify the distribution of the different tissues (new bone, soft tissue, residual graft and artifact) with the use of the two commercially available mineralized bone allograft particle sizes (small vs. large) after 6 months of healing. Hence, the primary outcome was the new bone formation, defined as the percentage of new bone area in the histomorphometric sections. Secondary outcomes were clinical and radiographic quantification of bone width gain after ridge augmentation. Clinical assessment of bone density at time of implant placement (6 months post-op) was also performed.

Primary Outcomes	Secondary Outcomes
Quantitative histomorphometric evaluation of new bone formation 6 months after GBR	Quantitative clinical comparison of ridge dimensions (in mm) 6 months after GBR
	Quantitative two- and three-dimensional radiographic comparison of dimensional changes 6months using cone beam computed tomography (CBCT) and a virtual implant planning software, coDiagnostiX™

Table 2: Primary and Secondary outcomes of the study

MATERIALS AND METHODS

Patient Selection

This study was reviewed approved by the Institutional Review Board (IRB) of University of Alabama in Birmingham (UAB), protocol # F161123001. The minimum number of patients needed to detect a clinically significant difference was determined by a power analysis performed by the statistician. Based on the amounts of vital bone reported in the clinical trial by Testori et al (Testori, Wallace et al. 2013) for both small- and large-sized particles 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' medical history and electronic records were reviewed and study examiners conducted clinical and radiographic examinations to determine their eligibility. If deemed eligible, study visits and objectives were explained to all participants and IRB approved written informed consent were obtained. The surgeries were performed by three experienced surgeons at UAB department of Periodontology. A Total of 22 seeking treatment at the UAB SOD Graduate Periodontology clinics were recruited to participate in this study according to the criteria in Table 3.

Inclusion Criteria	Exclusion Criteria
Patient related criteria	
• English speaking and Able to read and understand informed consent document • At least 18 years old • Planned for implant(s) to replace missing tooth or teeth in at least one quadrant of the mouth • Registered patient at UAB dental school • Willing and able to comply with the preoperative and postoperative diagnostic and clinical evaluations required.	• Systemic conditions contraindicating oral surgical procedures or adversely affecting wound healing • Significant medical conditions or habits expected to interfere with bone healing. • Poor compliance risk (i.e., poor oral hygiene, history of alcohol or drug abuse) • Smoking ≥10 cigarettes/day • Presence of active periodontal disease
Site related criteria	
• Insufficient alveolar ridge width for endosseous implant placement defined as ≤5mm as determined by cone beam computed tomography (CBCT) scan.	• Vertical loss of bone at edentulous ridge

Table 3: Inclusion and Exclusion Criteria

Randomization

A computer generated pre-determined block permuted randomization (provided by the statistician) was used to individually assign each participant to a surgical group as he/she was recruited and revealed to the surgeon on the day of surgery. The two groups are as follows:

- Group 1: Small particle (SP) bone allograft (0.25-1.0 mm)
- Group 2: Large particle (LP) bone allograft (1.0-2.0 mm)

The graft material utilized for all surgical procedures, for all patients, was obtained from one manufactured lot, from the same donor to account for variation in age, race, gender and related healing potential of different grafts (Maxxeus™ Dental, mineralized corticocancellous bone allograft, Community Tissue Services, Kettering, OH).

Screening/Baseline Visit

Upon enrollment into the study, patients were treatment planned by an interdisciplinary team consisting of a periodontist and a restoring dentist and corresponding residents. Cone Beam Computed Tomography (CBCT) scan was taken to optimize the treatment plan and determine the feasibility of implant placement following bone grafting. Surgical guides were prepared by the restoring dentist/resident to represent the future implant position. One trained examiner conducted clinical and radiographic exams to determine eligibility according to the above inclusion criteria. A calibrated examiner was available for all study visits when clinical measurements were required and was blinded with regards to the randomization. Another examiner performed the radiographic evaluations and was also blinded to the randomization process. Intra-examiner calibration was conducted to ensure reliability of measuring method.

Clinical parameters	Timing of measurements
Cone Beam CT Scan	Screening, 6 months post-op
Width of bone at the crest level and 4mm apical using a gauge(Pre-surgical) and a surgical stent (surgical)	Screening, Surgery (Pre-surgical), 6 post-op
Biopsy at the center of healed bone graft	6 months post-surgery

Table 4. Clinical parameters and their respective timing of measurements

Visit 1: Guided Bone Regeneration Surgery (**Fig. 8**)

Each 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. A loading dose of prophylactic antibiotics was dispensed at the time of surgery (Amoxicillin 2g, 30 minutes to one hour prior to surgery). If the patient was allergic to penicillin, Clindamycin 600mg was substituted. Patients were given a 0.2% chlorhexidine solution for 1 minute to rinse with in order to disinfect the surgical site to minimize the potential contamination from extraoral sources. A local anesthesia with 4% Articaine Chlorhydrate and epinephrine 1:100000, was applied. The flap design was made to ensure primary tension-free closure after the bone grafting procedure accommodate the dimensional increase after the augmentation. A crestal incision was made on the study quadrant using a 15c and 12b blade. A vertical incision was done at least one tooth away on both mesial and distal to the grafted area. Buccal and lingual full-thickness flaps was reflected to allow adequate access to the surgical site. Any anatomical structure was located prior to proceeding. A superficial

Periosteal releasing incision was made on the buccal surface to allow flap extension to achieve complete coverage of the barrier membrane and graft materials. After exposure of the bone the future implant sites were located with the use of the surgical guide and the ridge width were measured at the crest and 4 mm apical to the crest with standardized surgical calipers. The area of augmentation was decorticated using a high-speed hand piece with a #2 round bur perforating the cortical plate every 4 mm throughout the area needing the augmentation. The defect was grafted with the randomized bone allograft (SP or LP). The graft sites were covered with a resorbable collagen barrier membrane. The same type of membrane from the same manufactured lot was used for all defect sites; however, the membrane was trimmed to the volume of the graft, and care was taken to avoid contact with the edges of the adjacent teeth. The membrane was fixated with at least 4 surgical tacks for barrier stabilization. After the membrane was completely secured, the flap was mobilized to permit tension-free primary closure. All surgical sites were closed with vicryl sutures. Provisional fixed or removable appliances were relieved over the surgical sites prior to insertion. Standardized intrasurgical photographs were taken throughout the procedure.

Post-surgical analgesics were prescribed and/or dispensed as necessary. All subjects were dispensed Peridex® chlorhexidine mouth rinse and instructed to rinse twice daily for two weeks following the regenerative surgery, to help guard against possible infection. Prescriptions for relief of post-surgical discomfort, follow-up antibiotics (amoxicillin (875 mg) and clavulanic acid (125 mg) for 10 days, and written home care instructions was provided.

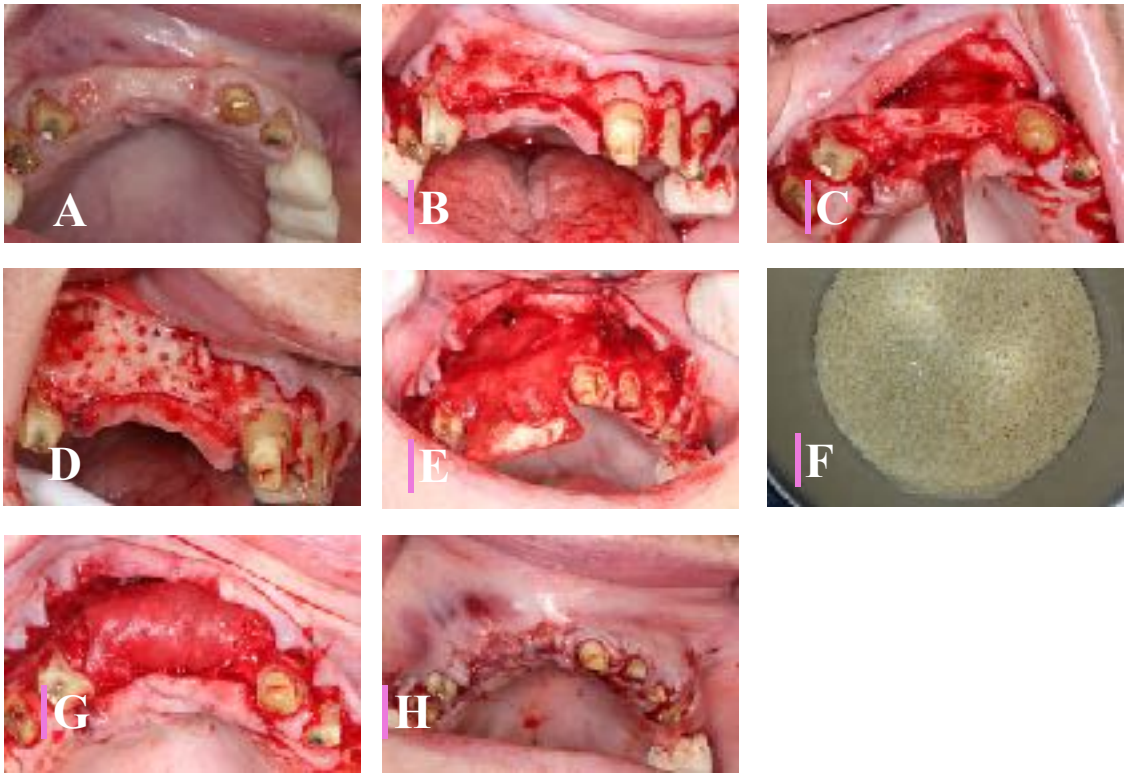


Fig. 8: GBR procedure randomized in the SP allograft group
(A) Initial presentation of the ridge
(B) Full thickness flap elevated after vertical and crestal incision
(C) Pre-Op Ridge: measurement at the level of the crest and 4mm apical to the crest
(D) Decortication of the ridge
(E) Fixation of the membrane using tacks
(F) SP allograft
(G) Membrane covering the grafted site
(H) Suturing the flap

Visit 2 (Follow-up): (**Fig. 9**)

The sutures were removed after two weeks after cleaning the sutures with a gauze soaked with Peridex® chlorhexidine mouth rinse. Surgical sites were evaluated for

healing status and postoperative instructions on resuming oral hygiene measures were instructed to patients.

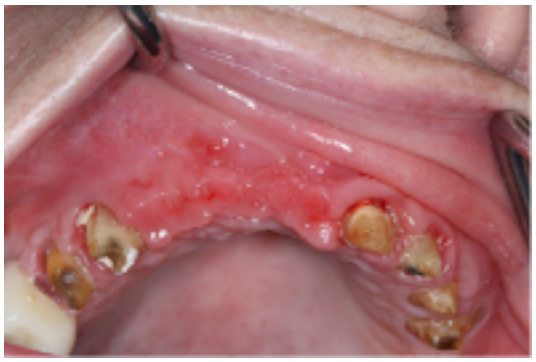


Fig. 9: Healing of GBR after 2 weeks

Visit 3: Bone Biopsy and Implant Placement

Six months post-ridge augmentation, a second CBCT scan was taken to evaluate the ridge width gain and to plan the optimal implant position. The surgical approach was similar to the procedures for the graft procedures. After exposure of the augmented bone ridge, the implant sites were located with the use of the surgical guide (prepared by the restoring dentist) and the ridge width was measured at the crest and 4 mm apical to the crest with the same standardized calipers. Prior to implant placement a bone biopsy was taken from the implant sites using a 2-mm internal diameter trephine **(Fig. 10)**. For the bone biopsy, the center of the new bone was identified, and the core was taken from the center of the new bone. Another option was to take the biopsy buccal to the implant placement, that had the biggest part of biopsy in augmented ridge. The biopsy was stored in the correct medium (10% Formalin) and was sent to the UAB Histomorphometry and

Molecular Analysis Core for histomorphometric analysis. The implant sites were located with the use of a surgical stent prepared by the restoring dentist. The implant preparation was completed and implants were placed according to manufacturer protocol.

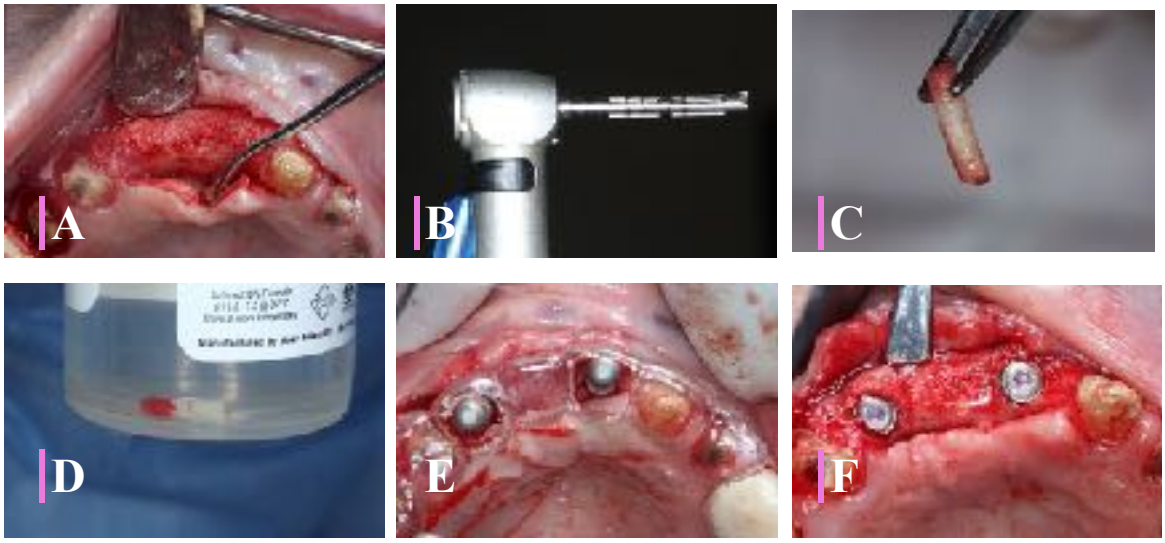


Fig 10: Implant Placement and Biopsy 6 months post GBR
(A) Post-op Ridge
(B) 2mm trephine
(C) Biopsy core
(D) Biopsy placed in 10% formalin
(E) Implant position based on a prosthetic guide
(F) Implant placement

Histomorphometric Analysis

Immediately following the bone biopsy at the center of the healed and regenerated ridge with a trephine, the specimens were placed in a formalin solution. Following fixation with 10% neutral buffered formalin for 48h, the bone biopsy specimens were

dehydrated, embedded in methylmethacrylate, 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 as smooth a surface a 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) by measuring the total surface of vital bone, residual graft particles, organic matrix and artifact/air components. Corresponding percentages was calculated for each of these tissues and compared between small and large particle grafts for ridge preservation and augmentation separately. These experiments were conducted at the UAB Histomorphometry and Molecular Analysis Core and all measurements made by an experienced lab technician blinded to the study protocol.

Clinical and Radiographic Measurements

All clinical measurements were taken by one experienced examiner blinded to the randomization process (small vs. large particles) before grafting and at time of implant placement, using a calibrated surgical caliper and UNC-15 periodontal probe and measurements rounded to the nearest 0.5 mm. Another examiner performed the radiographic evaluations and over time comparisons (before and after grafting) and was blinded to the randomization process. Bucco-lingual dimensions at the level of the crest and 4mm apical to the crest were measured clinically and radiographically. In addition,

ridge height changes were evaluated radiographically at the same locations using an implant planning software with a digital reference (digital implant).

Using coDiagnostiX Guided Surgery Software - Dental Wings, linear horizontal and vertical measurements of the healed grafted ridge were performed in comparison to the previously deficient ridge. For that purpose, the pre-op Cone beam CT scan (taken prior to the augmentation) was superimposed on the post-op Cone beam CT scan (taken at 6months after the augmentation) and a digital implant placed to standardize the locations of the measurements on the 2 scans **(Fig 11)**. These radiographic measurements mirrored the horizontal ridge width measurements (performed clinically) at two locations; at crest level and at 4mm apical to the crest. They also included a vertical measurement of the change in ridge height at the center of the ridge where the digital implant was planned in the software.

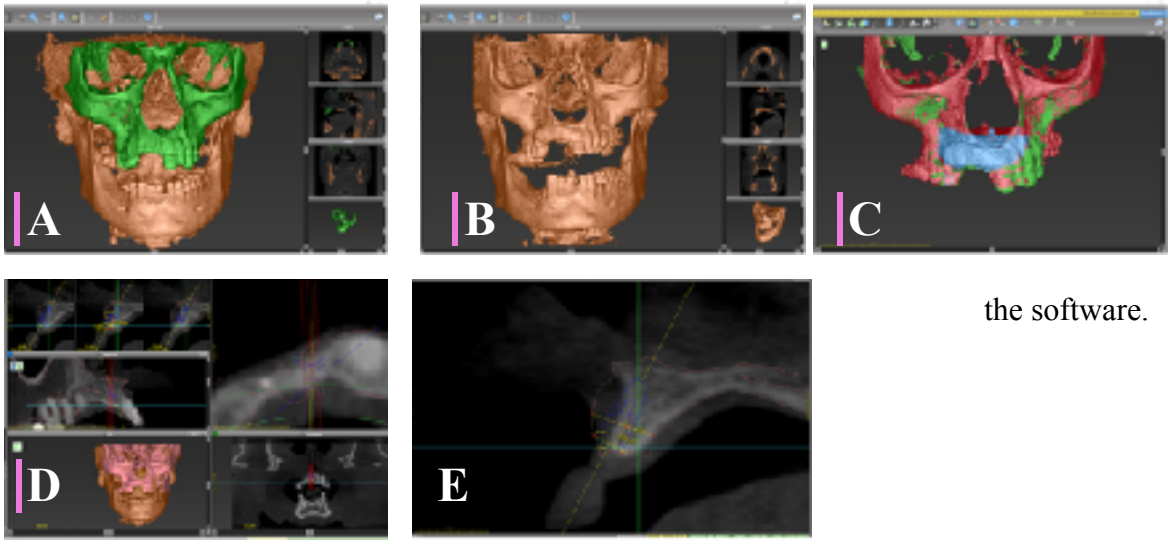


Fig 11: Radiographic Measurements using Co-Diagnostix
(A) Pre-op CBCT segmented
(B) Post-op CBCT
(C) Superimposition of the Pre and post op CBCTs
(D) Verification of alignment both CBCTs superimposed
(E) Radiographic measurements

Statistical Analysis

Power calculation was performed to determine the number of study participants. Assuming that vital bone formation with LP and SP will be as reported by Testori et al. (Testori, Wallace et al. 2013), 22 patients in each group (total of 44) will reach 0.90 statistical power to reject the null hypothesis of equal means with a significance level (alpha) of 0.05 using a two-sided two-sample unequal-variance t-test.

All the outcomes were summarized as mean and standard deviation (SD). For the primary outcome i.e. the new bone formation, a two-sample t-test was conducted to compare difference between the two groups. For the secondary outcomes i.e. the dimension changes of the extraction sites, both the clinical and the radiographic difference in changes between the groups were evaluated using two-sample t-test. A paired t-test was used to compared among all subjects the radiographic vertical loss at the facial, center 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 a two-sample t-test.

RESULTS

A total of 22 patients with 24 qualifying sites participated in the present study and were randomly allocated to receive either the small particle (SP) bone allograft (n = 12) or large particle (LP) bone allograft (n = 12). Each site comprised a single treatment area. Of the 22 subjects initially enrolled, a total of 17 patients completed the study. The participants comprised of 7 males and 10 females aged between 46 and 78 years old, 15 Caucasians and 2 African Americans. Two patients received grafts at bilateral sites; one patient had posterior left and right mandibular qualifying sites and the second patient had posterior right mandibular and posterior left maxillary qualifying sites.

Unfortunately, the 5 patients who were disqualified or withdrew from the study happened to be randomly assigned to the SP group. Two patients were no longer able to return for the core biopsy and implant placement due to developing significant medical problems unrelated to their participation in the study, namely cardiovascular problems. The other three patients were disqualified due to delivering removable prosthetic appliances over the grafted areas, which resulted in the complete failure of the ridge augmentation procedure, as evidenced by the CBCT information and surgical re-entry findings after 6 months of healing.

Consequently, a total of 17 patients and 19 sites (Table 5) were included in the data analyses. The group distribution was as follows; SP group (7 sites) and LP group (12

sites). The site distribution according to location in the mouth included the following: Anterior Mandible (1 site), Anterior Maxilla (2 sites), Posterior Mandible (14 sites) and Posterior Maxilla (2 sites). For the purposes of this study, each site rather than the patient was considered an independent unit.

Variable	Small (N=7)	Large (N=12)	p
Age	67.9 ± 5.7	66.3 ± 8.4	0.6770*
Race			1.0000**
AA	1 (14.3%)	1 (8.3%)	
Caucasian	6 (85.7%)	11 (91.7%)	
Sex			0.6562**
Female	5 (71.4%)	7 (58.3%)	
Male	2 (28.6%)	5 (41.7%)	
Site			1.0000**
Anterior mandible	0	1 (8.3%)	
Anterior maxilla	1 (14.3%)	1 (8.3%)	
Posterior mandible	5 (71.4%)	9 (75.0%)	
Posterior maxilla	1 (14.3%)	1 (8.3%)	

Mean ± SD or frequency (%); * t test; ** Fisher's exact test.

Table 5. Patients' and sites distribution

Ridge width changes at the crest

Clinically, both treatment groups resulted in significant bone gain after 6 months of healing (Fig. 12). GBR in the LP group achieved an average of 5.1 ± 1.7 mm and an average of 3.7 ± 1.3 mm in the SP group. A clinically significant greater ridge width gain (mean of 1.4 mm) at the level of the crest was demonstrated with the use of the LP

allograft when compared to the SP allograft. This difference between the reported gain of both groups neared statistically significance (p=0.0642).

Radiographically, the measured ridge width gain results were in accordance with the clinical measurements, including average gains of 5.1 ± 2.0mm in the LP group and 3.8 ± 1.3mm in the SP group (Fig. 13). However, no statistical significant difference was found between the groups (p=0.1494).

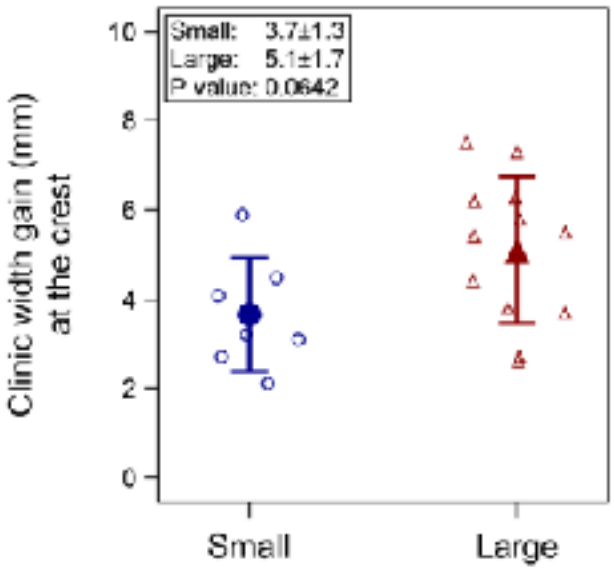


Fig. 12: Clinical width gain at the crest

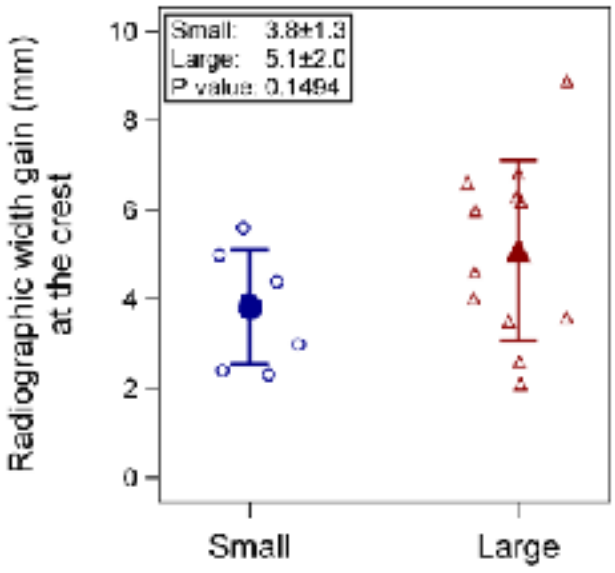


Fig. 13: Radiographic width gain at the crest

Ridge width changes at 4 mm apical to the crest

Clinically, the post-grafting measurements could not be obtained due to significant gain in width at this level of the ridge, prohibiting the use of the surgical calipers.

Radiographically, (Fig. 14) the bone gain at 4mm apical to the crest was comparable between the two groups. An average of 5.9 ± 2.2 mm was reported in the LP group and 5.1 ± 1.8 mm in the SP group. These results were statistically insignificant between the two groups (p=0.4480).

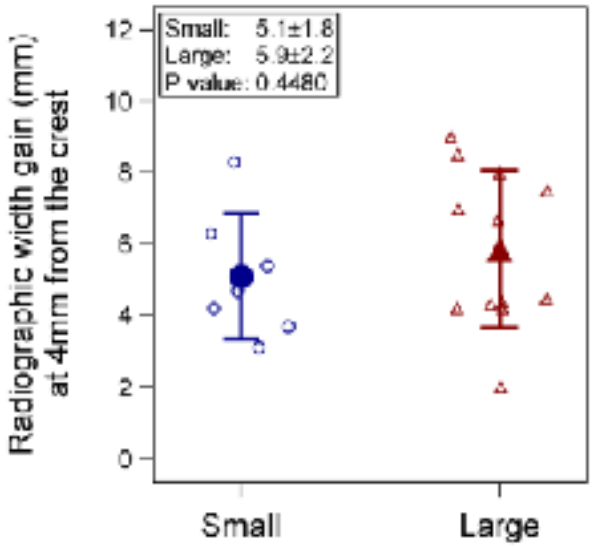


Fig. 14:Radiographic width gain at 4mm from the crest (mm).

Ridge height change at the crest:

The use of SP allografts was associated with a loss of vertical height at the level of the crest (mean of -0.4 ± 0.5mm) whereas and LP allografts resulted in a mean vertical gain (0.3 ± 1.0mm). However, these results were not statistically significant when the effect of both graft size was considered (p=0.1321) (Fig. 15).

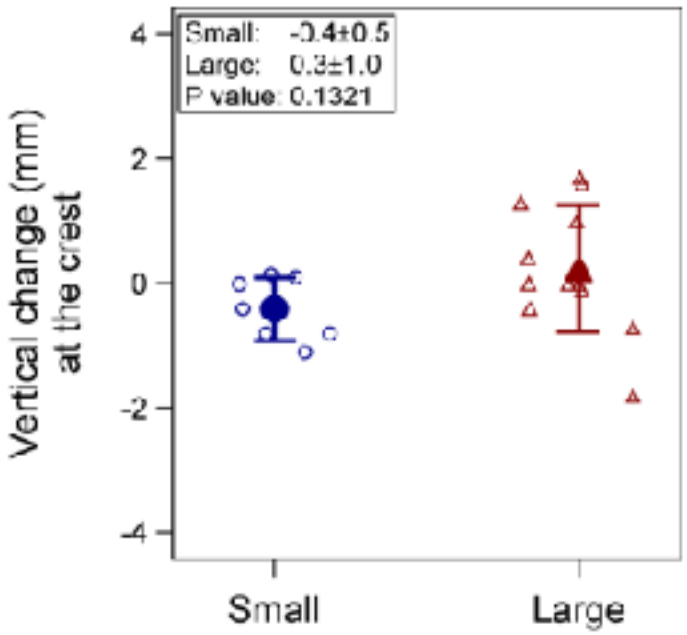


Fig. 15:Vertical change at the crest (mm)

Bone Density

Bone density as estimated by the surgeon at time of bone core biopsy:

Most sites exhibited a D1 and D2 bone density and the sites were distributed in the SP and LP allograft group as such: 4 out of 7 (57.1%) site in the SP group exhibited a D1 density and the remaining 3 sites (42.9%) exhibited a D2 bone Density. However, in the LP group 7 out of 12 sites (58.3%) exhibited D1 bone density, 4 sites (33.3%) revealed D2 density and only 1 site (8.3%) had a D3 bone density. Also, no statistical significance between the type of particle size and the bone density at the time of implant placement (p=1.0000) when using the Fisher's exact test (Fig. 16)

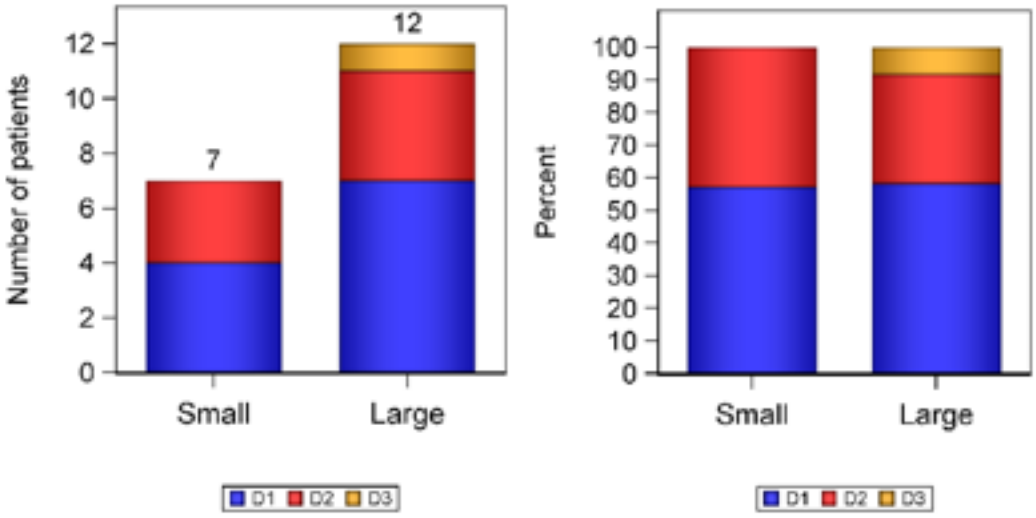


Fig. 16: Bone Density Outcomes

Correlation between clinical and radiographic measurements

Pearson correlation between Clinic width gain at the crest and radiographic width gain at the crest showed a very high correlation between the two measurements ($r = 0.86$, $p<0.0001$). Further correlations could not be evaluated for the other measurements as the ridge width change at 4mm apical to the crest and ridge height change were only evaluated radiographically(Fig 17)

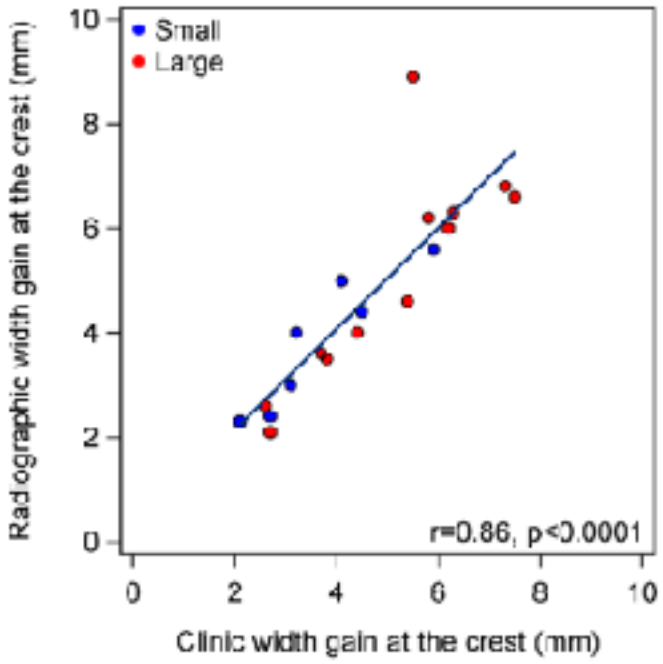


Fig. 17. Pearson correlation between Clinic width gain at the crest and Radiographic width gain at the crest.

Outcome	Small (N=7)	Large (N=12)	p
Clinic Width Gain at the crest (mm)	3.7 ± 1.3	5.1 ± 1.7	0.0642*
Radiographic Width Gain at the crest (mm)	3.8 ± 1.3	5.1 ± 2.0	0.1494*
Radiographic Width Gain at 4mm from the crest (mm)	5.1 ± 1.8	5.9 ± 2.2	0.4480*
Vertical change at the crest (mm)	-0.4 ± 0.5	0.3 ± 1.0	0.1321*
Bone Density			1.0000**
D1	4 (57.1%)	7 (58.3%)	
D2	3 (42.9%)	4 (33.3%)	
D3	0	1 (8.3%)	

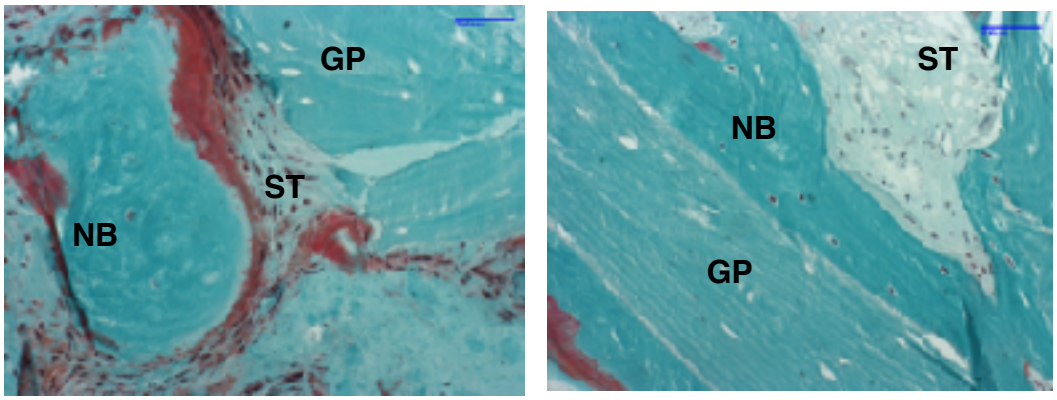
Mean ± SD or frequency (%); * t test; ** Fisher's exact test.

Table 6: Patients' outcomes.

Histology

At the time of implant placement a biopsy was harvested from the grafted site which occurred 6 month post augmentation. All biopsy cores (SP and LP groups) were divided into three zones: zone 1 corresponds to the coronal third of the core, zone 2 to the middle third, and zone 3 to the apical third of the biopsy. All biopsies revealed newly formed bone, residual allograft particles and dense, organized connective tissue encircled the graft particles. Some biopsies length was insufficient to divide into 3 zones due to non-intact biopsy cores. Allograft particles were identified by the separation lines and the absence of osteocytes in lacunae. The new bone in contact with the residual particles appeared viable with osteocytes in lacunae. Osteoblasts were present in conjunction with newly formed bone surrounding the FDBA particles. No acute or chronic inflammatory infiltrate was noticed in any of the biopsies. Statistical analysis was done to calculate the percentage of new bone, residual graft and connective tissue after measuring the surface area in comparison to the total biopsy surface area. Two total percentages were done, one comprised of the overall percentage for all biopsies in each group and the other represented the overall percentage of all biopsies after excluding the samples that contained only one zone (Fig. 18).

Fig. 18. Histology at X20



magnification (NB = new bone, GP = graft particle, and ST = soft tissue)

Histomorphometric analysis

Statistical analysis showed no significant difference in the percentage of new bone, residual graft particles and soft tissue between the two groups (SP and LP) or among the 3 zones between these groups. The only exception was the significant difference (p=0.05) found in the percentage of the soft tissue area in zone 1 between the SP group (29.2 ± 7.1 %) and the LP group (42.3 ± 15.2 %).

Table 7 shows the calculated percentage of new bone, residual graft particles and soft tissue in each zone and overall percentage. For the SP group, the mean new bone formed was 41.0 ± 10.1 %, mean residual graft was 33.6 ± 7.3 % and mean soft tissue was 25.5 ± 10.5 %. Zone 3 revealed the highest % of new bone in this group (49.8 ± 5.32 %) (Table 8) while zone 1 exhibited the least (37.2 ± 11.1 %). In the LP group, the mean new bone formed was 31.4 ± 14.8 %, mean residual graft was 38.3 ± 19.7 % and mean

soft tissue was 30.3 ± 13.8%. Zone 3 showed the highest percentage of new bone formed in this group (47.3 ± 13.6 %) while zone 1 was the least (132.6 ± 15.1 %) (Table 7).

Hence the amount of overall new bone formed was higher in the SP group. The amount of residual graft and connective tissue were higher in the LP group. Zones 1 and 2 showed higher % of new bone in the SP group however zone 3 of LP contained the larger percentage. However, the two-sample t-test revealed no statistical significant difference for all these measurements.

Outcomes	N (S vs L)	Small	Large	p**
Total % New Bone*	7 vs 12	41.0 ± 10.1	31.4 ± 14.8	0.1496
Total % Graft*	7 vs 12	33.6 ± 7.3	38.3 ± 19.7	0.4689
Total % Soft Tissue Area*	7 vs 12	25.5 ± 10.5	30.3 ± 13.8	0.4299

Table 7:. Overall Histomorphometric Analysis

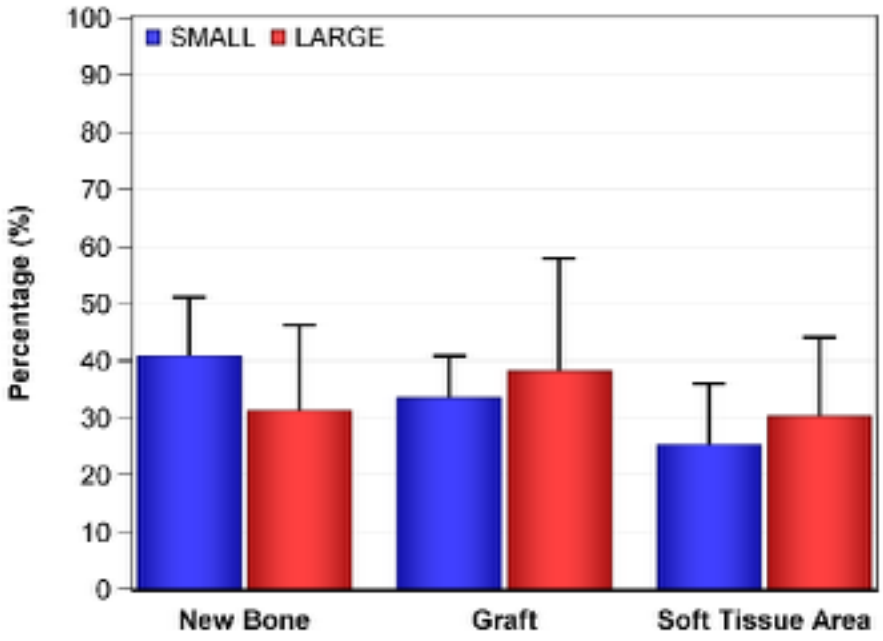


Fig. 19. Tissue distribution in core sections per group

Outcomes	N (S vs L)	Small	Large	p**
Zone 1 % New Bone	7 vs 9	37.2 ± 11.1	32.6 ± 15.1	0.7102
Zone 1 % Graft	7 vs 9	33.6 ± 10.1	23.1 ± 13.0	0.1010
Zone 1 % Soft Tissue Area	7 vs 9	29.2 ± 7.1	42.3 ± 15.2	0.0555
Zone 2 % New Bone	7 vs 9	45.0 ± 20.2	34.9 ± 16.6	0.2487
Zone 2 % Graft	7 vs 9	33.6 ± 17.8	38.3 ± 20.7	0.6405
Zone 2 % Soft Tissue Area	7 vs 9	21.4 ± 16.8	27.8 ± 15.5	0.4452
Zone 3 % New Bone	2 vs 6	49.8 ± 5.3	47.3 ± 13.6	0.8140
Zone 3 % Graft	2 vs 6	32.6 ± 4.7	33.4 ± 12.1	0.9332
Zone 3 % Soft Tissue Area	2 vs 6	17.6 ± 0.6	19.7 ± 15.0	0.8521

* From the tables that only contain the total percentages;

** T test

Table 8: Histomorphometric Analysis per zone

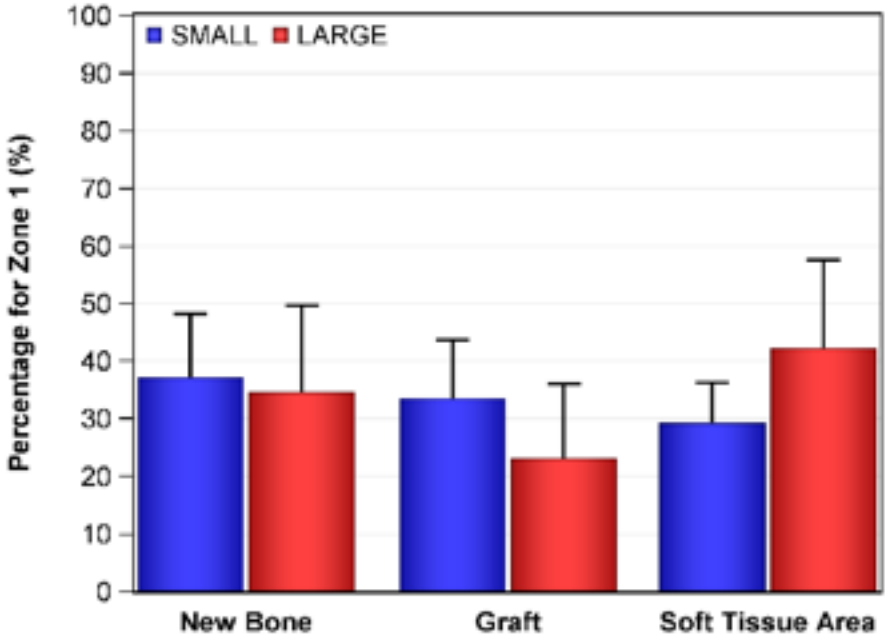


Fig. 20. Tissue distribution in the coronal zone

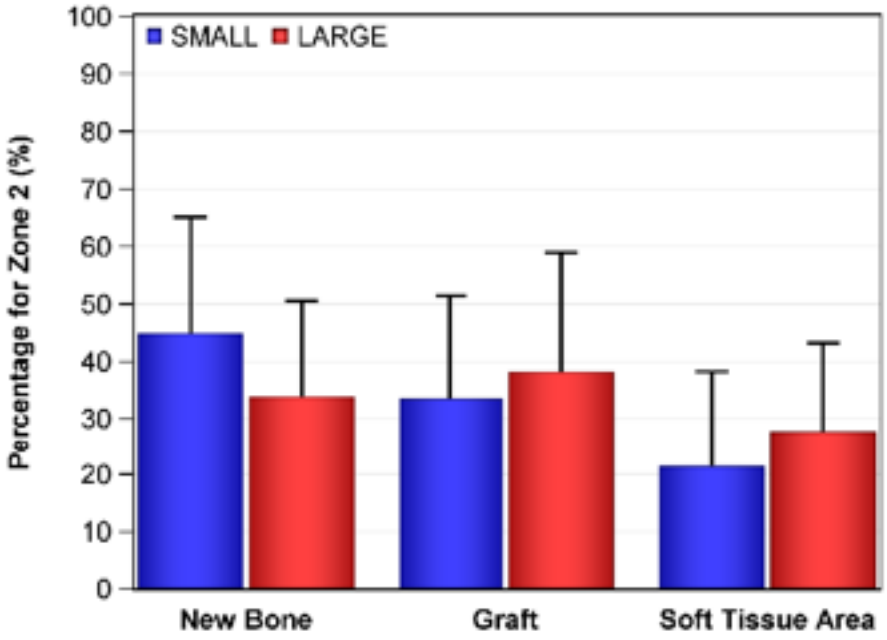


Fig. 21. Tissue distribution in the middle zone

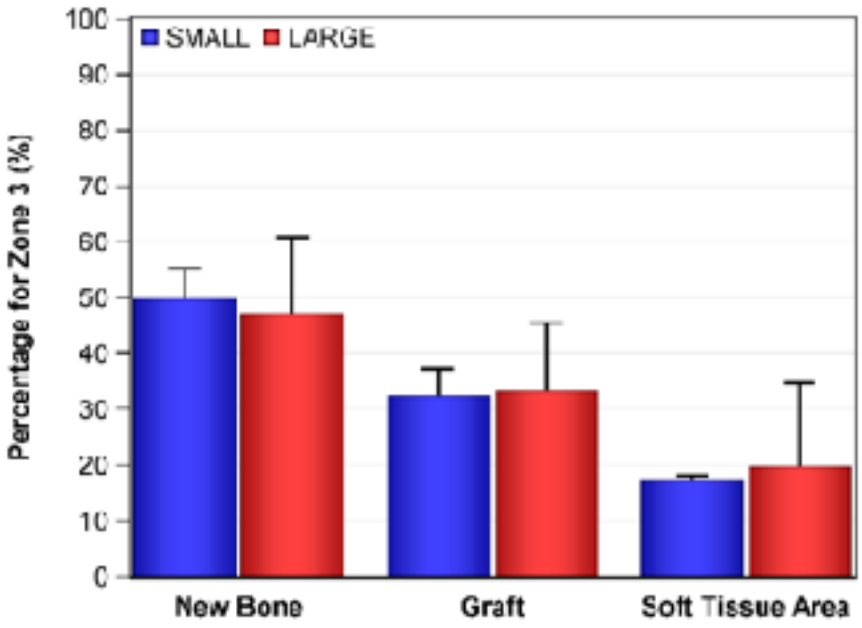


Fig. 22. Tissue distribution in the apical zone

DISCUSSION

GBR has become a major treatment option for horizontal and vertical ridge augmentation with low complication rates and high implant survival rate(Esposito, Grusovin et al. 2006). The “knife-edge” shaped ridge, also known as class IV in the Cawood and Howell (Cawood and Howell 1988) ridge classification system, represents a significant deficiency that undoubtedly requires an augmentation prior to implant placement. Several materials have been proposed to be used in GBR procedures. The use of bone grafting materials and barrier membranes to treat knife-edged defects with horizontal augmentation may lead to less morbidity when compared to other treatment modalities. Resorbable and non-resorbable membranes have been used in GBR (Buser, Ingimarsson et al. 2002, Hammerle, Jung et al. 2008).To achieve the necessary ridge volume, autogenous bone or bone substitutes are placed underneath the barrier membrane to prevent collapse of the augmented volume. The effect of particle size on the clinical and histological outcomes of lateral ridge augmentation has been scarcely studied or reported in the literature. Therefore, the aim of this study was to clinically and histologically compare the amount and quality of the bone gained after lateral ridge augmentation procedures when using small (250-1000μm) versus large (1000-2000μm) particle size cortico-cancellous bone allografts at 6 months following surgical intervention.

Due to time constraints for patient enrollment, as well as the application of strict inclusion and exclusion criteria, the sample size of this clinical trial was very small resulting in a limited statistical power. A total of 22 subjects (24 sites) were enrolled, among which 17 completed the study for a total of 19 sites. This limitation was compounded by the fact that patients that withdrew or were disqualified throughout the study belonged to the same randomized group, which resulted in unequal numbers for comparison (SP=7 sites and LP=12 sites).

The present study demonstrates that the combination of particulated cortico-cancellous bone allografts with a fixated resorbable collagen membrane can be used safely and effectively for horizontal augmentation of knife-edged ridges. Healing of the ridge augmentations were uneventful in this study. The selected collagen membrane was associated with good soft tissue healing with no membrane exposures or infections reported during the healing phase. Similar outcomes for soft tissue healing have been reported for both non-resorbable expanded polytetrafluoroethylene (e-PTFE) and resorbable synthetic and collagen membranes (Urban, Caplanis et al. 2009, Urban, Jovanovic et al. 2009). While non-resorbable e-PTFE membranes are still considered the gold standard in GBR, the need for surgical re-entry for membrane removal and possibly re-grafting as a result of an infection related to spontaneous membrane exposures and associated soft tissue problems, has favored the increased use of resorbable membranes.

On the other hand, resorbable membranes lack rigidity and space maintenance characteristics, which can be overcome by secure fixation of the membrane on both the lingual/palatal and the buccal side. Membrane fixation also allows for increased stability

and compaction of the graft material while avoiding its random spread underneath the membrane in the adjacent tissues. Titanium tacks were used in this study to stabilize the collagen membranes. A systematic review and meta analysis by Wessing et al (Wessing, Lettner et al. 2018) showed that, despite the lack of statistical significance, the amount of augmented bone was higher when the collagen membrane was fixated (mean=4.25mm) in comparison to no fixation (mean =2.94 mm). This finding suggests that the space under the membrane should be maintained to prevent the collapse. The membrane used in this study, Mem-Lok® Pliable is a dense, uniform, single layer and non-cross-linked membrane derived from highly purified porcine tissue with a resorption time of 12-16 weeks according to the manufacturer. No membrane exposures were noted in the present study. This finding is in contrast with the results of Wessing et al. who reported membrane exposure rates of 28.62% for cross-linked membranes and 20.74% for non–cross-linked membranes. Differences may be due to surgical technique, tension-free closure and type of selected graft and or collagen membranes.

It has been reported that spontaneous membrane exposure leads to less new bone formation (Nowzari and Slots 1995). When a resorbable membrane is spontaneously exposed, it disintegrates causing loss of the barrier function at the exposed site; however, part of the membrane remains functional within the tissues (Simion, Misitano et al. 1997, Tal, Kozlovsky et al. 2008)**.** Current literature slightly favors the use of non–cross-linked membranes due to the lower exposure rates. There were no publications reporting the difference in bone gain in patients with and without membrane exposure (Tal, Kozlovsky et al. 2008).

Decortication was also part of the surgery in this study. The principle of decortication is to perforate the alveolar corticalis to induce trauma which allow better access for the progenitor cells to the grafted site and ensure revascularization and nutrition especially in dense bone cases (Wessing, Lettner et al. 2018).

Using surgical calipers clinically and a superimposition method in co-Diagnostix™ software radiographically, measurements were made at the level of the crest and at 4mm apical to the crest to assess ridge width changes. Due to excessive width gain at 4mm apical to the crest, clinical measurements were not performed and replaced with radiographic measurements only. In this clinical trial, the locations of the measurements were standardized by recording the distance from adjacent teeth (used as fixed references) to the exact location of the ridge that was measured at time of grafting, at time of biopsy and implant placement as well as on the pre- and post-augmentation CBCTs superimposed on the software. In addition, a digital implant was placed in the initial scan using coDiagnostiX™ that corresponded to the clinical measurement location and was used as a fixed digital reference shared in common for both CBCTs. The strong correlation ($r=0.86$, $p<0.0001$) demonstrated between clinical and radiographic width measurements seems to validate this novel radiographic methodology. Therefore, future studies can reliably use the described digital protocol for accurate assessment of horizontal and vertical ridge changes when similar procedures are planned.

In this randomized trial, there was a mean horizontal bone gain of 3.8 ± 1.3 mm and 5.1 ± 1.8 mm for the SP group versus 5.1 ± 2.0 mm and 5.9 ± 2.2 mm for the LP group at the level of the crest and at 4mm apical to the crest respectively, with some sites gaining up to 9 mm. Using the LP bone graft resulted in more gain of horizontal width when compared with the SP group. However, the two sample t-test revealed no significant difference (p=0.1494).

In a systematic review and meta-analysis by Sanz-Sánchez et al (Sanz-Sanchez, Ortiz-Vigon et al. 2015), an average of 3.90 mm bone width gain was reported after lateral augmentation procedures. One of the included studies, that reported the greatest amount of mean increase in ridge width (5.68 mm) , utilized a mixture of particulate autogenous bone graft and anorganic bovine bone mineral covered with a fixated collagen membrane after 8–9 months (Urban, Nagursky et al. 2011). In contrast, the study reporting on the least increase in ridge width (1.10 mm) utilized particulate synthetic graft and non-resorbable membrane. When FDBA was used with a non resorbable expanded PTFE barrier, it resulted in a mean ridge width increase of 3.2 ± 1.0 mm observed at the 6- month reentry in one of the other included studies (Feuille, Knapp et al. 2003).

The results of the present study also concur with those of Beitlitum et al. (Beitlitum, Artzi et al. 2010) who evaluated the outcomes of ridge augmentation when FDBA was used with or without autogenous graft. Their outcomes demonstrated an average width gain of ~5 mm when FDBA was used solely and 3.6 mm when mixed with autogenous. The use of a cross-linked collagen without any fixation was reported in their study.

When the size of the graft particles was considered used in this study, there was a trend towards greater ridge width gain (+1.4 mm) at the level of the crest with the use of large particles (1.0 to 2.0 mm) when compared to the small particles (0.25-1.0 mm). Statistical differences neared significance (p=0.0642). However no statistical significant difference at 4 mm apical to the crest between large and small particle size.

To our knowledge, this is the first human study that investigates the influence of bone graft particle size on clinical and histologic ridge augmentation outcomes. Some existing studies in a rabbit model have conducted comparisons of small versus large graft particles when using autogenous or alloplast materials (Kon, Shiota et al. 2009)(Pallesen, Schou et al. 2002). It is important to highlight that the designation of small and large-sized particles may differ significantly among studies and graft materials. In the Kon et al. study, autogenous grafts were utilized as small (150 to 400 μm), large (1.0 to 2.0 mm), and a mixture containing equal weights of both large and small bone particles (Kon, Shiota et al. 2009). Histology and micro-CT were performed at 4 and 8 weeks post-grafting. The authors reported that large particles achieved the best outcomes in the preservation of bone height and volume. Furthermore, there was a significant volume reduction in the mixed group between 4 and 8 weeks. The same authors showed that small particles of the autogenous graft were completely resorbed with a 49% reduction in the graft volume at 8 weeks whereas the volume reduction in the large particle group was 3% only (Kon, Shiota et al. 2009). Using completely different particle sizes, another rabbit study reported that 0.5- to 2 mm particle size grafts resulted in more new bone formation compared with super large 10-mm-size autogenous bone graft particles in

rabbit calvaria defects (Pallesen, Schou et al. 2002). The authors theorized that the improved outcomes with the small particles may be related to a lack of release of growth factors from large particle size grafts and to a more difficult incorporation of the large particles into the sites (Pallesen, Schou et al. 2002).

Histomorphometric analysis in the current study revealed that smaller FDBA graft particles achieved higher percentage of new bone (41%) when compared to the larger FDBA graft particles (31.4%). In contrast, there was a lesser amount of residual graft particles (33.6%) when small particles were used in comparison to 38.3% of residual graft with the large group. None of these differences were considered statistically significant.

A previous study reported on the histomorphometric outcomes with the use of FDBA with non-resorbable barriers (e-PTFE) for ridge augmentation in 10 patients (Feuille, Knapp et al. 2003). The used FDBA included only particles <1.0 mm in size and resulted in an average 47.6% new bone formation with a mean of 52.4% of residual graft particles in harvested biopsies after 6 months of healing. It must be noted that there was no mention of a soft tissue (or connective tissue) component when evaluating the biopsies in that study. This finding renders any direct comparison with our study rather difficult.

The results of the current study are however in agreement with those reported by Cammack et al. comparing FDBA to DFDBA in ridge and sinus augmentation procedures after 6 to 36 months of healing. A mean percentage of 41.89% of new bone was reported in the FDBA group (Cammack, Nevins et al. 2005). The lack of differentiation in graft particle size as well as the largely variable healing phase render direct comparisons with

the current findings less than adequate. Nonetheless, the range of reported new bone formation seems to concur with existing comparable literature despite differences in graft material, barrier membrane, surgical technique and healing time.

In the current study, the biopsy core was further divided into three zones (Fig. 23) and the difference between the three zones in each group and between the two groups (small vs. large) were calculated. The highest percentage of new bone was found in zone 3 in both groups, which corresponded to the apical third of the biopsy, i.e. the closest to the native bone. Zone 3 contained greater new bone (SP=49.8% , LP=47.3%) than zone 2 (SP=45%, LP=33.9%), which in turn was larger than zone 1 (SP=37.2%, LP=32.6). From a biological standpoint, it is expected that the most apical third of the biopsy comprises greater new bone tissue as it is the closest in proximity to native bone. It is almost possible that the most apical part of the biopsy engaged the older ridge which represents more vital bone than the grafted part of the ridge

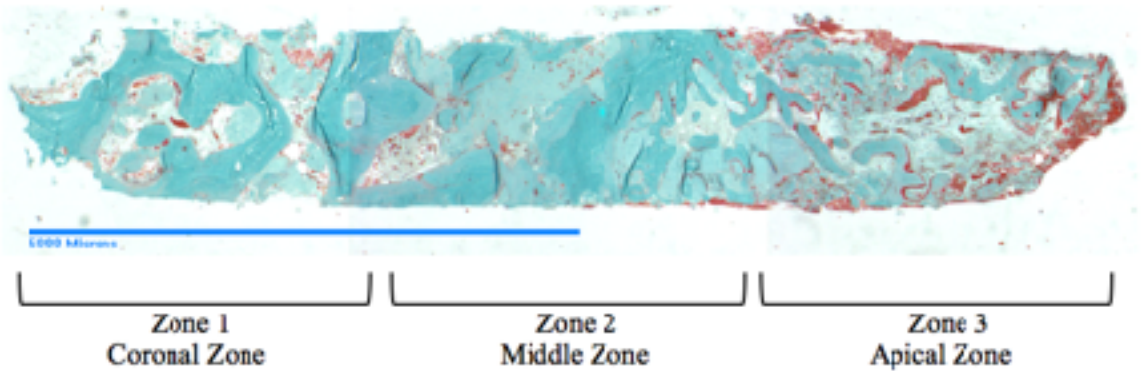


Fig.23: Biopsy core

The current histologic results agree with published outcomes in a study investigating particle size in an animal model where higher density of newly formed bone was in the small particle group (Zhou, Zhang et al. 2011). However, a direct comparison is not possible owing to the difference in study model (animal vs. human and type of bone defect) as well as differences in particle size designation. It is hypothesized that the larger surface area created by the more numerous small particles allows for more bone to form around those particles due to the increase in the contact length between the bone and the small particles. An increase in the surface area along with an increase in the osteoclastic activity leads to a better osteogenic induction (Shapoff, Bowers et al. 1980). Therefore, it is possible that the particle size might play a role in the osteogenic activity. Furthermore, the inter-particular space might also play an important role. It was claimed that the size of the pore is comparable to the size of the spaces in between the particles and it was proven that spaces between the small particles were significantly larger than those between larger particles (Shapoff, Bowers et al. 1980). So these larger spaces allow more ingrowth of the capillaries. The small number of samples in the current study does not allow for drawing any definitive conclusions in that regard.

The current study used a novel radiographic methodology in the measurement of ridge dimensional changes in bucco-lingual width and height following GBR. To our knowledge, previous lateral ridge augmentation studies have not reported on possible ridge height changes. Although there was no statistical significant difference between the SP and LP groups ($p=0.1321$), the SP group resulted in a mean vertical loss of the ridge

-0.4 ± 0.5mm while the LP group resulted in a mean vertical gain 0.3 ± 1.0 mm. These vertical changes ranges between -1.7 to 1.8mm. Despite the limited number of samples in this study, these results should be taken into consideration especially in anterior cases where loosing around 2mm of ridge height might cause significant esthetic challenges. Furthermore, no statistical significant differences between SP and LP group regarding the bone density outcomes.

From a clinical observation standpoint, differences could be detected between the sites augmented with SP vs LP grafts at time of implant placement. Sites augmented with LP grafts resulted in more uneven and rough ridge that needed minor osteoplasty prior to placing implants. However, the number of sites treated with SP were less due to patient withdrawal or disqualification affecting this group. In all sites treated with SP and LP, the grafts showed good incorporation with the newly formed ridge. This is supported by the available histologic evidence of the augmentation area showing that the residual allograft particles were highly connected by a dense network of newly formed bone. This may further support the use of particulated allograft materials solely or mixed with other types of bone grafts.

One strength of this study design is that the bone graft material used for all subjects from both the SP and LP groups came from one single donor, in order to account for the variation in healing potential that may be related to the age, race or gender of the donor (Schwartz, Somers et al. 1998). Another unique feature of this study was the use of co-Diagnostix software to perform the radiographic measurements. The pre-op Cone beam CT scan (taken prior to the augmentation) was superimposed on the post-op Cone beam

CT scan (taken after the augmentation) using this software. The software allowed a precise superimposition and a simultaneous evaluation of ridge dimensional changes with limited to no errors in the reproducibility of the location of measurements. This study supports the future use of this methodology in similar study designs.

Implants survival rates were not part of this study. Several studies have shown that implant survival rate in grafted bone is similar when placed in native bone (Arvidson 1998, Jung, Pjetursson et al. 2008). In a split mouth study, implants showed a survival rate of 100%for implants placed simultaneously with GBR at 5 years followup. A systematic review of augmentation procedures showed that after 12-60 months followup implant survival rate ranged between 93% and 100% (Jensen and Terheyden 2009). More long term studies showed be done in order to check the amount of remodeling of grafted bone around implants.

CONCLUSION

Within the limitations of this study, it demonstrated that particle size of bon allografts might influence the dimensional changes in lateral ridge augmentation procedures. There was a trend for greater ridge width gain when large particles were used in comparison to small particles with near statistical significance despite the small number of patients and sites. There was also a slight gain in ridge height with the large particles whereas a slight loss of ridge height was observed with the small particles with no statistical differences between the groups.

Histologically, both groups demonstrated new bone formation percentages that are comparable to previously published reports on ridge augmentation procedures. There was a trend for more new bone with small particles but the small sample size did not allow for statistical significance.

A novel radiographic methodology was presented in the current study and was demonstrated to be reliable owing to its strong correlation with the clinical measurements. This technique also allowed for accurate radiographic measurements of ridge height changes. Based on the outcomes of this study, this non-invasive and simple methodology should be used in similar future studies.

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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/sw

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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APPENDIX B

Procedure Checklist

Visit-1 - Ridge Augmentation Procedures Checklist

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-op CT scan obtained (CBCT 1) Pre-treatment antibiotics dispensed Vital signs measured		
Anesthesia accomplished		
Ridge dimensions measured at crest and at 4 mm apical to crest		
Ridge dimensions measured 4 mm below crest		
Standardized clinical photographs taken		
Post-augmentation procedures completed in accordance with randomized group therapy		
Post-operative prescriptions written Written post-operative instructions given Print-out of the EDR procedural note		

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

Visit-1 - Ridge Augmentation

1: Areas Augmented: Site 1 #_____ Site 2 #_____ Site 3 #_____

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

3: Type and dimensions of membrane used:
_____

4: Tacks used ☐ Y ☐ N If YES, Location: _____

5: Ridge dimensions measured at the c# at crest _____ 4 mm below crest
_____

_____ # at crest _____ 4 mm below crest

_____ # at crest _____ 4 mm below crest

6: Surgical Comments *(if any)*:
_____
_____

_____
_____

Please attach label from each graft vial used

Visit-3 - Bone Biopsy and Implant Placement

I: 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)* Ridge dimensions at crest					*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 dimensions 4 mm apical to crest					

Please attach label from each implant used