

**PROSPECTIVE, COMPARATIVE ASSESSMENT OF ALVEOLAR RIDGE
PRESERVATION USING DIFFERENT BONE GRAFTING MATERIALS
(SYMBIOS CORTICAL-CANCELLOUS GRANULE MIX; SYMBIOS
OSTEOGRAF LD-300; OSTEOGRAF/N-300) FOLLOWING TOOTH
EXTRACTION**

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CLINICAL STUDY PROTOCOL

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PROTOCOL SYNOPSIS

Principal Investigator

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Co-Investigators

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Anthony Prudenti, DDS

Study Coordinator

Gidgetta Jenkins

Study center(s) and number of subjects planned

The study will include a total of 80 subjects recruited at the University of North Carolina, USA

Study timetable

Estimated date of first subject Q4 2015
enrolled

Estimated date of last subject Q3 2016
completed

Objectives

The primary objective of this study is to investigate volumetric osseous changes of an extraction socket at 3 months following a ridge preservation procedure using three different commercially available grafting materials (allograft, xenograft, and alloplast).

Secondary objectives include calculating linear horizontal and vertical bone and soft tissue changes.

Study design

The study is designed as a randomized controlled clinical trial. Immediately following tooth extraction, patients will be assigned to one of four treatment groups for ridge preservation. Groups will include the following:

- Group A (Symbios demineralized cortical-cancellous granule mix, Symbios OsteoShield Collagen Resorbable Membrane) (Allograft)
- Group B (Symbios OsteoGraf/LD-300, Symbios OsteoShield Collagen Resorbable Membrane) (Alloplast)
- Group C (OsteoGraf/N-300, Symbios OsteoShield Collagen Resorbable Membrane) (Xenograft)
- Group D (Symbios OsteoShield Collagen Resorbable Membrane only)

Target subject population

Patients in need of extraction and ridge preservation within zones 4-13, 20-22, and 27-29 will be included.

Investigational products

All products are intended to be used in an on-label procedure.

Symbios demineralized cortical-cancellous granule mix is an allograft bone graft material.

Symbios OsteoGraf LD-300 is an alloplastic bone grafting material composed of a low density synthetic hydroxyapatite.

OsteoGraf/N-300 is a xenoplastic bone grafting material that is a bovine-derived microporous hydroxyapatite.

Symbios OsteoShield Collagen Resorbable Membrane is a resorbable tissue matrix composed of Type-I Bovine collagen taken from the Achilles tendon. It presents as a multi-layer membrane which guides the healing of bone and the surrounding tissue, while the inner layers help prevent cellular and bacterial down growth into the site of bone preservation.

Duration of treatment

The study will be a 3 month follow-up. The treatment period includes tooth extraction followed by a ridge preservation procedure. Follow-up visits following the ridge preservation will occur at 14 days, and 3 months.

Outcome variables

Efficacy

Primary outcome variable:

- Volumetric osseous dimensional changes at 3 months

Secondary outcome variables:

- Horizontal and vertical dimensional changes in site (CBCT, cast measurements)
- Soft tissue dimensional changes (CBCT, cast measurements)

Patient reported outcomes

- Not applicable

Health economics

- Not applicable

Pharmacokinetics

- Not applicable

Safety

- Not applicable

Genetics

- Not applicable

Statistical methods

All primary and secondary outcome measurements will be analyzed by Wilcoxon rank sum test.

1 INTRODUCTION

1.1 Background

Following tooth extraction, a reduction in the alveolar ridge dimension occurs as a consequence of the wound healing process (1, 2). These resorptive changes preferentially affect the buccal bone, resulting in a horizontal shift of the bone volume to the lingual (3). Studies indicate that osseous resorption of 29-63% of the initial horizontal ridge width may occur, with much of the remodeling occurring within the first 3 months following extraction (2). When implant replacement is planned, this reduction in bone width and the displacement of the ridge crest may challenge ideal dental implant, resulting in the need for further bone augmentation procedures at time of implant placement and/or compromised implant positioning.

Implant positioning must facilitate esthetics and functional restoration. As such, maintenance of the alveolar architecture is desirable, although not detrimental, in helping to achieve this proper implant positioning. Attempts to prevent the dimensional ridge alterations associated with extraction socket healing have resulted in the development of ridge preservation procedures, whereby membranes are placed over the extraction socket and/or regenerative biomaterials are placed within the extraction socket, in an effort to maintain the alveolar ridge. While an implant can still be placed in an extraction site that has not undergone a ridge preservation procedure, systematic reviews suggest that ridge preservation techniques can be used to reduce the amount of horizontal and vertical ridge changes following tooth extraction (4, 5), possibly reducing the need for additional bone grafting procedures, and facilitating ideal implant placement. Currently ridge preservation procedures are not regarded as the standard of care. The majority of ridge preservation evidence, is restricted to linear measurements on radiographs or dental casts, which may not take into account volumetric or dimensional hard and soft tissue changes.

Membranes and bone replacement grafts are commonly used in ridge preservation procedures. A variety of bone graft options are available, including autogenic, allogenic, xenogenic, and alloplastic options, none of which have shown any superiority over another. The use of autogenic bone, however, requires a second surgical site, leading to increased treatment time and added patient morbidity. Instead, allografts, xenografts, and alloplasts are favored for ridge preservation procedures due to their convenience. Similarly, allogenic, xenogeneic, and alloplastic membranes have been used to seal extraction sockets, promoting a more ideal healing pattern, and reducing the amount of bone loss (6). What is unclear, however, is the effect of these different grafting materials in maintaining the ridge. Clarification of their unique influences, if any, of the maintenance of the post-extraction alveolus is necessary to help guide treatment decisions.

1.2 Rationale

This study is intended to provide statistically robust evidence that Symbios demineralized Cortical cancellous mix, Symbios OsteoGraf LD-300, and OsteoGraf/N-300 combined with Symbios OsteoShield Collagen Resorbable Membrane can adequately support the alveolus during ridge augmentation

procedures, reducing the dimensional changes of both the alveolus and the overlying soft tissues. Additionally, a comparison between each material will be made, providing further evidence of each materials' ability to preserve the alveolus. An additional comparison of the use of the Symbios OsteoShield Collagen Resorbable Membrane with or without supportive graft material will be made. These comparisons are intended to define in objective terms the response of the hard and soft tissues to ridge augmentation using four different protocols currently used in clinical practice. To fulfill the goals of this project, the clinical study will enroll sufficient patients to perform statistical analyses of primary objectives.

A common and non invasive approach used for site assessment following a grafting procedure is the use of cone-beam computed tomography (CBCT) images (7). Several studies have demonstrated the reproducibility and accuracy of CBCT(8-11); concluding that there was no significant difference between the radiographic and clinical measurements. CBCT is now acknowledged by the American Academy of Oral and Maxillofacial Radiology, for the pre-surgical implant planning and augmentation procedures as the imaging modality of choice for preoperative cross-sectional images of potential implant sites (12). CBCT may be used to measure the alveolar bone condition prior to implant therapy and following ridge preservation. Therefore, when serial CBCT data is available, the direct volumetric assessment of ridge preservation outcomes will be possible.

2 STUDY OBJECTIVES

2.1 Primary objective

The primary objective is to compare the volumetric changes in the osseous dimensions following a post-extraction ridge preservation procedure. Three different bone-grafting materials will be used, along with comparison to a non-grafted membrane only group. Data will primarily be obtained from DICOM images acquired by CBCT imaging. A surface based model will be constructed through image segmentation from both the pre- and post-operative CBCT images. The models will then be aligned/superimposed using a surface registration algorithm. Both images will be cropped to represent the same region of interest and the volumes will be calculated and compared.

Hypothesis: No significant differences in ridge volume maintenance are anticipated among the three grafting groups. The combined use of particulate graft material with a resorbable collagen membrane will maintain a greater ridge volume than the use of a resorbable collagen membrane alone.

2.2 Secondary objectives

Secondary objectives of the study are to evaluate and compare:

- Buccolingual dimensional changes in site (CBCT)
- Vertical dimensional changes in site (CBCT)
- Soft tissue contour changes associated with healing post extraction (digitized cast measurements superimposed on CBCT images)

3 STUDY PLAN AND PROCEDURES

3.1 Overall study design and flow chart

The study is designed as a randomized controlled prospective clinical trial.

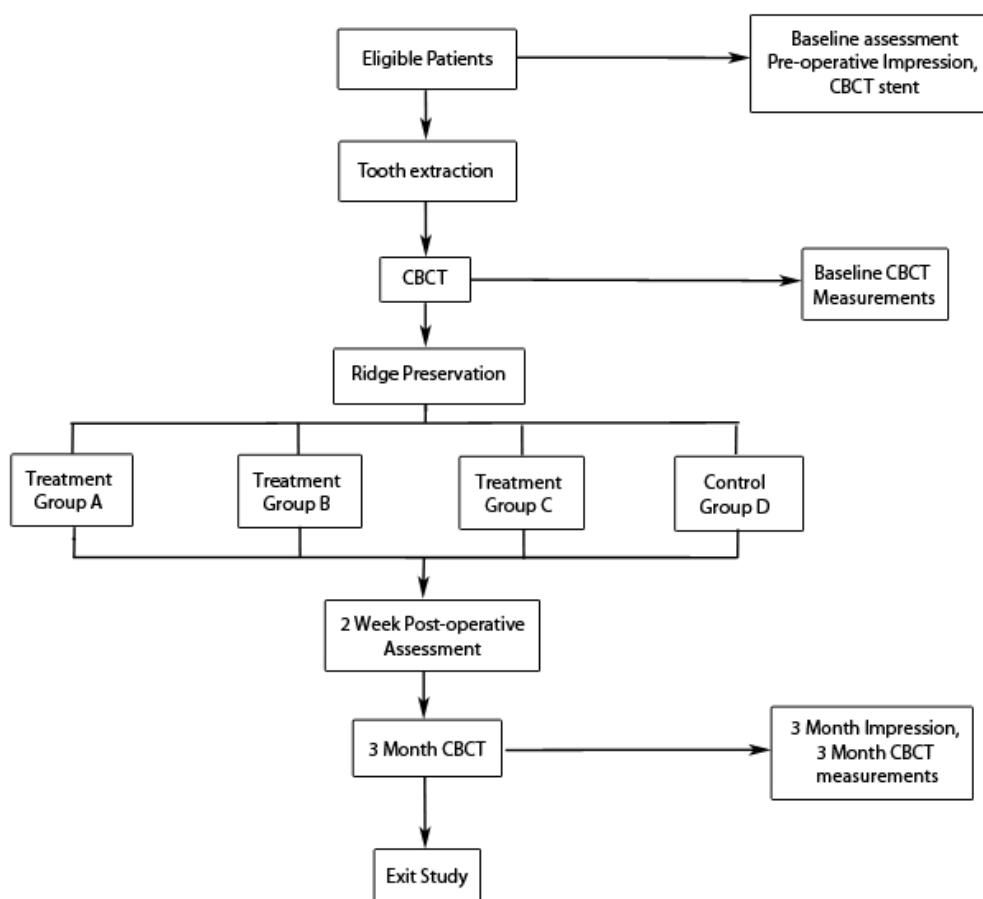
The study population will consist of individuals requiring an extraction with socket preservation. Sites within zones 4-13, 20-22, 27-29 will be included.

80 subjects will be included. Each subject will be randomized into one of three bone graft treatment groups or one membrane only treatment group:

- Group A (Symbios demineralized cortical-cancellous granule mix, Symbios OsteoShield Collagen Resorbable Membrane) (Allograft)
- Group B (Symbios OsteoGraf/LD-300, Symbios OsteoShield Collagen Resorbable Membrane) (Alloplast)
- Group C (OsteoGraf/N-300, Symbios OsteoShield Collagen Resorbable Membrane) (Xenograft)
- Group D (Symbios OsteoShield Collagen Resorbable Membrane only)

The study will be a 3 month follow-up with 4 clinic visits.

Figure 1 Study flow chart



3.1.1 Visit 1: Screening

Subjects in need of a single tooth extraction and ridge preservation of a single tooth bound by adjacent teeth that are deemed eligible by meeting inclusion and exclusion criteria will be considered for treatment in this study. The tooth requiring extraction must lie within the region 4-13, 20-22, 27-29 (maxillary or mandibular first premolar or anterior tooth, excluding mandibular incisors).

Before any assessment or examination is carried out the subject must have been informed orally and in writing about the study, and have signed the appropriate clinical and study consent forms. Informed consent for the research study will be given and signed on paper, as well as HIPAA authorization. Subjects will also be asked to sign informed consent documents.

Individuals meeting all inclusion and exclusion criteria and that have signed all appropriate consent forms will be further evaluated. A clinical exam will entail evaluation of general dental and systemic health. It will also include a clinical and radiographic assessment of the tooth to be removed and the adjacent teeth, which is standard of care. Pre-existing radiographs (e.g. panoramic and intraoral) can be used but must not be older than 6 months. An impression will be made using an elastomeric impression material of the planned extraction site to allow fabrication of a temporary tooth (standard clinical requirement). This impression will also serve an additional research purpose, providing baseline tissue data. Subjects will immediately be scheduled for Visit 2 provided that they fulfill all inclusion and exclusion criterion.

A radiographic stent for research purposes will be fabricated using the model obtained from the initial impression to aid in image alignment, superimposition and identifying the points of measurement for the study objectives.

After pre-surgical evaluation and planning, patients will be enrolled and randomly allocated treatment into treatment group A, B, or C; or into group D.

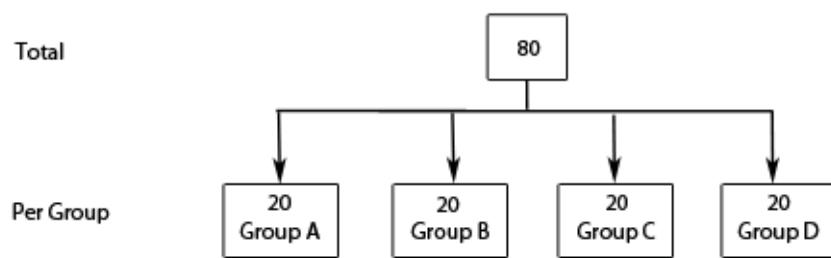
This visit includes all procedures, which are standard clinical protocol, with additional usage of the clinical impression for research purposes.

3.1.2 Visit 2: Extraction, Initial CBCT, and Socket Preservation Surgery

Randomization and Stratification

Subjects meeting all inclusion and none of the exclusion criteria will be randomly allocated to group A, B, C, or D, through the blind drawing out of a folded piece of paper containing the assigned treatment group (20 pieces indicating group, A, B, C and D) by the PI or co-investigators. Subjects will be randomized into treatment groups immediately following tooth extraction.

Figure 2 Randomization and stratification



Pre-Surgical, Surgical, and Post-Surgical Care (at surgical appointment)

Pre-surgical, surgical and post-surgical care will be given at the discretion of the Investigator and recorded in appropriate sections in the CRF:

- Antibiotics
- Analgesics
- Anaesthesia
- Anxiolysis

Antibiotics:

Pre-surgical antibiotic prophylaxis will be provided for patients at risk for infective endocarditis or with total joint replacement according to current guidelines provided by the American Dental Association, American Heart Association (13), and the American Academy of Orthopaedic Surgeons (14).

Post-surgical antibiotic coverage will be provided to patients. It is anticipated that a 7-day course of 500mg Amoxicillin every 8 hours or 300mg Clindamycin every 6 hours will be provided.

Analgesics:

Post-surgical analgesics will be provided, most likely consisting of 800mg Ibuprofen every 6-8 hours and 5/325mg Acetaminophen/Hydrocodone every 4-8 hours.

Anxiolysis:

Options will be discussed with the patient and a decision will be made to reduced anxiety using 1-2mg Ativan.

Anesthesia:

20% benzocaine topical anesthesia will be maintained for 1 minute. Infiltration anesthesia using 2% lidocaine with 1:100,000 and 2% lidocaine with 1:50,000 epinephrine will be provided. It is anticipated that 1.8 to 3.6cc will be used.

Post-surgery, infiltration anesthesia with 0.5% bupivacaine with 1:200,000 will be provided. It is anticipated that 1.8cc will be used.

All medications used are standard clinical practices.

Pre-Surgical Procedures

Buccal and crestal photographs of the planned surgical site will be obtained using a Canon 50D digital camera body with a Canon 100mm f/2.8 macro lens and a Canon MR-14EX macro ring flash.

All pre-surgical procedures including photographs taken are standard clinical practice in the School of Dentistry clinics.

Surgical Procedure

A circumferential sulcular incision will be placed around the teeth to be extracted. Periotomes and luxators will be used to extract the tooth with minimal trauma to the adjacent tissues. The socket wall will be examined for any dehiscence greater than 3mm or fenestrations. If these are present, the patient will be excluded from the study. Gauze will be placed in the area to produce wound hemostasis

Immediately following tooth extraction, the patient will have a small field-of-view cone beam computed tomography (CBCT) image produced whilst wearing the radiographic stent. Images will provide pre-preservation data for horizontal and vertical bone locations.

Following CBCT imaging, a ridge preservation procedure will be completed as follows:

- Group A: Symbios demineralized cortical-cancellous granule mix reconstituted in sterile saline will be used to augment the socket and will be covered by a Symbios OsteoShield Collagen resorbable membrane.
- Group B: Symbios OsteoGraf LD-300 reconstituted in sterile saline will be used to augment the socket and will be covered by a Symbios OsteoShield Collagen resorbable membrane.
- Group C: Symbios OsteoGraf/N-300 reconstituted in sterile saline will be used to augment the socket and will be covered by a Symbios OsteoShield Collagen resorbable membrane
- Group D: Socket covered with Symbios OsteoShield Collagen resorbable membrane and no grafting material, allowing socket to heal.

Grafted sockets will be closed with a barrier membrane using 5-0 chromic gut suture. It is anticipated that a cross mattress and/or simple loop sutures will be employed.

All surgical procedures listed above are standard clinical procedures, including preparation and utilization of grafting materials list above.

Post-Surgical Procedures

Buccal and crestal photographs of the surgical site will be obtained, which is standard clinical practice in the School of Dentistry.

Standard UNC post-operative instructions will be given which includes the use of a Chlorhexidine rinse 2 times daily for 2 weeks.

This visit includes a CBCT for research purposes only. The remainder of treatment is standard clinical protocol.

3.1.3 Visit 3: Post-Operative Visit (14 days \pm 2 days)

The stage of healing will be clinically assessed. Remaining suture material will be removed. Buccal and crestal photographs of the surgical site will be obtained using a Canon 50D digital camera body with a Canon 100mm f/2.8 macro lens and a Canon MR-14EX macro ring flash.

AEs/ADEs will be recorded.

This visit is standard clinical protocol

3.1.4 Visit 4: Cone Beam Computed Tomography Imaging (12 weeks \pm 7 days)

Small field-of-view cone beam computed tomography (CBCT) imaging whilst wearing the radiographic stent will be obtained for all patients 3 months following surgical treatment. Images will provide post-ridge preservation data for horizontal and vertical bone assessments. Buccal and crestal photographs of the surgical site will be obtained. An elastomeric impression of the site will be obtained.

AEs/ ADEs will be recorded.

This appointment contains a CBCT, photographs and an impression which is standard clinical protocol when planning future implant placement. Additionally the CBCT and impression will be used for research purposes.

Additional visits may be scheduled at the discretion of the investigator and recorded as extra visits in the CRF.

Figure 3 Appointment flow chart

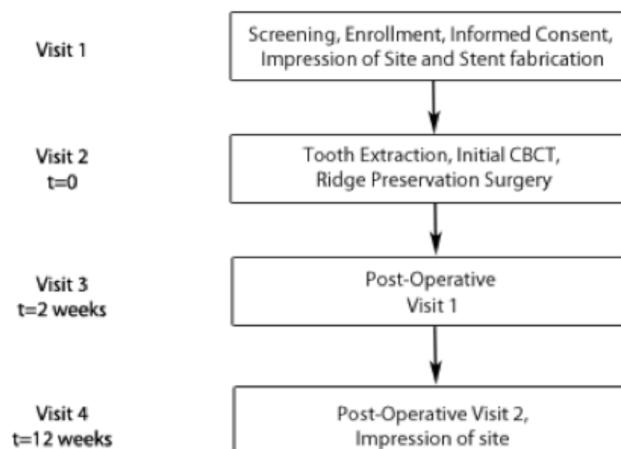


Table 1 Study plan

Visit Number	1	2	3	4
Visit Description	Screening, Impressions	Extraction, initial CBCT, Ridge Preservation	Post-op	Post-op, Impressions, final CBCT
Visit Window		0	2w	12w
Patient Information				
Informed consent	X (R)			
Patient demographics	X (C)			
Medical/surgical history	X, (C)			
Oral examination	X (C)			
Inclusion/exclusion criteria	X (R)			
Radiographic examination	X (C)			
Treatment				
CBCT		X (R)		X (C)
Clinical photography		X (C)	X (C)	X (C)
Tooth Extraction		X (C)		
Site Preservation		X (C)		
Suture removal			X (C)	
Impression	X (C)			X (C)
Other				
Adverse events/Adverse device effects		X	X	X
Summary of additional patient requirements per appointment solely for research purposes		CBCT		

**All standard of care procedures will be used for research purposes.

Standard of Care Clinical requirement=C

Research requirement =R

3.2 Rationale and risk/benefit assessment

Limited data is available regarding volumetric changes of the alveolus following tooth extraction and ridge preservation procedures. This has direct effects on subsequent dental implant treatment. Successful ridge preservation enhances proper implant placement. Furthermore, it is necessary to better define the effects of different grafting materials on the clinical outcomes following ridge preservation. This study has been designed to treat a clinical scenario commonly encountered in clinical practice

The public will benefit from the study results, which will help to define the knowledge of bone healing following extraction and ridge preservation with different grafting materials. This will help aid clinicians in the selection of materials to optimize their clinical outcomes of treatment. It will also further characterize the effects of these materials on soft tissue healing while comparing its outcomes to other commonly used materials. Data acquisition includes minimally invasive collection methods, both before and after the extraction and ridge preservation procedure.

3.2.1 Study selection record

Investigator(s) will keep a record of subjects who were considered for enrollment but were never enrolled (e.g., subject screening log). This information is necessary to establish that the subject population was selected without bias.

3.2.2 Inclusion criteria

For inclusion in the study subjects must fulfill all of the following criteria:

1. Provision of informed consent
2. ≥ 18 years and ≤ 75 years
3. Good physical health (ASAI/II)
4. Extraction of maxillary premolar, canine or incisor, or mandibular premolar and canine required (4-13, 20-22, 27-29)
5. Teeth adjacent (mesial and distal) to study site must consist of two stable natural teeth with minimal restorations, without signs of periodontal bone loss (> 3 mm) and/or significant soft tissue deficiencies

3.2.3 Exclusion criteria

Any of the following is regarded as a criterion for exclusion from the study:

1. Buccal plate dehiscence and/or fenestration >3 mm at study site following extraction
2. Untreated rampant caries and uncontrolled periodontal disease
3. Inadequate oral hygiene (estimated plaque score $>20\%$)

4. Smokers using more than 10 cigarettes or equivalent per day
5. Smokeless tobacco use or e-cigarette use
6. Compromised physical health and/or uncontrolled or severe systemic diseases including:

ASA III/IV

Metabolic bone disease

History of malignancy

History or radiotherapy or chemotherapy for malignancy in the past 5 years

History of autoimmune disease

Long-term steroid (20mg cortisol or equivalent for 2 weeks duration in past 2 years) or antibiotic therapy (Antibiotic therapy exceeding 2 weeks in past 1 year)

Uncontrolled diabetes (HbA1c ≥ 7)

Known alcohol or drug abuse

7. Systemic or local disease or condition that would compromise post-operative healing
8. Use of any substance or medication that will influence bone metabolism
9. Pregnancy at time of screening
10. Unable or unwilling to return for follow-up visits for a period of 3 months
11. Unlikely to be able to comply with study procedures according to Investigators judgement
12. Involvement in the planning and conduct of the study
13. Previous enrolment or randomization of treatment in the present study

3.2.4 Restrictions

Subjects will be advised of the following restrictions during the study period:

- To avoid disruption of wound healing during the initial study period the subject should have a restricted diet for at least 3-5 days and will be instructed to avoid manual oral hygiene in site for 2 weeks (printed instructions will be distributed to the subjects at Visit 2)
- For current smokers, interim cessation will be encouraged and no more than 10 cigarettes per day are allowed

3.2.5 Discontinuation of subjects from treatment or assessment

3.2.5.1 Criteria for discontinuation

Subjects may be discontinued from study treatment and assessments at any time. Specific reasons for discontinuing a subject from this study are:

- Voluntary discontinuation by the subject who is at any time free to discontinue his/her participation in the study, without prejudice to further treatment
- Safety reasons as judged by the investigator
- Severe non-compliance to protocol as judged by the investigator
- Incorrect enrolment (i.e., the subject does not meet the required inclusion/exclusion criteria for the study)
- Subject received further dental therapy on study site or adjacent teeth without consent of investigator (e.g. new crown, periodontal surgery, or orthodontics on adjacent teeth)
- Subject lost to follow-up

3.2.5.2 Procedures for discontinuation

Subjects who discontinue should always be asked about the reason(s) for their discontinuation and the presence of any AEs or ADEs. If possible, they should be seen and assessed by an investigator(s). Ongoing AEs and ADEs should be followed up.

A subject will be classified as lost to follow up only if, he/she has failed to return to the required study visits and his/her dental status remains unknown, despite multiple attempts to contact the subject via telephone, fax, email, certified letter and through subject locator agencies (if allowed per national regulation).

3.3 Treatments

3.3.1 Identity of investigational product and comparators

All procedures involved in this research study are standard of care procedures for patients requiring dental extraction and desiring dental implant treatment in the future. All graft materials and their combinations used in this study are widely used in the dental procession for patient care. All components are commercially available.

Symbios demineralized cortical-cancellous granule mix is a human allograft product obtained from the Musculoskeletal Transplant Foundation (MTF), which mimics the natural bone anatomy, providing space maintenance and surface area for bone formation. The material contains 80% cortical and 20% cancellous bone, displaying a particle size of 200-1000 microns.

Symbios OsteoGraf LD-300 is a 100% pure synthetic hydroxyapatite bone product that shows 56% porosity and high surface area. It is conducive to solution-mediated resorption, producing a readily available source of calcium for bone regeneration. This low density resorbable grafting material presents as 250-420 micron particles, and is available in 0.5, 1.0 and 3.0 gram vials.

Symbios OsteoGraf/N – 300 is a sintered natural anorganic bovine-derived microporous hydroxyapatite, displaying a particle size of 250-420 microns. The hydroxyapatite is conducive to cell-mediated resorption and provides a scaffold for new bone growth, holding the space until host bone takes over. There are no extractable proteins present in the product. The product is available in 1.0 and 3.0 gram vials.

Symbios OsteoShield Collagen Resorbable membrane made from Type-I bovine collagen from the Achilles tendon. It has a resorption time of between 26 and 38 weeks. It is a multi-layered membrane, which assists in healing of the bone and surrounding tissues, while the inner layers help prevent cellular and bacterial down growth. The membrane is available in 15x20mm, 20x30mm and 30x40mm.

3.3.2 Labeling

All grafting materials and membranes will be labelled as follows:

- “Reference number”
- “Lot number”
- “Expiration date”

The reference number and lot numbers will be recorded in clinical records (source data) and CRF.

3.3.3 Storage

All investigational products must be kept in a secure place under appropriate storage conditions. A description of the appropriate storage and shipment conditions are specified on the study product label and product information.

3.3.4 Accountability

Distributed study products will be used for this study in accordance with the Clinical Study Protocol. All product deliveries will be confirmed by the investigator or delegate.

3.4 Method of assigning subjects to treatment groups

Subject numbers (subject ID) will be consecutively allocated in series at day of inclusion (Visit 1). Subjects will receive numbers starting at 101. Enrollment will continue until 80 subjects have been allocated a subject ID. If a subject discontinues, the subject number will not be reused.

Subjects will be randomized strictly sequentially at day of extraction and ridge preservation (Visit 2). At Visit 2 all study sites in all subjects are considered equivalent. The randomization schedule will be generated using a validated system under the responsibility of the investigatory team. 80 pieces of folded paper will contain the allocated treatment group (20 each of group A, B, C and D) and contained in an enclosed container. One piece of paper will be blindly selected by the PI or co-investigators, and the treatment group will be revealed to the clinician only following extraction of the tooth.

The randomization code papers will be stored in an enclosed and concealed container and stored by PI and co-investigators.

3.5 Pre-study, concomitant and post-study treatment(s)

In the case of dehiscence, fenestration, infection, or other surgical complications encountered during treatment and/or healing, appropriate site preservation procedures and/or debridement and/or infection control steps will be implemented.

4 MEASUREMENTS OF STUDY VARIABLES AND DEFINITIONS OF OUTCOME VARIABLES

4.1 Screening and demographic measurements

The following data will be recorded via a standard CRF:

- Date of birth
- Height and weight (Body Mass Index)
- Sex
- Race
- Relevant medical and surgical history
- Medication
- Oral examination
 - Periodontal disease
 - Bone loss
 - Caries
 - Mobility
 - Crown
 - Post/core
 - Endodontic treatment
 - Existing periapical radiolucency
- Tobacco use
- Reason for tooth extraction
- Previous bone graft, soft tissue graft, or apical surgery

4.2 Efficacy

4.2.1 Primary outcome variable

4.2.1.1 Volumetric osseous changes

The amount of change in bone volume will be calculated using data from DICOM images acquired by CBCT using 3D software.

4.2.1.1.1 Methods of assessment

CBCT images will be obtained at baseline (Visit 2) and 3 months following alveolar ridge augmentation (Visit 4). Using 3D imaging software, the bone will be segmented to produce an STL file. Following this, the segmented images will be superimposed and aligned through a fiducial point registration algorithm, and at the same time, the same region of interest will be cropped in each scan and exported as a separate model. The volume of the baseline and 3 month scan will be calculated and compared.

4.2.1.1.2 Derivation or calculation of variable

The changes in the bone volume between baseline (Visit 2) and 3 months (Visit 4) will be measured electronically using 3D analysis software. The change for each study site will be compared longitudinally and the average for each treatment group will be calculated and compared among treatment and control groups.

4.2.2 Secondary Outcome Variables

4.2.2.1 Osseous dimensional changes

The amount of horizontal and vertical bone changes will be calculated using data from DICOM images acquired by CBCT using 3D software.

4.2.2.1.1 *Methods of assessment*

CBCT images will be obtained at baseline (Visit 2) and 3 months following alveolar ridge augmentation (Visit 4). Using 3D analysis software, the CBCT images will be aligned via superimposition through fiducial point registration, to permit evaluation of bone changes within the bound edentulous space.

4.2.2.1.2 *Derivation or calculation of variable*

The mid-alveolar point (middle of the extraction site) positioned on a tangent drawn from the mesial and distal adjacent tooth CEJs will be used as a reference point for assessment of dimensional bone changes in the Z-axis (Bone width) at 3, 6 and 9mm as measured from the height of the original buccal zenith (with the aid of a radiographic stent. Measurements will be taken from at these points from the buccal plate to the lingual plate. Changes in the Y-axis (bone height) will also be assessed. The changes in the bone dimensions between baseline (Visit 2) and 3 months (Visit 4) will be measured. The change for each study site will be compared longitudinally and the average for each treatment group will be calculated and compared among treatment groups.

4.2.2.2 Soft tissue dimensional change

The amount of soft tissue change will be assessed through the use of dental casts at two time points following extraction and ridge preservation, and 3 months.

4.2.2.2.1 *Methods of assessment*

Elastomeric impressions will be made of the site at baseline, and 3 months following extraction. The impression will be poured in dental stone, and the cast will be scanned using an optical/laser scanner to produce a digitized model. The digital models will then be aligned in 3D software and superimposed on one another and the relevant CBCT image utilizing the fiducial point registration algorithm.

4.2.2.2.2 *Derivation or calculation of variable*

The mid-alveolar point (middle of the extraction site) positioned on a tangent drawn from the mesial and distal adjacent tooth CEJs will be used as a reference point for assessment of dimensional bone changes in the Z-axis (soft tissue width) at 3, 6 and 9mm as measured from the height of the original buccal zenith and indicated on the radiographic stent. Measurements will be taken from at these points from the buccal contour to the underlying buccal plate. Changes in the Y-axis (soft tissue height) will also be assessed. The changes in the soft tissue dimensions between baseline (Visit 2) and 3 months (Visit 4) will be measured. The change for each study site will be compared longitudinally and the average for each treatment group will be calculated and compared among treatment groups.

4.3 Patient reported outcomes

Not applicable

4.4 Health economic measurements and variables

Not applicable

4.5 Pharmacokinetics

Not applicable

4.6 Safety measurements and variables

The methods for collecting safety data are described below.

4.6.1 Adverse Events

4.6.1.1 Definitions

The definitions of AEs, ADEs and Serious Adverse Events (SAEs) are given below. It is of the utmost importance that all staff involved in the study are familiar with the content of this section. The principal investigator is responsible for ensuring this.

Adverse Event

An AE is any untoward and unintended medical occurrence in a subject. This definition does not imply that there is a relationship between the AE and the medical device under investigation. An AE which is possibly related, is one that may have been caused by the medical device, or treatment, however there is insufficient information to determine the likelihood of this possibility.

- Possibly related: temporal relationship of the onset of the event, relative to the use/administration of the medical device, is reasonable but the event could have been due to another, equally likely cause.
- Non-related (unlikely): temporal relationship of the onset of the event, relative to the use/administration of the medical device, is not reasonable or another cause can itself explain the occurrence of the event.

Ambiguous cases should be considered as possibly related.

Adverse Device Effect

An ADE is any untoward and unintended response to a medical device. This definition includes any event resulting from insufficiencies or inadequacies in the instructions for use of the medical device or any event that is a result of a user error. This definition also includes treatment- or procedure-related events. ADEs can only occur from the time of medical device use/administration. Here the event is related to the use of the medical device where there is a probable/definite relationship that the event may have been caused by the medical device, or treatment.

- Probably related: temporal relationship of the onset of the event, relative to the use/administration of the medical device, is reasonable and the event is more likely explained by the medical device/treatment than by any other cause.
- Definitely related: temporal relationship of the onset of the event, relative to the use/administration of the medical device, is reasonable and there is no other cause to explain the event.

Serious Adverse Event

A SAE is an AE/ADE occurring during any study phase of the medical device that fulfills one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-subject hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above

If a non-related AE becomes serious, i.e. fulfilling one or more of the above criteria, action should be taken as for any other SAE.

4.6.1.2 Recording of Adverse Events and Adverse Device Effects

At each visit, scheduled or unscheduled, the subject will be asked an open question; "Have you had any health problems since the previous visit?"

All health problems, reported by the subject or found at the clinic visit, where the investigator believes the event "possibly" has been caused by the investigational medical device or treatment, must be recorded in the CRF as AEs, specifying time of onset, action taken, outcome and whether it constitutes a SAE or not. Non-related AEs must be recorded in the CRF together with a short description and whether it constitutes a SAE or not.

All health problems, reported by the subject or found at the clinic visit, where the investigator believes the event "probably/definitely" has been caused by the investigational medical device or treatment, must be recorded in the CRF as AEs and ADEs, specifying time of onset, action taken, outcome and whether it constitutes a SAE or not.

Should a pregnancy occur, it must be reported in accordance with the procedures described in Section 9.2, Pregnancy. Pregnancy in itself is not regarded as an AE

unless there is a suspicion that a medical device under study may have interfered with the effectiveness of a contraceptive medication.

4.6.1.3 Reporting of Serious Adverse Events

Timelines for reporting of SAEs

Investigators and other study site personnel must inform the ethics committee and, if necessary, the appropriate regulatory authorities of any SAE that occurs during the course of the study within the timeline set by the code of federal regulations.

4.7 Genetics

Not applicable

5 DATA MANAGEMENT

5.1 Data handling

A web-based electronic data capture system (REDCAP) will be used. The eCRF will be completed for each included patient. The completed eCRFs will not be made available in any form to third parties without written permission from the principal investigator/sponsor.

All data from source documents will be entered into the eCRF database. The data entry will be verified by means of double data entry or proofreading. Before database lock, both manual and computerized validation tests will be performed and, if necessary, data queries will be sent to the investigator for clarification. Should questionable data be detected during the validation process, written queries will be raised as result of this validation. The study site personnel are required to resolve any such queries. The eCRFs will be retained for 3 years following completion of the study as required by law and then disposed of according to the standard operating procedure of the School of Dentistry for patient information.

5.2 Record retention

To enable evaluations and/or audits from regulatory agencies, the investigator agrees to keep records in the Investigator's Study File (ISF), including the identification list of the participating patients, all original signed Informed Consent forms, and detailed records of medical device disposition. To comply with international regulations, the investigator should retain the records for 3 years following completion of the study as required by law and then disposed of according to the standard operating procedure of the School of Dentistry for patient information.

6 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

6.1 Statistical evaluation – general aspects

A comprehensive Statistical Analysis Plan (SAP) may be prepared before database lock. If prepared, the SAP will be kept as an appendix to the Data Management Plan. When using the terminology descriptive statistics it means that number of patients, mean, median, standard deviation, minimum and maximum values will be presented for continuous data and frequencies and percentages for categorical data.

Descriptive statistics will be given for each variable in the study and p-values may be complemented by confidence intervals as appropriate. A p-value less than 0.05 will be considered “statistically significant”.

6.1.1 Demographics and other baseline characteristics

Demographics and other baseline characteristics will be presented by means of descriptive statistics (by group and in total). Continuous variables will be presented by means of number of observations (N), minimum (min), median, maximum (max), mean, and standard deviation (SD). Discrete variables will be presented by frequency and percentage. Difference in patient demographics between the treatment groups will be assessed using the Chi Square test to assess the similarity in treatment groups, ensuring that the outcomes are not influenced by difference in treatment group demographics.

6.1.2 Covariates and prognostic variables

No covariates are judged to influence the outcome of the primary or any of the secondary variables, however these will be assessed using the Chi Square test.

6.1.3 Handling of dropouts and missing data

Patients dropping out from the trial prior to study end will not be replaced. Data totally missing will not be estimated.

6.1.4 Subgroup analyses

No subgroup analyses are planned.

6.2 Method of statistical analysis

6.2.1 Primary objective

The primary objective is to compare dimensional bone changes from baseline (Visit 2) to three months (Visit 4) occurring in a single bound extraction site following ridge preservation using three different grafting materials and membrane, and one no graft membrane only group.

Assume the change is denoted C_A , C_B , C_C , and C_D , for group A, B, C, and D respectively. The null-hypothesis is then to test if:

$H_0: C_A = C_i$ ($i = B, C$, and D respectively)
can be rejected and hence
 $H_1: C_A \neq C_i$ ($i = B, C$, and D respectively)
accepted.

H_0 will be tested by means of the Wilcoxon rank sum test. A p-value less than 0.05 will be regarded statistically significant.

6.2.2 Secondary objectives

Both the dimensional soft tissue changes from baseline (Visit 2) to three months (Visit 4) and linear soft tissue and osseous changes occurring in a single bound extraction site following ridge preservation using three different materials will be compared.

Assume the change (in the mid-buccal horizontal soft tissue dimensions from baseline to three months) is denoted C_A , C_B , C_C , and C_D , for group A, B, C, and D respectively. The null-hypothesis is then to test if:

$H_0: C_A = C_i$ ($i = B, C$, and D respectively)
can be rejected and hence
 $H_1: C_A \neq C_i$ ($i = B, C$, and D respectively)
accepted.

H_0 will be tested by means of the Wilcoxon rank sum test. A p-value less than 0.05 will be regarded statistically significant.

6.3 Determination of sample size

A sample size of 20 participants per treatment group was selected following power calculations and allowing for 10% patient dropout (has dropout rate been factored into our sample size of 20 patients per group). A sample size of 18 patients was calculated for the primary outcome variable (horizontal bone changes) with the assumption that the detectable difference would amount to 0.5mm³ with a standard deviation of 0.5. The type I error probability was set at 0.05 and the statistical power was set at 80%.

6.4 Statistical analyses during the course of the study

The primary and secondary objectives will be analyzed when all data from the 3 month follow-up visit has been collected, entered, verified, and validated (partial clean file).

7 STUDY MANAGEMENT

7.1 Audits and Inspections

Authorized regulatory authorities or an ethics committee may visit the center to perform audits or inspections, including source data verification. The purpose of an audit or inspections is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, ISO 14155, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements.

7.2 Training of staff

The principal investigator will maintain a record of all individuals involved in the study (medical, nursing and other staff). He or she will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information of relevance to the performance of this study is forwarded to the staff involved.

7.3 Changes to the protocol

Study procedures will not be changed without the agreement of the principal investigator.

If it is necessary for the study protocol to be amended, the amendment and/or a new version of the study protocol (Amended Protocol) must be notified to or approved by each Ethics Committee, and if applicable, also the local regulatory authority, before implementation. Local requirements must be followed.

If a protocol amendment requires a change to Informed Consent Form, then the Ethics Committee must be notified. Approval of the revised Informed Consent Form by the Ethics Committee is required before the revised form is used.

7.4 Study timetable

Before a subject's enrollment in the study and any study-related procedures are undertaken the following should be fulfilled:

- Approval of the clinical study protocol
- Approval of the study by the Ethics Committee
- Approval of the study, if applicable, by the regulatory authority

8 ETHICS

8.1 Ethics review

The final study protocol, including the final version of the Informed Consent Form, must be approved or given a favorable opinion in writing by an Ethics Committee as appropriate.

The Principal Investigator is responsible for informing the Ethics Committee of any amendment to the protocol in accordance with local requirements. In addition, the Ethics Committee must approve all advertising used to recruit subjects for the study.

The Principal Investigator is also responsible for providing the Ethics Committee with reports of any SAEs (or ADEs) from any other study conducted with the investigational product.

Progress reports and notifications of SAEs (and ADEs) will be provided to the Ethics Committee according to local regulations and guidelines.

8.2 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ISO 14155 and ICH/Good Clinical Practice and applicable regulatory requirements.

8.3 Informed consent

The principal investigator(s) will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated informed consent must be obtained before conducting any procedure specifically for the study. The principal investigator(s) must store the original, signed Informed Consent Form in the ISF. A copy of the signed Informed Consent Form must be given to the subject.

8.4 Subject data protection

The Master Informed Consent Form will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation. Pursuant to this wording, subjects will authorize the collection, use and disclosure of their study data by the Investigator and by those persons who need that information for the purposes of the study.

The Master Informed Consent Form will explain that study data will be stored in a computer database, maintaining confidentiality in accordance with national data legislation.

9 PROCEDURES IN CASE OF EMERGENCY

9.1 Medical emergency

In the case of a medical emergency you may contact the Clinical Study Team Leader. If the Clinical Study Team Leader is not available, contact the Study Coordinator, see below:

Role in the study	Name	Address & telephone number
Principal Investigator	Jonathan Reside, DDS, MS	UNC School of Dentistry 111 Brauer Hall CB #7450 Chapel Hill, NC 27599-7450 Tel: (919) 537-3727
Study Coordinator	Gidget Jenkins	UNC School of Dentistry 9.1.1.1.1 School of Dentistry 9.1.1.1.2 UNC Chapel Hill 9.1.1.1.3 Prosthodontics 9.1.1.1.4 CB #7450 Chapel Hill, NC 27599-7450 Tel: 919-537-3968

The principal investigator(s) is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. **A medical emergency usually constitutes an SAE and should be reported as such.**

9.2 Pregnancy

Many physiologic changes can be observed in the pregnant patient and should be considered when planning dental treatment. It has been suggested to avoid elective dental procedures in the 1st and 3rd trimesters due to risk of spontaneous abortions, pre-term births, increased patient discomfort, and increased likelihood of intraoperative complications (15). The ultimate goal in dental therapy during pregnancy should be to educate the patient on the importance of oral hygiene and plaque control, perform periodontal maintenance therapy and treat emergency situations.

According the American College of Obstetricians and Gynecologists, fetal risks of anomalies, growth restriction, or abortions are not increased with radiation exposures of less than 5 rad (50,000mSv), a level which is well above the range of exposure for diagnostic purposes (16). CBCT radiation exposure levels depends on field of view and manufacturer, and has been shown to fall within the range of 69 to 560 mSv when a medium field of view CBCT was used (17). These dosages are well

below the deterministic threshold for fetal abnormalities as outlined above. Despite the very low risk of adverse pregnancy outcomes, adherence to the ALARA principle or radiology will be adhered to.

Although pregnancy is not a specific exclusion criteria for the study, all potential female subjects of reproductive age will be asked to complete a urine pregnancy test prior to enrollment in the study. Patients who are pregnant at the time of screening will be excluded from the study and referred back to their primary dental provider for further dental treatment. Patients who become pregnant during the course of the study will be asked to notify study personal. As the initial research indicated CBCT would have been completed prior to the patient knowing they were pregnant, and the second CBCT is a standard clinical requirement prior to implant placement, the patient will not be placed at any additional risk if they remained in the study, than if they were not taking part in the study. The patient does have the right to withdraw themselves from the study. Patients will be appropriately educated about the possible effects of dental treatment during pregnancy, along with the effects of ionizing radiation exposure as outlined below.

Pregnancy itself is not regarded as an AE/ADE unless there is a suspicion that the medical device under study may have interfered with the effectiveness of a contraceptive medication.

Congenital abnormalities/birth defects and spontaneous miscarriages should be reported as SAEs. Elective abortions without complications should not be handled as AEs/ADEs.

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