The Influence of Bone Allograft Particle Sizes on the Quantity and Quality of New Bone Formation in Grafted Extraction Sockets and Edentulous Ridges

Study Protocol & Statistical Analysis Plan

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The Influence of Bone Allograft Particle Sizes on the Quantity and Quality of New Bone Formation in Grafted Edentulous Ridges

1. 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 the buccal/palatal, apical/coronal, mesial/distal dimensions. Several methods for augmenting the alveolar ridge in preparation for implant placement have been described. Autogenous bone grafts, which are 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 of the bone gained after lateral ridge augmentation procedures performed using small (100-300µm) versus large(1000-2000µm) particle size bone cortico-cancellous allograft (Maxxeus™ Dental, Community Tissue Services, Kettering, OH) at 6 months following surgical intervention.

2. Introduction/Background:

Dental rehabilitation of partially or totally edentulous patients with oral implants has become a routine treatment modality in the last decades, with reliable long-term results(1-7).However, early loss of the teeth due to trauma or periodontitis often leads to deformities in these resulting edentulous ridges. Studies have demonstrated that bone resorption will occur secondary to tooth extraction(8-10). This tends to occur over a 12 month period, most notably in the first 4 months following extraction (Fig. 1) and, depending upon location, may range up to 5–7 mm bucco-lingually. In addition, 2–4 mm of vertical height loss frequently accompanies the horizontal loss and usually is seen when multiple adjacent extraction sites are combined(8-10).

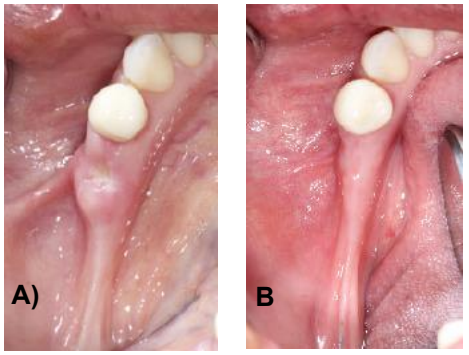

Fig. 1. Ridge resorption following tooth extraction. (A) 1 week after tooth extraction (B) 12 weeks after extraction

Unfavorable local conditions of the alveolar ridge, may provide insufficient bone volume or unfavorable vertical, horizontal, and sagittal inter-maxillary relationships, which may render implant placement impossible or incorrect from a functional and esthetic viewpoint (11).Several methods for augmenting the alveolar ridge in preparation for implant placement have been described. Among these techniques, guided bone regeneration (GBR) has probably generated the most interest(12,13).The concept of GBR (Fig. 2) is based upon the use of a barrier membrane to exclude rapidly growing soft tissue cells from a bony defect and, more importantly, to maintain a space for the slower process of bone formation. Bone grafts or bone substitutes are commonly used in GBR procedures to provide support for the barrier membrane, for additional space maintenance, and/or for their osteoconductive / osteoinductive properties.

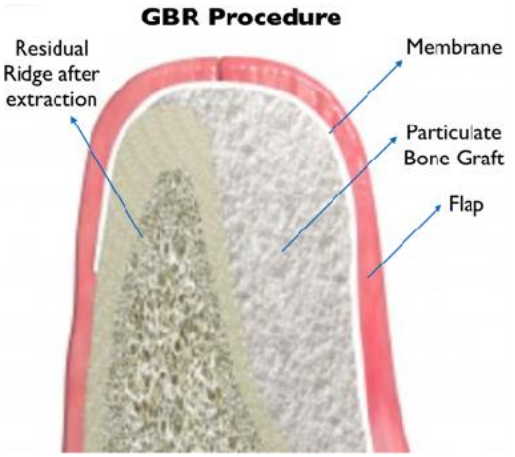

Fig. 2. GBR concept

Autogenous bone grafts are still considered the gold standard in bone regeneration procedures(14). 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(15-16). Bone-substitute materials 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. Bone-substitute materials can be categorized in three groups: (1) allogenic, from another individual within the same species; (2) xenogenic, from another species; or (3) alloplastic, synthetically produced.

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(14,16,17). Freeze-dried bone allograft (FDBA) is commonly used today and has shown success, both clinically and histologically in augmenting deficient ridges. A graft material that promotes a high percentage of new vital bone is beneficial for implant placement and stability. Because particulate mineralized freeze-dried bone allograft (FDBA) is effective in producing dense new bone, it is often preferred to decalcified freeze-dried bone allograft (DFDBA) for lateral ridge augmentation prior to implant placement(18,19). However, there is a paucity of documentation to verify this clinical impression. Both clinical and histologic results indicate a beneficial effect from the addition of DFDBA to GBR procedures in humans(20).This combination results in the formation of viable new bone at 9 months(21) . Histologic examination of biopsy samples from the sub-membranous hard tissue reveals particles of DFDBA partially surrounded by un-inflamed connective tissue and by vital bone adjacent and adhered to the DFDBA particles(22). The histologic results of a study comparing FDBA and DFDBA showed that in DFDBA sites, only the particles near the host bone are involved in the mineralization processes, while in FDBA sites, even the particles that are farthest from the host bone are lined by

osteoblasts, actively secreting osteoid matrix and newly formed bone(23). This suggests more of an osteo-conductive effect with FDBA than DFDBA.

The effect of particle size (Fig. 3) on the clinical and histological outcomes of lateral ridge augmentation (insufficient edentulous ridge width) has been scarcely studied or reported in the literature. Shapoff et al (24) did a study Rhesus Monkeys to determine if particle size should be considered as a factor for evaluation of osteogenic activity of FDBA.He found that there is a significantly more new bone formation associated with small particle FDBA when mixed with autogenous marrow than that of the large particles.Also, he found that there is a marked resolution of small graft particles in the new bone formed. He concluded that small particles FDBA enhance osteogenesis when mixed with autogenous marrow by increasing the number of pores.

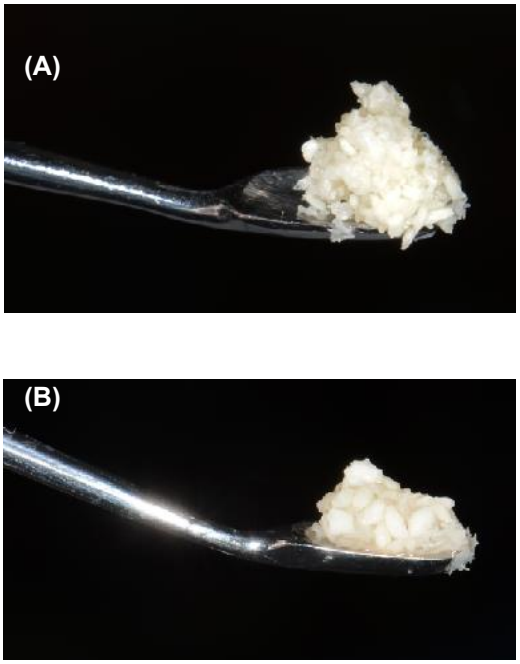

Fig. 3. Maxxeus Allograft ;
(A)Small particles (100-300µm).(B)Large particles (1000-2000µm).

In a prospective randomized controlled clinical trial, Testori et al. (25) 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 in 13 patients. For each patient, one subantral compartment was grafted with 100% large particle Bio-Oss and the contralateral subantral 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 superior-distal area of 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 is observed. Hence, the histologic results of this study reaffirm the osteoconductive ability of the ABBM (Bio-Oss) when used as the sole grafting material in maxillary sinus augmentation and the histomorphometric results 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. (26) The authors related the difference between studies to the small sample size included in both. (25)

3. Objectives/Specific Aims:

The aim of this study is to histologically and clinically compare the amount of the bone gained after lateral ridge augmentation (edentulous ridge) procedures performed using small versus large particle size bone cortico-cancellous allograft (Maxxeus™ Dental, Community Tissue Services, Kettering, OH).

Histomorphometric outcomes(Primary Outcomes):

-evaluate the quality of bone regenerated with the use of the two available bone allograft particle sizes

-evaluate whether the size of commercially available bone allografts influences the outcomes of guided bone regeneration (GBR) procedures

Clinical and Radiographic outcomes(Secondary Outcomes):

-quantify the amount of bone augmentation achieved by the two available bone allograft particle sizes by direct clinical measurements

-quantify the amount of bone augmentation achieved by the two available bone allograft particle sizes by radiographic measurements (CBCT)

-evaluate the handling characteristics of the two available bone allograft particle sizes.

4. Materials and Method:

Study population

A total of 50 patients seeking treatment at the UAB SOD Graduate Periodontology clinics will be recruited to participate in this study according to the criteria in Table 1.
If deemed eligible, study visits, objectives, risks and benefits will be explained to all participants and IRB-approved written informed consent will be obtained.. One trained calibrated examiner will be available for all study visits when clinical measurements are required. Photographs will be taken during all visits using a SLR digital camera in a 1:1 ratio.

Randomization

Randomization will take place on the day of surgery via sealed envelopes indicating the surgical approach the patient will receive, as follows:

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

Table 1. Study Inclusion and Exclusion Criteria

<table>
<tr><th>Inclusion Criteria</th><th>Exclusion Criteria</th></tr>
<tr><td rowspan="3">Patients:
□ English speaking
□ At least 18 years old
□ Able to read and understand informed consent document
□ Need implants to replace missing tooth or teeth in at least one quadrant of the mouth.
□ Patient at UAB dental school
□ willing and able to comply with the preoperative and postoperative diagnostic and clinical evaluations required.</td><td>• Systemic conditions contraindicating oral surgical procedures or adversely affecting wound healing
• significant medical conditions or habits expected to interfere with bony healing.</td></tr>
<tr><td>Smoking >10 cigarettes/day</td></tr>
<tr><td>Patient is a poor compliance risk (i.e., poor oral hygiene, history of alcohol or drug abuse)</td></tr>
<tr><td>Insufficient alveolar ridge width for endosseous implant placement defined as 5mm or less as determined by bone sounding and cone beam computed tomography (CBCT) scan.</td><td>Vertical loss of bone at edentulous ridge</td></tr>
</table>

Screening/baseline visit:

Upon enrollment into the study the patient's medical history and electronic records will be reviewed. Patients will be treatment planned by an interdisciplinary team consisting of a surgeon and a prosthodontist or restoring dentist. Cone Beam Computed Tomography (CBCT) scan will be taken to optimize the treatment plan and determine the feasibility of implant placement following bone grafting. Clinical examiners will conduct clinical and radiographic exams to determine eligibility according to the above inclusion criteria. Study visits and objectives will be explained to all participants. IRB approved written informed consent will be obtained from all the participants.

Surgical visit (Visit 1):(Fig. 4)

A course of prophylactic antibiotics will be dispensed at the time of surgery (mostly Amoxicillin 2 g or Clindamycin 600 mg, 30 minutes to one hour prior to surgery). After preparation and isolation of the surgical area, anesthesia will be accomplished by intravenous or inhalation sedation (when indicated) plus local anesthesia.

The graft material utilized for all surgical procedures, for all patients, will be 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, cortico-cancellous bone allograft, Community Tissue Services, Kettering, OH).

For patients that present with an edentulous ridge insufficient to house one or more dental implants, requiring a lateral ridge augmentation procedure:

A crestal incision will be made on the study quadrant. A superficial split thickness will be made on the facial surface to allow flap extension to achieve complete coverage of the barrier membrane material. The secondary periosteal flaps will be fully reflected to allow adequate access to the surgical site. The surgical site will be debrided and degranulated. After exposure of the bone the implant sites will be located with the use of the surgical stent and the ridge width will be measured at the crest and 4 mm apical to the crest with the a standardized caliper. The area of augmentation will be 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 defects will be grafted with the randomized bone allograft (SP or LP). The graft sites will be covered with a resorbable barrier membrane. The same type of membrane from the same manufactured lot will be used for all defect sites; however, the membrane will be trimmed for the individual defects during surgery. All surgical sites will be closed with the same, vicryl PGA Hu-Friedy Perma Sharp® suture.

Post-surgical analgesics will be prescribed and/or dispensed as necessary. All subjects will be dispensed Peridex® chlorhexidine mouth rinse and instructed to rinse twice daily for two weeks following the regenerative surgery, to help guard against possible infection. Pre-surgical antibiotics will also be dispensed and prescriptions for relief of post-surgical discomfort, follow-up antibiotics and written home care instructions will be provided. Immediately following the surgery the surgeon will complete a handling characteristics survey on the properties of bone graft material.

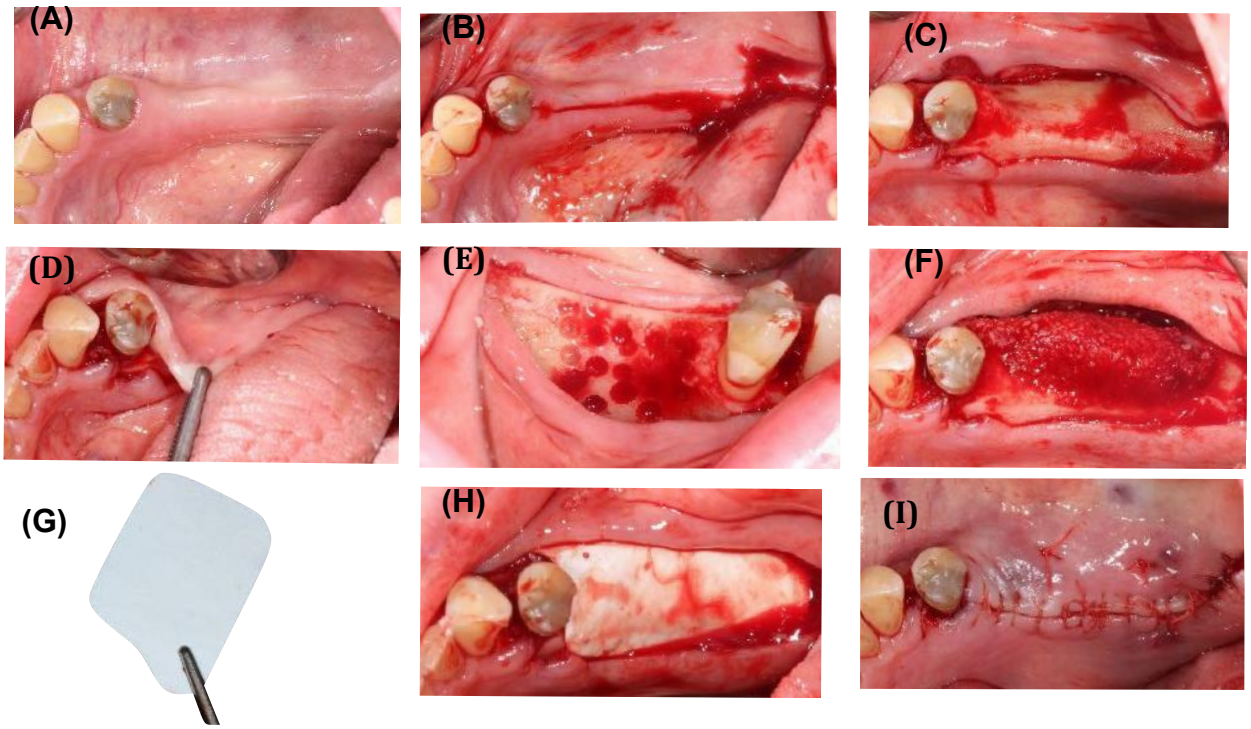

Fig. 4. Surgical steps .(A) Clinical appearance of horizontal ridge defect, (B) Crestal and vertical incisions, (C) Full thickness flap elevation .notice horizontal defect, (D) Mucoperiosteal release,(E) Bone decortication, (F) Particulate bone graft placed to rebuild defect,(G) bioresorbable collagen membrane shaping,(H) Placing the collagen membrane over grafted area,(I) Suturing

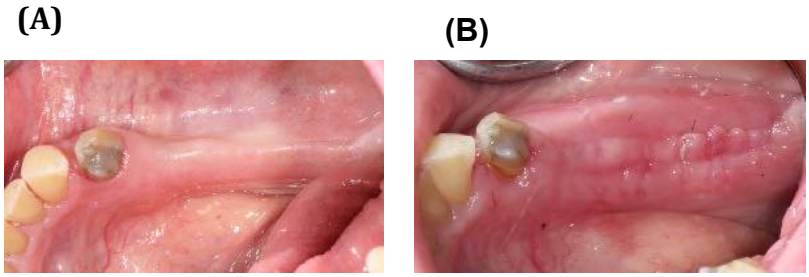

Fig. 5 . (A) initial appearance (B) 2 weeks follow-up and suture removal

Visit 2 (Follow-up): (Fig. 5)

The sutures will be removed after two weeks after cleaning the sutures with a gauze stacked with Peridex® chlorhexidine mouth rinse . Surgical sites will be evaluated for healing status and postoperative instructions on resuming oral hygiene measures will be instructed to patients.

Visit 3 (Bone Biopsy and Implant Placement):

Six months post-ridge augmentation, a second CBCT scan will be taken to evaluate the success of the grafting procedures and to plan the optimal implant position. The sites will be exposed for surgical placement of implants. The surgical approach will be similar to the

procedures for the graft procedures. After exposure of the bone the implant sites will be located with the use of the surgical stent (prepared by the restoring dentist) and the ridge width will be measured at the crest and 4 mm apical to the crest with the same standardized caliper. If ridge width permits implant placement, root form dental implants will be placed. Prior to implant placement a bone biopsy will be taken from the implant sites using a 2 mm internal diameter trephine(Fig. 6). For the bone biopsy, first the new bone will be measured, next the center of the new bone will be identified, and finally the core will be taken from the center of the new bone. The biopsy will be stored in the correct medium and will be sent to the Histomorphometry and Molecular Analysis Core for histomorphometric analysis. The implant preparation will be completed and implants will be placed according to manufacturer protocol.

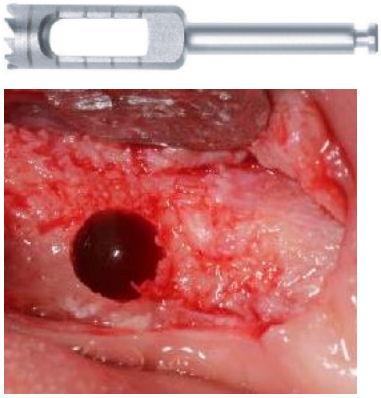

Fig.6 Bone Biopsy taken prior to implant placement using a trephine

All clinical measurements will be taken by one experienced examiner blinded to the randomization process (small vs. large particles) before grafting and at time of implant placement.Another examiner will perform the radiographic evaluations and will also be blinded to the randomization process. Intra-examiner calibration will be conducted to ensure reliability of measuring method.

Sample size rationale:

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, 22 patients in each group (total of 44) will reach 0.90 statistical power. Accounting for a 8 patient loss-to-follow up, 50 patients will be enrolled in the study.

Histomorphometric Analysis

Immediately following the bone biopsy at the center of the healed and regenerated ridge with a trephine (2-mm internal diameter), the specimen is placed in a formalin solution. Following fixation with 10% neutral buffered formalin for 48h, the bone biopsy specimens will be 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 will be stained with Sanderson's Bone Stain and imaged for quantification of bone formation. Histomorphometry will be 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 will be calculated for each of these tissues and compared between small and large particle grafts for ridge preservation and augmentation separately.

These experiments will be conducted at the UAB Histomorphometry and Molecular Analysis Core and all measurements made by an experienced blinded lab technician

Table 2. Clinical parameters and their respective timing of measurement

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

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The Influence of Bone Allograft Particle Sizes on the Quantity and Quality of New Bone Formation in Grafted Extraction Sockets

1. Abstract

Ridge preservation (socket grafting) at the time of tooth extraction is a commonly performed procedure that prevents extensive ridge resorption and allows for optimal implant placement. Ridge preservation can be achieved with different graft materials from autogenous, allogenic, xenograft, and alloplast sources. Due to its success in space maintenance, rapid turnover, biocompatibility, and the lack of need to harvest from another site, small-sized bone allograft materials (especially freeze-dried bone allografts, FDBA) have become increasingly popular. However, the effect of particle size on the clinical and histological outcomes following site preservation at the time of tooth extraction has not been well studied or reported in the literature. This randomized trial aims to clinically and histologically compare the amount and quality of the bone gained following ridge preservation procedures when using small (250-1000µm) versus large (1000-2000µm) particle size bone cortico-cancellous allografts at time of dental implant placement, 3 months following surgical intervention.

2. Introduction/Background

Studies have demonstrated that bone resorption occurs secondary to tooth extraction with the majority of dimensional changes occurring within the first 3 to 6 months [1-5]. The buccal wall of the socket (Fig. 1) tends to be resorbed to a greater degree than the lingual wall because the coronal aspect of the buccal plate is often comprised of bundle bone, a very thin layer (≤1mm in most cases) of bone [6]. Cardaropoli et al. [1] reported a meaningful negative correlation between baseline buccal wall thickness and ridge width change at sites receiving tooth extraction but no ridge preservation procedures. Vertical height loss frequently accompanies the horizontal loss and usually is seen when multiple adjacent extraction sites are combined (Fig. 2). Some have described a loss of up to 50% of the overall ridge width without ridge preservation at time of tooth extraction [7].

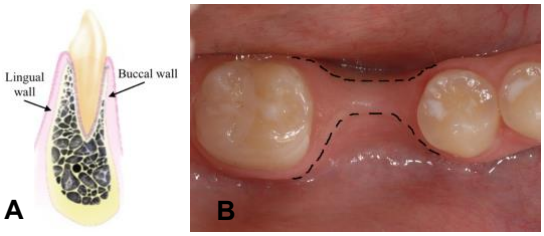

Fig 1. A. Socket anatomy, **B**. Loss of buccal bone following tooth extraction.

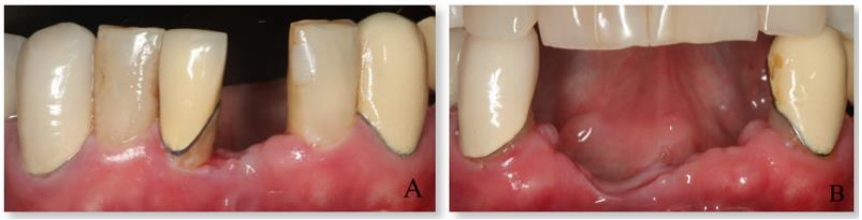

Fig. 2 A. Ridge shape and volume before extraction of mandibular anterior teeth. **B**. Ridge shape and volume after extraction without socket grafting of 3 adjacent teeth. Note vertical loss of ridge height.

Implant dentistry has become a successful routine treatment modality for partially or totally edentulous patients [8-14]. Socket grafting at the time of tooth extraction (referred to as ridge preservation) tends to maintain the bone volume necessary for the esthetic and functional placement of a dental implant [5, 7, 15-19]. In post-extraction ridge preservation procedures, a systematic review by Jambhekar et al. [5] reported on the histologic outcomes and/or changes in the bucco-lingual dimension and buccal wall height for a total of 117 extraction sockets grafted with allograft in 5 studies. For the re-entry point at implant placement at or beyond 12 weeks, the mean loss of bucco-lingual 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 [5]. These average dimensional changes are dramatically less than those observed following tooth loss in the absence of socket preservation.

Allografts are the most widely used bone replacement grafts for intra-oral applications in the US. Certified tissue banks use typically proprietary techniques for processing and packaging these allografts, to ensure their safety and

their clinical availability. Two of the most commonly used allografts in dentistry are freeze-dried bone allografts (FDBA) and demineralized freeze-dried bone allografts (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, released bone morphogenetic proteins (BMPs) from DFDBA give the allograft the added benefit of osteoinductivity by stimulating the host osteoprogenitor cells to differentiate into osteoblasts and begin new bone formation [20].

Eskow and Mealey [16] histologically evaluated in a randomized clinical trial the new bone formation following ridge preservation with cortical FDBA compared with cancellous FDBA. All bone graft materials were obtained from the same donor and a standard particle size of 250 to 1,000 μm was chosen for the study. A greater percentage of residual graft material was found in the cortical group compared with the cancellous group and as the percentage of new bone formation increased, the percentage of residual graft material decreased. No difference was found in the percentage of new bone formation between the two groups when core biopsies were harvested an average of 18 weeks after extraction and socket grafting.

Whitman and Mealey [19] reported on the differences in healing after tooth extraction and ridge preservation of non-molar teeth using DFDBA at 8 to 10 weeks of healing compared with 18 to 20 weeks of healing. There was no difference in ridge dimensional changes between sites healing for an average of 9 weeks compared with 19 weeks. There was a significantly different percentage of new vital bone between the short-term group and the long-term group. The short-term group had a mean of 32.63% vital bone compared with 47.41% in the long-term group. However, the authors stated that it was uncertain if the percentage of vital bone at an implant site would directly affect implant survival or success.

In another ridge preservation randomized clinical study, Wood and Mealey [15] histologically evaluated and compared the healing of non-molar extraction sockets grafted with DFDBA versus FDBA. There were no statistically significant differences in the changes in ridge dimensions after ridge preservation is performed with DFDBA versus 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.

The effect of particle size on the clinical and histological outcomes of ridge preservation has been scarcely studied or reported in the literature. Hoang and Mealey [21] conducted a ridge preservation randomized clinical trial after molar extractions. The objectives were to histologically and clinically compare human demineralized bone matrix (DBM) putty with one size of bone particles (SPS) to human DBM putty with two different sizes of bone particles (multiple particle sizes [MPS]). After 20 weeks of healing, core biopsies were obtained during implant placement and analyzed for the percentage area of vital bone, residual graft particles, and non-mineralized structures (connective tissue/other non-mineralized tissue [CT]). Changes in alveolar ridge dimensions were also determined. The SPS group had a mean of 49% vital bone, 8% residual graft, and 43% CT. The MPS group had 53%, 5%, and 42%, respectively. 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 with no reported statistically significant differences. The authors concluded that addition of larger bone particles to DBM putty did not offer additional benefit in the preservation of alveolar bone after the extraction of molar teeth. However, this conclusion is based on the effect of a mixture of different particle sizes and a small number of patients. Most commercially available grafts are categorized in either small or large particle sizes.

The use of large sized particles of anorganic bovine bone matrix resulted in significantly more vital bone formation than small particle grafts (26.77% ± 9.63% vs 18.77% ± 4.74%, respectively) in a prospective randomized controlled clinical trial of bilateral sinus augmentations [22]. 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 [23]. The authors related the difference between studies to the small sample size included in both (<15 patients). Due to the conflicting information and paucity of the literature on this topic, this proposed study will help 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.

3. Objectives/Specific Aims

The aim of the study is to clinically and histologically analyze the amount of the bone gained after ridge preservation procedures performed using small (250-1000μm) versus large (1000-2000μm) particle size bone cortico-cancellous allograft (Maxxeus™ Dental, Community Tissue Services, Kettering, OH) at time of implant placement, 3 months following surgical intervention.

Specific objectives include:

A. To compare the bone quantity (bucco-lingual ridge width) between the two bone allograft particle sizes by direct clinical measurements
B. To compare the bone quantity (bucco-lingual ridge width and apico-coronal ridge height) between the two bone allograft particle sizes by 3-dimensional radiographic measurements (CBCT scan)
C. To evaluate the quality of bone regenerated with the use of the two bone allograft particle sizes via histological and histomorphometric analyses.

Primary outcome: histologic and histomorphometric bone core analyses (from the implant preparation site).
Secondary outcomes: clinical and radiographic dimensional changes of the edentulous ridges.
Null Hypothesis: Large-sized particle bone allografts will achieve similar histologic and clinical outcomes as small-sized particle allografts.

4. Materials and Methods

Patients presenting to the graduate periodontal clinic at the University of Alabama at Birmingham School of Dentistry will be screened according to the following criteria:

Inclusion criteria	Exclusion criteria
• English speaking, able to read and understand informed consent document	• Less than 18 years old
• Hopeless non-molar tooth or teeth that are planned to be replaced with dental implants after ridge preservation	• Smokers/tobacco users (>10 cigarettes/day)
• Socket with residual 4 walls following minimally invasive tooth extraction (a dehiscence ≤ 3 mm may be included)	• Patients with systemic pathologies or conditions contraindicating oral surgical procedures or adverse affecting wound healing as uncontrolled diabetes
• Healthy adjacent teeth must be present and not planned for extraction	• Poor compliance risk (i.e., poor oral hygiene, history of alcohol or drug abuse)

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, 22 patients in each group (total of 44) will be required to reach 0.90 statistical power. Accounting for 8-patient loss-to-follow up, 50 patients will be enrolled in the study.

All clinical measurements are being taken by one experienced examiner blinded to the randomization process (small vs. large particles) before grafting and at time of implant placement. Another examiner will perform the radiographic evaluations and will also be blinded to the randomization process. Intra-examiner calibration will be conducted to ensure reliability of measuring method.

Screening/baseline visit:
Patients' medical history and electronic records will be reviewed. A treatment plan will be developed by an interdisciplinary team consisting of a periodontist and a restoring dentist and respective residents. Study examiners will conduct clinical and radiographic exams to determine eligibility according to the above criteria. If deemed eligible, study visits and objectives will be explained to all participants and IRB approved written informed consent will be obtained.

Surgical visit - Study visit 1:
Patients will be randomized on the day of surgery to receive either Small Particle (SP) (Group 1) or Large Particle (LP) bone allograft (Group 2) by permuted block randomization approach to ensure the same number of patients in each group, using computer-generated random number list. A course of prophylactic antibiotics will be dispensed at the time of surgery (Amoxicillin 2 g or Clindamycin 600 mg, 30 minutes to one hour prior to surgery). The graft material utilized for all surgical procedures, for all patients, will be 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, cortico-cancellous FDBA, Community Tissue Services, Kettering, OH). Following local anesthesia, tooth or teeth will be gently elevated using a minimally traumatic technique that prevents loss of supporting bone. Socket(s) will be curetted, irrigated and inspected following extraction and the presence of intact walls or minimal dehiscence (≤3mm) will be verified. The randomized bone allograft (SP or LP) will be hydrated in saline and then placed in the socket and covered with a collagen dressing. Vicryl sutures will be used to achieve stability of the collagen dressing over the graft material (Fig. 3). Standardized photographs will be taken to document the procedure.

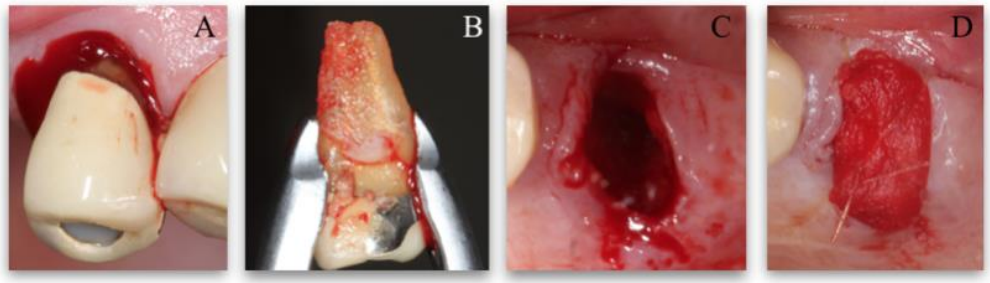

Figure 3.
A. Tooth is elevated, **B**. Extracted tooth, **C.** Socket is curetted, irrigated and inspected for presence of bone walls **D.** Graft is placed in the socket and covered with a collagen dressing and sutures.

Cone Beam Computed Tomography (CBCT) scan will be taken immediately after socket preservation and will serve for baseline measurements.
Post-surgical antibiotics will be prescribed for the first post-operative week and analgesics will be prescribed as necessary. All subjects will be dispensed Peridex® chlorhexidine mouth rinse and instructed to rinse twice daily for two weeks following the surgery, to help guard against possible infection. Verbal and written home care instructions will be provided.

Follow-up visit- Study visit 2
The sutures will be removed after two weeks. Surgical sites will be evaluated for healing status and postoperative instructions on resuming oral hygiene measures will be instructed to patients.

Bone Biopsy and Implant Placement - Study visit 3

Three months' post-socket grafting, a second CBCT scan will be taken to evaluate the success of the grafting procedures and to plan the optimal implant position. The site(s) will be exposed for surgical placement of the implant(s). After exposure of the healed edentulous ridge, the implant sites will be located with the use of the surgical stent (prepared by the restoring dentist) and the ridge width will be measured at the crest and 4 mm apical to the crest with the same standardized caliper. If ridge width is deemed adequate, a dental implant of appropriate size will be placed. Prior to implant placement a bone biopsy will be taken from the implant site using a 2-mm internal diameter trephine. The biopsy will be stored in formalin and sent to the UAB Histomorphometry and Molecular Analysis Core for histomorphometric analysis.

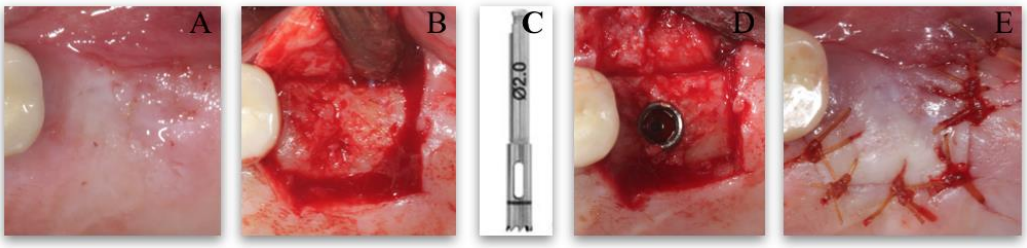

Fig 4. **A.** Site after 3 months of healing, **B.** Flap elevation, **C.** Core biopsy trephine, **D.** Implant placement, **E.** Flap replaced and sutured.

Histomorphometric Analysis

Each specimen will be fixated with 10% neutral buffered formalin for 48 hours prior to be dehydrated and embedded in methylmethacrylate. It will be 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 will be stained with Goldner's Trichrome stain and imaged for quantification of bone formation. Histomorphometry will be 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 will be calculated for each of these tissues and compared between small and large particle grafts for ridge preservation and augmentation separately. These experiments will be conducted at the UAB Histomorphometry and Molecular Analysis Core and all measurements made by an experienced blinded lab technician.

Clinical and Radiographic Measurements

Direct measurements (in mm) of the bucco-lingual ridge width will be taken following tooth extraction at the largest area of the socket orifice, using a UNC-15 periodontal probe. This measurement will be repeated at time of implant placement, 3 months after socket preservation.

Using baseline and implant placement CBCT data, the 2 scans will be superimposed in a virtual implant planning software (coDiagnostix) to evaluate 3-D volumetric changes (in cc). In addition, a virtual tooth (planned in optimal position at the implant site) will serve as reference point for standardized assessment of 2-dimensional ridge width and height changes (in mm) from the time of grafting until time of implant placement.

Statistical Analysis

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, 22 patients in each group (total of 44) will be required to reach 0.90 statistical power. Accounting for 8-patient loss-to-follow up, 50 patients will be enrolled in the study.

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.

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