# A Prospective Study of Bone Augmentation Techniques in Extraction Sockets

Study Protocol & Statistical Analysis Plan

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Study Synopsis

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| **Study Title** | A Prospective Study of Bone Augmentation Techniques in Extraction Sockets |
| **Rationale** | In an effort to establish clinical evidence, this prospective, randomized clinical trial will assess and compare the efficacy of two different post-extraction bone grafting applications, Bone Allograft and Bone Allograft/Amnion, in 20 eligible adult subjects.<br><br>The study will assess the efficacy of each application to aid socket healing and preserve alveolar ridge integrity prior to full reconstruction with root-form endosseous dental implants.<br><br>The amniotic allograft is a cryopreserved allograft tissue matrix derived from human placental tissues recovered from live healthy donors. Amniotic tissue is an abundant source of collagen that provides an extracellular matrix to act as a natural scaffold for cellular attachment in the body. Collagen provides a structural tissue matrix that facilitates cellular migration and proliferation in vivo. The product was developed using a proprietary technique that morselizes the amnion in an effort to preserve its osteogenic properties in an injectable form. The "micro-scaffold" created by the morselized tissue matrix includes the residual proteins, growth factors, chemical compounds and cells naturally present in the placental tissues. The amniotic allograft growth factors are thought to enhance formation of a supportive scaffold for regeneration, to facilitate interactions between cell types, and to influence anti-inflammatory, anti-microbial and immunoprivilege activity.<br><br>Follow-up evaluations will assess osseous and soft-tissue healing at 1, 2, 4, and 7 weeks. Cone-Beam CT scans will be obtained immediately post-grafting and after 7 weeks.<br><br>At 8 weeks, a biopsy will be harvested from one site per subject and implants will be surgically positioned into the regenerated extraction sockets. Torque measurements at implantation will be recorded.<br><br>Implants will be uncovered 4-6 months post-implantation and subjects will be referred for permanent prosthetic attachment.<br><br>The study will evaluate the longitudinal outcome of early reconstruction with dental implants via two annual post-implant follow-up evaluations to be completed at 12 and 24 months following permanent prosthetic attachment.<br><br>Standardized, intra-oral periapical radiographic images will be obtained pre-surgery, immediate post-implant insertion, 7 weeks following implantation, when implants are uncovered, at permanent restoration, and at each follow-up evaluation.<br><br>Clinical photographs will be taken at each clinical visit or at investigator discretion. |
| **Objectives** | To evaluate and compare post-treatment healing of hard and soft tissues in maxillary or mandibular aesthetic zones following procedures utilizing test and control grafting materials.<br><br>To evaluate longitudinal clinical outcome of dental implants surgically inserted into regenerated bone 8 weeks after tooth extraction and grafting. |
| **Null** | The following null hypothesis will be tested: there is no difference in post-extraction healing patterns between extraction sockets grafted with Bone Allograft (BA) and extraction sockets grafted |

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| **Hypotheses** | with Bone Allograft + Amnion Allograft. |
| **Randomization** | Participants will be stratified for smoking status and randomized to one of two treatment groups:<br><br>Test Group-1: Atraumatic extractions followed by Bone Allograft (BA) and collagen plug<br><br>Test Group-2: Atraumatic extractions followed by Bone Allograft/Amnion Allograft (AA) and collagen plug |
| **Procedures** | Only treatment of non-adjacent sites will be included in the database. Each participant may have up to four non-adjacent sites selected for inclusion as study treatment sites.<br><br><u>Extractions</u><br>Atraumatic extractions and graft procedures following the randomization table and standardized surgical procedures.<br><br>Three-dimensional images, acquired through Cone Beam Computed Technology (CBCT), will be captured immediately post-grafting. Prophylactic antibiotics will be dispensed, prescriptions for post-surgery antibiotics and analgesics (prn) will be written. Antibacterial mouth wash will be dispensed; written and verbal patient home-care instructions will be provided.<br><br>To preserve anterior aesthetics, during the healing phase, an Essix retainer will be made for each participant and inserted immediately after the extractions/grafting procedures.<br><br><u>Follow-up evaluations</u><br>Performed at week-1, week-2, week-4, and week 7 will include:<br>Assessment of post-surgical healing<br>Assessment of adverse experiences<br>Measurement of clinical parameters<br>Clinical photographs<br>Suture removal at week 2<br>A CBCT scan will be completed at week-7<br><br><u>Biopsy and Implant Placement</u><br>A biopsy, and surgical placement of implants completed at week-8<br>Prophylactic antibiotics dispensed<br>Pre-surgical oral rinse<br>One bone-fill biopsy per patient, harvested by trephine (placed in fixative for later histological analysis)<br>Measurement of clinical parameters<br>Clinical photographs<br>Surgeon's subjective observation of osseous fill quality (D-1-D-4).<br>Measurements of torque at implantation will be recorded<br>Immediate post-surgical intra-oral radiographs<br>Prescriptions for post-surgery antibiotics and analgesics (prn)<br>Antibacterial mouth wash and ice-packs dispensed<br>Written and verbal home-care instructions<br><br><u>Post-Insertion Follow-up</u><br>Implant surgical follow-up assessment and suture removal will be performed after two-weeks. |

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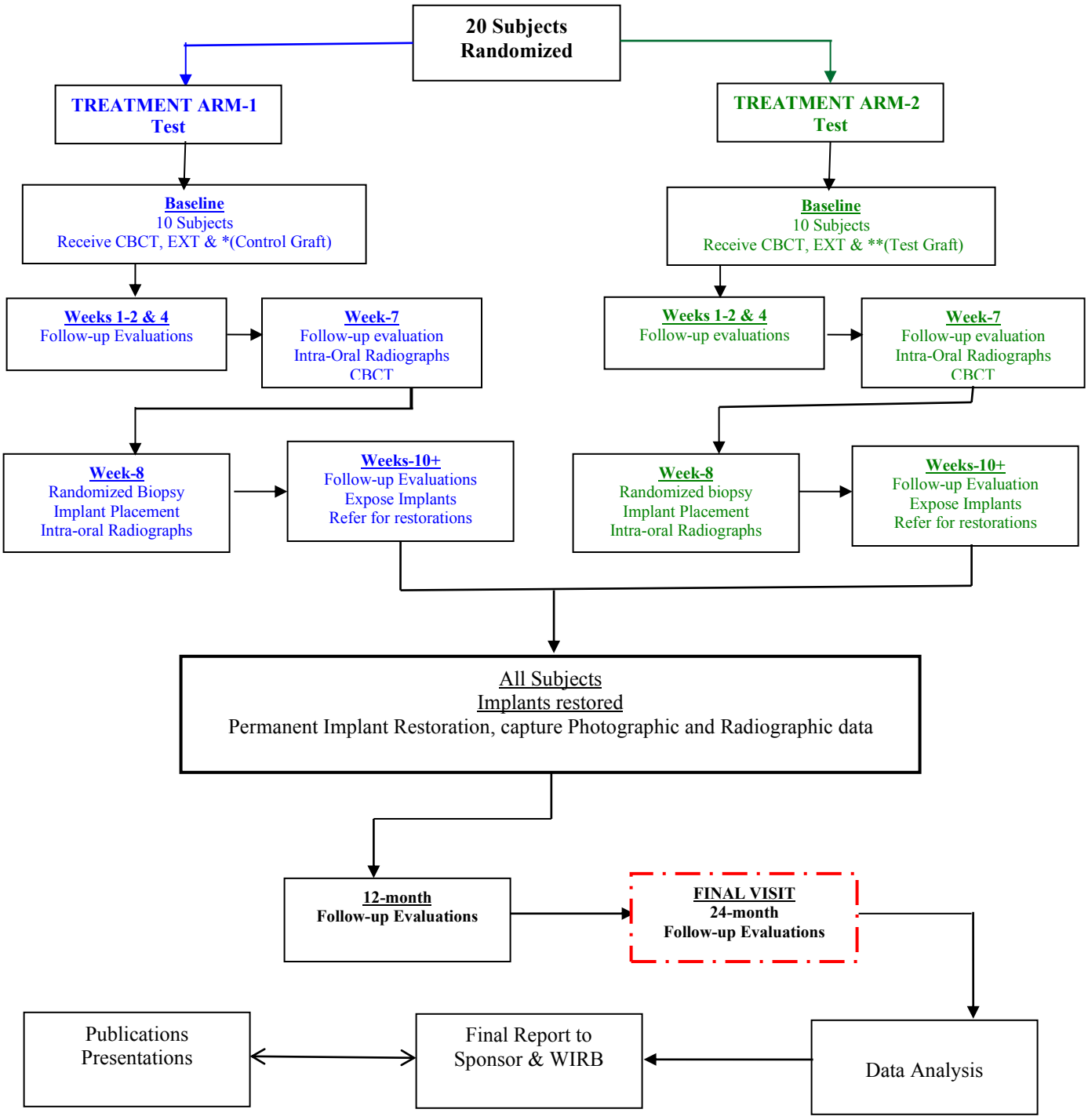
| | Implants Exposed<br>Mandibular implants will be exposed after approximately 4 months; maxillary implants will be uncovered after approximately 6 months.<br><br>Implant Restoration<br>Implants will be restored in the conventional manner with aesthetically pleasing cemented single crown restorations by one of the investigators. Photographs and radiographs will be obtained at permanent restoration.<br><br>Longitudinal Follow-up<br>Two annual follow-up evaluations will be completed at 12 months and 24 months from the date the permanent restoration is attached to the implant. Each assessment will include:<br>Standardized intra-oral periapical dental radiographs<br>Clinical photographs<br>Measurement of clinical parameters<br>Assessment of soft-tissue contours<br>Assessment of overall aesthetics<br>Assessment of implant/restoration interface |
| **Population** | Twenty healthy adult patients (10 per group) of any ethnicity or gender whose treatment plan includes extraction of mandibular and/or maxillary pre-molars and/or maxillary anterior teeth and their replacement with root-form dental implants and who meet the entrance eligibility criteria will be consecutively recruited for enrollment in the study. See inclusion/exclusion criteria – Table 1. |
| **Outcomes** | Histology and histomorphometric analyses (site biopsies)<br><br>Soft-Tissue aesthetic assessments (Jempt Indices and photographs)<br><br>Soft Tissue Health (PI & GI, and photographs)<br><br>Bone Support to Implants (standardized PA radiographs and image analysis)<br><br>Radiographic image analysis of osseous architecture changes at study sites over time |
| **Statistical Analyses Plan** | Two-tailed *t* tests will be used to compare clinical characteristics between the test and control therapies for continuous variables with a normal distribution. Nonparametric (Wilcoxon) tests will be used otherwise.<br><br>For categorical variables, inter-group comparisons will be conducted using chi-square tests or Fisher's Exact Test, as deemed necessary.<br><br>To determine whether the groups experienced equivalent changes in bone growth from baseline to follow-up, paired *t* (or Wilcoxon signed-rank) and McNemar's tests will be used for continuous and categorical variables, respectively. The $\alpha$ level will be set at 0.05.<br><br>Statistical analysis will be carried out using SAS software version 9.2 (SAS Institute, Inc, Cary, North Carolina). |




**Test Graft: Amniotic Allograft
*Control Graft: Bone Allograft

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## 1.0 BACKGROUND AND SIGNIFICANCE

1.1 Tooth Extraction Socket Preservation

Tooth extraction is often associated with resorption of alveolar ridge height and width and with compromised soft tissue contours.[1-8] An increasingly common periodontal practice combines skilled surgical technique with extraction site grafting. These procedures are thought to retard resorption of the socket volume while lending some control over bone-fill – thereby preserving the integrity of the alveolar ridge for future reconstruction with dental implants.

1.2 Amniotic Allograft

The study will assess the efficacy of amniotic allographs used to graft extraction sockets prior to reconstruction with dental implants. The amniotic allograft is a cryopreserved allograft derived from the placental tissues recovered from live healthy donors.[9] It has demonstrated osteogenic properties in both animal studies and clinical applications.

Amniotic fluid has been shown to contain multipotential cells that can differentiate into all three germ layers of the human body, including osteoblasts that can subsequently initiate bone formation with the deposition of osteoid. A plethora of growth factors such as Epidermal Growth Factor (EGF), Transforming Growth Factor beta (TGF-β), Fibroblast Growth Factor (FGF), Platelet Derived Growth Factor A&B (PDGF A&B) are associated with amniotic tissue and fluid. All these growth factors interact with different cell lines through their transmembrane receptors and may act to accelerate not only bone formation but also soft tissue healing. The product was developed using a proprietary technique that morselizes the amnion in an effort to preserve its osteogenic properties in an injectable form. The "micro-scaffold" created by the morselized tissue matrix includes the residual proteins, growth factors, chemical compounds and cells naturally present in the placental tissues. The amniotic allograft also contains a series of extracellular proteins such as fibronectin, laminins, vitronectin and cytokines as well as collagen that create a supportive scaffold that facilitates the communication and interaction amongst cells.[10]

In addition to this, amniotic tissue and fluid is characterized by anti-inflammatory,[10] anti-microbial[11] and immunopriviledge actions.[12,13,14] Anti-inflammatory activity is expressed by the suppression of the pro-inflammatory cytokines such as interleukin-1α and interleukin-1β as well as by the inhibitors of metalloproteases that are secreted from polymorphonuclear cells and macrophages. Anti-microbial property is evident with the presence and release of β-defensins, a group of antimicrobial peptides. The immunopriviledge action is associated with the expression Human Leukocyte Antigens, HLA-G that eliminates the acute rejection by serving as a ligand for the inhibitory receptors of the natural killer cells and the macrophages. Moreover, there is no expression of HLA-A, -B, -D, and -DR antigens on the cell surface of the amniotic embryonic cells and consequently there is no immune response.

Lastly, amniotic tissue and fluid has angiogenic ability that is demonstrated by the secretion of angiogenic factors by the amniotic epithelial and stromal cells. Vascular Endothelial Growth Factor (VEGF), Interleukin-8 (IL-8), Angiogenin, Interferon-γ, Interleukin-6 (IL-6), basic Fibroblast Growth Factor (bFGF), Epidermal Growth Factor (EGF) and Platelet-Derived Growth Factor (PDGF) are produced. However the amniotic epithelial cells express some anti-angiogenic factors that they may affect to some degree the net angiogenic potential of the amniotic allograft.[15]

1.3 Reconstruction with Dental Implants

Dental implants have been used in dentistry for decades in an attempt to reestablish function and aesthetics to areas of the mouth where natural dentition is lost.[16, 17] Materials and designs of dental implants[18, 19, 20] have evolved over the years to arrive at the optimal combination of materials, shape and surface texture that would induce permanence to the restoration.

1.4 A Rigorous Test

In an effort to establish further clinical evidence, the current study will assess and compare the efficacy between two randomly assigned different bone grafting applications in non-adjacent aesthetic zone extraction sockets. Placing implants into augmented bone represents the most rigorous test of potential bone-preserving therapies.

## 2.0 INVESTIGATIONAL DESIGN

2.1 Purpose

The purpose of this prospective, randomized clinical trial is to compare healing of extraction sockets treated with Bone Allograft and sockets treated with Amnion-enhanced Allograft. The study will also compare the efficacy of early reconstruction with dental implants placed into the regenerated bone.

2.2 Objectives of the Investigation

The overall objective of this investigation is two-fold:

1. To evaluate outcomes with respect to osseous and soft tissue healing of grafted extraction sockets between Bone Allograft (control therapy) and Bone Allograft/Amniotic allograft (test therapy).

2. To evaluate longitudinal stability of dental implants after placement into grafted extraction sockets.

2.3 Hypotheses and Specific Aims

The trial will test two hypotheses:

Hypothesis-1: Grafting with Bone Allograft is an efficacious method of enhancing the development of new bone fill in extraction sockets.

Hypothesis-2: Bone Allograft/Amniotic allograft will aid osseous healing and will preserve alveolar ridge dimensions of extraction sockets more effectively than extraction sites grafted with Bone Allograft alone.

Specific Aims

1: Compare the efficacy of bone grafting with and without enrichment with amnion.

2: Compare alveolar ridge dimensions between groups after 7 weeks of post-extraction healing.

3: Compare soft tissue healing between groups at week-1, week-2, week-4 and after 7 weeks of post-extraction healing.

4: Assess quantitative bone fill between groups after 7 weeks of post-extraction healing

5: Compare qualitative bone fill between groups after 7 weeks of post-extraction healing.

6: Assess and compare the relationship of osseous healing and implant stability at insertion across groups after 8 weeks of post-extraction healing.

7: Compare implant stability between groups at 12 months and 24 months post-restoration.

8: Assess changes in longitudinal crestal bone level between groups at 12 and 24 months after permanent prosthetic attachment.

## 3.0 STUDY POPULATION

3.1 Entrance Criteria

Twenty healthy adult patients (10 in each group) of any ethnicity or gender whose treatment plan includes extraction of maxillary anterior teeth and/or mandibular and/or maxillary pre-molars and their replacement with root-form dental implants will be consecutively recruited for enrollment in the study.

Participants must be at last 19 years old with demonstrated ability to understand the proposed treatment recommendations and prognosis and be able to provide informed consent, in English, without the aid of ad-hoc translation from another language. Decisionally-impaired adults and/or minors, who cannot consent for themselves, will not be enrolled; women who are pregnant or lactating at the time of enrollment will not be included. Women will be tested for current pregnancy status.

Prior to enrollment, prospective participants must agree to be available for the entire duration of the study, be compliant with needed study-related evaluations and demonstrate a willingness to comply with strict time-line requirements. Certain health and medication exclusions apply (table 1).

Table 1 Entrance Criteria

| **Inclusion** | **Exclusion** |
|---|---|
| Existence of one or more non-adjacent teeth in the aesthetic zone that are scheduled for extraction | Pregnancy or lactating at the time of enrollment |
| Healthy enough to undergo the proposed therapy without compromise to their existing health status | Previous malignant neoplasm |
| Demonstrated willingness to comply fully with protocol time-line and procedural requirements | A known hypersensitivity to DBM |
| At least nineteen years old | A known hypersensitivity to Titanium metal |
| Able to read and understand written English without the aid of ad hoc interpretation | Any health condition that in the opinion of the clinical investigators may adversely affect bone healing |
| Able to cognitively understand the proposed study therapy and possible prognosis | Any medication that in the opinion of the clinical investigators may adversely affect bone healing |
| Able to consent for their own inclusion in the study | Any indication of an inability to make autonomous decisions |

3.2 Visit Sequence

The study will include a minimum of four time-sensitive clinic visits over seven consecutive weeks; dental implants will be placed 8 weeks after extraction/grafting procedures. Following implant

placement, additional visits, necessary for health maintenance will be completed. Implants will be uncovered following routine practice and at the investigator's discretion. The implants will be restored and the number and sequence of visits will be scheduled at the restoring dentist's discretion. Participants will return for two follow-up visits at 12 and 24 months after permanent prosthetic attachment.

## 4.0 SUMMARY OF STUDY PROCEDURES

4.1 Enrollment and Treatment Planning

After completing an individualized, private consent discussion and obtaining informed consent, a pre-surgical assessment will be completed. Each enrolled participant will be seen for an initial evaluation to include assessments of medical and dental conditions that will verify compliance with entrance criteria and for collection of demographic data.

Participants will be assigned a unique identification number. Information will be recorded on baseline case report forms (CRFs) developed specifically for this study.

Each participant may have up to four non-adjacent sites selected for inclusion as study treatment sites. Through consultations with prosthodontic clinicians, the documented treatment plan will be reviewed and revised if necessary to develop a therapy that will be of most benefit to the participant.

4.2 Randomization

Participants will be stratified for smoking status and randomized to one of two extraction/grafting groups. In the case of multiple tooth sites within one participant, all the tooth sites will be assigned to receive the same graft material.

4.3 Randomized Groups

Control Group 1: Atraumatic extractions followed by grafting with Bone Allograft mixed with sterile saline and collagen plug.

Test Group 2: Atraumatic extractions followed by grafting with Bone Allograft mixed with Amnion graft and collagen plug.

4.4 Pre-surgical Procedures

Conventional oral impressions will be made and a temporary Essix retainer will be fabricated. The Essix retainer, a removable prosthesis, will protect the healing tissue in an aesthetically pleasing manner but will not impede the clinical observation of study sites. Study casts may also be used to fabricate a surgical guide prior to implant surgery.

4.5 Surgical Extractions

All surgeries will be performed by qualified clinicians/investigators in an appropriately equipped surgical operatory located in the Periodontal Clinic at UAB School of Dentistry.

Prior to extraction surgery, participants will be given a loading dose of antibiotics based on their medical history and concomitant medications. A suggested prophylactic regimen follows:

2 gm of Amoxicillin 1 hour prior to the procedure followed by 500 mg (TID) for 7 days
Patients with reported allergy to Amoxicillin:
600mg Clindamycin 1 hour prior to the procedure followed by 300 mg (TID) for 7 days

The facial area will be scrubbed with 2% Clorhexidine soap and the oral cavity disinfected by rinsing for 1 minute with a 0.12% chlorhexidine gluconate antimicrobial oral rinse. Prior to extractions clinical photographs will be taken from the buccal and occlusal aspects. Photographs will be taken in a 1:1 ratio using a Nikon D-7000.

After preparation and isolation of the surgical area, anesthesia will be achieved in the region by regional block or local infiltration. Conscious sedation may be added at the surgeon's discretion to help manage patient anxiety.

The teeth will be extracted as atraumatically as possible, without raising a flap, utilizing periotomes, luxators and the Easy Extrac system. In both groups, the integrity of the remaining socket walls will be assessed and measurements recorded on the surgical CRF. The sockets will be debrided and a bleeding surface created by decorticating the socket walls with either a curette or a surgical round burr.

4.6 Grafting Extraction Sockets
After extractions, sockets will be grafted according to each participant's assigned randomization group.

*Group-1*: Each socket will be grafted with Bone Allograft mixed with sterile saline.

4.6.1a Irrigate sockets with sterile saline.

4.6.1b Pack the graft into each of the study sites using mild pressure achieving complete fill of the sockets.

4.6.1c Trim a 3 mm section of collagen plug and secure it over the grafted areas with 5.0 Monocryl crossing mattress sutures.

*Group-2:* Each socket will be grafted with Amnion Graft mixed with Bone Allograft

4.6.2a Irrigate sockets with sterile saline.

4.6.2b Pack the graft into each of the study sites using mild pressure achieving complete fill of the sockets.

4.6.2c Trim a 3 mm section of collagen plug and secure it over the grafted areas with 5.0 or 6.0 Monocryl crossing mattress sutures.

After completion of procedures, the Essix retainer will be fitted and placed.

A baseline, post-grafting CBCT scan will be made to include images of each study site. Prescriptions for anti-bacterial mouth wash, antibiotics, and pain relief will be provided based on individual patient

needs. An ice-pack will be dispensed. Verbal and written home care instructions will be given and the patient will be scheduled for a follow-up appointment.

4.7 Surgical Extraction Data Collection

The following data points will be collected and recorded on the surgical CRF: A description of the integrity of each of the socket walls will be recorded as follows.

4.7.1 Measurements of the Mesio-distal distance and the buco-lingual distance of the socket walls.

4.7.2 Measurements of dehiscence and fenestration defects made with a UNC-15 periodontal probe in relation to the adjacent socket crest and rounded up to the nearest millimeter.

4.7.3 Wound Measurements – Mesio-distal distance and bucco-lingual distance of the soft tissue wound margins after suturing made with a UNC-15 periodontal probe and rounded to the nearest millimeter.

4.8 Post-Extraction Evaluations

Participants will return to clinic for oral evaluations as close to 7-days after the extraction/grafting procedure as possible and again at 14 days; sutures will be removed at the 14-day visit. Additional follow-up visits will be scheduled approximately 4 weeks and 7 weeks following grafting. Healing will be closely monitored and appropriate data recorded on follow-up CRFs.

At each visit, medical history will be reviewed and any changes documented. Information regarding adverse events will be captured and recorded following IRB and Federal reporting guidelines. At the 7-week post-grafting visit a second CBCT scan will be obtained.

4.9 Tissue Biopsy and Implant Placement

After reviewing the CBCT scans the investigator will determine whether or not the sockets have enough bone-fill to enable the surgical placement of implants. The diameter and length of the implants placed will be determined by the surgeon/investigator based on the tooth location, anatomy, and bone volume determined from the CT scan prior to placement. Immediately prior to implant placement, a tissue biopsy will be harvested from the center of one grafted site. Implants will be placed following standard procedures outlined in the Manual of Procedures.

4.10 Implants Exposed

The implants will be exposed following the established recommendations and at the discretion of the implanting surgeon.

4.11 Implant Restoration

Each implant will be restored in the conventional manner with an aesthetically pleasing single crown restoration. When the permanent restorations are attached intra-oral radiographs and photographs will be obtained.

Table 2 – Study Procedures Visits -1 — 8

| **Procedures** | **V- (-1)** Screening | **V-1** Pre-Tx | **V-2** EXT & Grafting | **V-3** Week 1 | **V-4** Week 2 | **V-5** Week 4 | **V-6** Week 7 CBCT | **V-7** Week 8 Implant placed | **V-8** 2 week Post-Op Evaluation |
|---|---|---|---|---|---|---|---|---|---|
| Consent Discussion/Obtained | X | | | | | | | | |
| Medical History Review | | X | X | X | X | X | X | X | X |
| Tx Plan Review | | X | | | | | | | |
| Impressions | | X | | | | | | | |
| Essix Retainer | | X | | | | | | | |
| Randomized to Tx Groups | | X | | | | | | | |
| Biopsy site selection | | X | | | | | | | |
| Pre-Surg Antibiotics | | | X | | | | | X | |
| Pre-Surg Oral Rinse | | | X | | | | | X | |
| Tooth Extraction | | | X | | | | | | |
| Tx Group Procedure | | | X | | | | | | |
| Clinical Photographs | | | X | X | X | X | X | X | X |
| CBCT Scan | | | X | | | | X | | |
| Periapical Radiographs | | | | | | | | X | |
| Post-Surg Instructions/Prescriptions | | | X | | | | | X | |
| Suture Removal | | | | | X | | | | X |
| AE's recorded | | | X | X | X | X | X | X | X |
| Bone Biopsy | | | | | | | | X | |
| Implant Placement | | | | | | | | X | |
| Torque Measurement | | | | | | | | X | |

Table 3 Study Procedures Visits 9 —12

| **Procedures** | **V-9** Implant Exposed | **V-10** Implant Restored | **V-11** 12 month | **V-12** 24 month |
|---|---|---|---|---|
| Medical History Review | X | X | X | X |
| Clinical Photographs | X | X | X | X |
| Periapical Radiographs | | X | X | X |
| AE's recorded | X | X | X | X |
| Implants Exposed | X | | | |
| Sutures Removed (14 DAYS) | X | | | |
| Implant Restored | | **X** | | |
| Soft-tissue assessments | | | X | X |
| Pocket Depth Measurements | | | X | X |

## 5.0 CONE BEAM COMPUTED TOMOGRAPHY

Cone-Beam Computed Tomography (CBCT) will be used in this trial to obtain a 3-dimensional database of each participant's anatomy from the volumetric tomography. Axial, sagital, and cross-images will be reconstructed to measure tooth socket volume. CBCT imaging is used in state-of-the-art dentistry to aid treatment planning of complex implant cases and is considered best practice in dental imaging. The CBCT produces a highly accurate scan of the head and neck. The image can then be visualized as a virtual reproduction of the jaw that is accurate to within a tenth of a millimeter (0.10mm). A CBCT scan allows the dental surgeon to view the anatomy in many different planes and

provides precise knowledge of the disposition of anatomical structures. These views enable the dental surgeon to select an implant with the most appropriate configurations to fit the anatomy of each individual patient.

Participants in this study will have 2 CBCT scans made; the first will aid in the initial treatment planning, the second scan will be used to assess bony healing of the grafted sites prior to implant placement. The images will be captured using I-Cat, Cone Beam CAT scanning equipment, manufactured by Imaging Sciences and designed especially for imaging the skull in the dental setting. CBCT scans are acquired through a series of rapid pulses in a single pass, exposing the patient to radiation for the equivalent of 3 seconds per image. These scans compare favorably to hospital-based CT scans of the head and neck area where exposure is 30 times greater.

A Cone Beam Computed Tomography (CBCT) scan will be made at baseline (immediately post-grafting), and at 7 weeks after the grafting.

## 6.0 TISSUE BIOPSY AND SURGICAL PLACEMENT OF IMPLANTS

6.1 Patient Preparation and Surgical Implant Placement

Prior to implant surgery the subjects will be given a loading dose of antibiotics based on their medical history and concomitant medications. Local anesthesia will be achieved in the region by regional block or local infiltration. Conscious sedation may be added at the surgeon's discretion to help manage a patient's anxiety. The facial area will be scrubbed with 2% Clorhexidine soap and the oral cavity disinfected by rinsing for 1 minute with a 0.12% chlorhexidine gluconate antimicrobial oral rinse prior to the procedure.

Once the treatment areas are sufficiently anesthetized a crestal incision will be made and full-thickness flaps will be raised. With the aid of a surgical guide, the proposed implant sites will be identified; measurements of the alveolar ridge will be captured, using a UNC-15 periodontal probe and recorded on the implant surgery CRF.

6.2 Implant Selection

The diameter and length of the implant will be determined by the surgeon based on the tooth location, anatomy, and bone volume determined from the CT scan prior to placement.

6.3 Drilling & Biopsy Technique

A standard drilling technique will be utilized except for a modification in the depth of the osteotomy and biopsy obtained from study sites described below. Copious amounts of sterile irrigant will be used in the osseous drilling procedure to prevent heating and thermal necrosis of the bone and surrounding tissue. The drill speed may be adjusted up to a maximum of 1500 rpm during the procedure. Thread forming or taping may be indicated in very dense bone and should be at a maximum of 30 rpm.

The surgeon will start with the 2mm diameter trephine drill for study sites and then proceed to increase the width with progressive depth drill depending on the implant diameter to be used at the site. The trephine with bone contained within will be harvested from the center core of one study site, per patient and will be immediately placed in fixative for later histological analysis. Vials will be labeled

with the participant's ID and the date of biopsy; laboratory technicians will be blinded to knowledge of participant group assignment.

6.4 Implant Placement

The sterile blister pack containing the implant will drop onto a sterile field and after thorough irrigation of the site the implant will be inserted directly into the prepared bone site. After implant placement the insertion torque will be measured with a torque wrench and recorded on the case report forms. A cover cap will be placed on the implant and the surgical wound sutured.

6.5 Immediate Post-Operative Procedures

Standardized intra-oral periapical dental radiographs will be made of each study site. Written and verbal post-operative instruction will be provided to each participant. An antimicrobial mouth rinse and ice packs will be dispensed. Antibiotics will be prescribed for 7 days; analgesics will be prescribed on an as needed basis at the discretion on the surgeon.

6.6 Implants Exposed

Implants will be exposed upon the investigator's determination that the implants are sufficiently integrated to withstand the forces of loading. In general, mandibular implants will be uncovered and restoration initiated approximately 4 months after placement; maxillary implants will be uncovered and restoration initiated approximately 6 months after placement.

Prior to exposing the implants subjects will be given a loading dose of antibiotics based on their medical history and concomitant medications. Local anesthesia will be achieved in the region by regional block or local infiltration. Conscious sedation may be added at the surgeon's discretion to help manage a patient's anxiety. The facial area will be scrubbed by 2% Chlorhexidine soap and the oral cavity disinfected by rinsing for 1 minute with a 0.12% chlorhexidine gluconate antimicrobial oral rinse prior to the procedure.

Once the treatment areas are sufficiently anesthetized a crestal incision will be made to reveal the top of the implant and an abutment will be attached. The incision will be closed with sutures and the temporary prostheses will be adjusted. Clinical photographs will be taken before, during and after the procedure; following the procedure and standardized intra-oral radiographic images of each implant site will be captured.

6.7 Post-Surgical Evaluation

Participants will return to clinic for oral evaluations approximately 14 days post-surgery; sutures will be removed at the 14-day visit. At this visit, medical history will be reviewed and any changes documented. Subjects will be referred to the prosthodontic investigators for restoration of the implants.

## 7.0 PERMANENT RESTORATION OF IMPLANTS

Implant restoration will be completed according to the prosthetic guidelines. Each implant will be restored in the conventional manner with an aesthetically pleasing single crown restoration. The restoring dentist will select restoring materials based on his discretion and the individual patient needs. Prosthetic restoration of the implants will require numerous visits to the restorative dentist over a

period of one to two months. The restoring dentist will advise when additional visits are necessary. Photographs and radiographs will be taken of each area when the final restoration is cemented.

## 8.0 LONGITUDINAL FOLLOW-UP

Post-Restoration Follow-up Evaluations (12-months and 24-months after the implant is restored)
Participants will return to the clinic at 12 and 24 months following permanent implant restoration. Evaluation of implant function and surrounding tissue health will be determined based on an interview with the participant, clinical examination of the study sites and an analysis of radiographic and photographic data.

At each visit, study personnel will review and update the participant's health history and record any adverse experiences; a study dentist will complete a thorough periodontal evaluation of the implant area and provide an assessment of the prosthetic attachment interface. A set of standardized periapical radiographs and clinical photographs will be made.

Case report forms will be used to record the following observations for each implant site:

- Overall soft tissue health
- Plaque and gingivitis scores (modified Löe and Sillness)
- Pocket depth and clinical attachment level
- Implant Stability
- Retention and stability of prosthetic attachment
- Overall aesthetics (soft tissue contours)
- Assessment of reported pain
- Implant survival

## 9.0 RADIOGRAPHIC AND PHOTOGRAPHIC SURVEYS

9.1 Intra-oral Radiographic Images
Standardized intra-oral periapical digital radiographic images will be obtained of each site prior to implant placement, when the implants are placed, when the implant is exposed, at permanent implant restoration and at twelve and twenty-four months after the implants are restored. A minimum of six images per study site over the duration of the study will be obtained. Additional images may be obtained based on the study dentist's determination of need.

Radiographic images will be used to monitor osseous health at the bone-implant interface, to verify implant position, to assess the quality of implant-attachment interface and to capture measurements of longitudinal osseous support to the implants. Consistently careful technique will yield images with similar geometry and with constant density leading to a valid and dependable analysis.

9.2 Radiographic Image Acquisitions
Longitudinal measurement of osseous support to the implant sites is reliant upon serial images of consistently similar quality that accurately image existing anatomy with minimal distortion over time. Ideal images for this study will center the targeted sites with at least 2mm of adjacent anatomy visible on each side of the implant.

9.3 Standardized Radiography

During this study, ensuring parallelism and standardization of periapical radiographs for measurement of osseous support will rely on consistent use of a Rinn bite-block and aiming ring and careful positioning of sensor in the oral cavity.

Thermoplastic impression tabs will be used to acquire an impression of the adjacent teeth in the area of interest, the impression will be attached to a Rinn bite-block; this will enable accurate positioning of the film during sequential exposures. After the initial exposure, the bite-block-impression will be disinfected, labeled with the subject ID and tooth number, and stored until needed for future exposures. Images will be acquired through the sequence of events listed below:

9.3.1 Select the area of interest
9.3.2 Assemble the needed materials
9.3.3 Prepare the thermoplastic tabs in a warm water bath according to package direction
9.3.4 Attach tabs to the bite block, insert the sensor and attach the Rinn aiming-ring apparatus
9.3.5 Position bite block/sensor in the oral cavity
9.3.6 Align the X-ray machine tube and flush against the aiming ring
9.3.7 Expose the image
9.3.8 Review the image for accurate positioning and quality
9.3.9 Label the image with subject ID and tooth number and save to a JPEG file for future use.
9.3.10 Disinfect the bite-block, label with subject ID and tooth number, and store for future use

As commonly practiced in the dental setting, participants will be draped with a protective apron with an attached thyroid collar while the radiographic images are captured. Dental X-ray equipment used in this trial is routinely inspected by the Department of Public Health. Only well-trained, qualified personnel will operate the imaging systems.

9.4 Clinical Photography

Each study site will be monitored with digital photography to assess the soft tissue healing. Photographic assessment will be based on images taken at 1:1 ratio with a Nikon D-7000 digital camera. Buccal and occlusal views will be obtained at the initial visit (prior to extraction), after completion of the grafting procedures, at 2 and 4 weeks, immediately prior to biopsy at 8-weeks, after implant insertion, at each prosthetic restorative visit, when the final restoration is attached and at four semi-annual follow-up visits.

## 10.0 ADVERSE EVENT REPORTING

Participants will be interviewed at each visit and prompted to discuss any adverse events; such events will be recorded. In the event of a serious adverse event, the IRB will be notified per posted requirements and in full compliance with federal guidelines for research in human subjects. An event, whether study-related or not, will be assessed based on description of the event/pathology, onset, duration, severity, analgesics or other medication taken, and possible causality.

## 11.0 CRITERIA FOR SUCCESS

11.1 Grafts

The success criteria for this investigation are determined as follows: Grafts will be deemed successful if there are no reported symptoms or clinical signs of post-application allergy, toxic reactions or evidence of gross local or systemic infections.

Histological and histomorphometric analyses will provide evidence of the relative success or failure of graft materials to enhance formation of new osseous tissue in extraction sockets. Results will be compared and between experimental groups.

A site that fails to meet the stated criteria for success, or where the width of the ridge remains too narrow for implant insertion will be re-grafted (an offer that patients may decline). The site may receive an implant at a later date but the site will be exited from the study results.

11.2 Implant Survival

At the 12 and 24-month follow-up evaluations, participants will be interviewed to determine if any pain or numbness has been experienced since implant placement or has developed since the previous interview. The implant sites will be examined for any evidence of implant failure including implant suppuration and erythema.

If signs or symptoms of implant failure are observed at any time during the course of the study, a periapical radiograph will be obtained and inspected for signs of radiolucency. The investigator will make assessment of implant status and if a failure is determined, will be reported on the appropriate CRF. At the investigator's discretion a medical and/or surgical attempt to rescue the implant may be made.

11.3 Implant Success

An implant will be considered successful as long as it remains immobile when tested with instrument pressure at follow-up evaluations and offers stable anchorage to a functional prosthetic attachment. A successful implant shows no evidence of fracture or signs of peri-implant radiolucency on an intraoral radiograph (using a paralleling technique strictly perpendicular to the implant bone-interface), or persistent or irreversible signs and/or symptoms of pain, infection, neuropathies or paresthesia.

## 12.0 COSTS TO PARTICIPANTS

12.1 Procedure Fees

No fees will be charged to participants for teeth extraction, socket grafting materials, the implant devices, implant surgery, CBCT scans, radiographs, photographs, bone biopsy analysis, or clinical evaluations for the entire study.

Participants will be expected to pay a **$1,000 fee for each implant restored.** This one-time-only fee will cover costs related to laboratory fees and materials for the fabrication of the implant restoration prosthesis. This fee is to be billed to participants prior to the initiation of any surgical procedures.

Study visits are not designed to take the place of routine dental care. Over the course of the study, participants will be expected to continue with regular dental check-ups with a dentist of their choice. Should the need arise patients who do not have a regular dentist of record will be referred in the appropriate manner for any non-study related dental treatment. Costs for non-study related treatment will be charged to the participant's account or to their insurance provider in the usual manner per dental school policy and will not be paid for from the study account

12.2 Payment to Participants

Participants will be paid **$50** after completing the 12-month visit and **$50** after completing the final study visit at 24 months following implant insertion.

## 13.0 OUTCOMES AND ANALYSES

The study is structured to provide both subjective and objective data; study sites will be followed, data will be captured at placement and at 12 and 24 months after implants are placed.

13.1 Objective Data

Bone biopsies preserved in the trephines and stored in formalin will be submitted to UAB's CMBD Core Laboratory. Trephine core 2x6 mm will be stained with hematoxylin and eosin and processed for histologic and histomorphometric analysis. Amount of bone fill and remaining bone graft (% of new bone fill, % of residual graft and % of soft tissue) material will be determined and compared across groups. Torque measurements will be recorded for each site at implant placement.

Cone Beam Computed Technology (CBCT) will be used to capture quantitative natural bone fill and bone fill following grafting applications at each extraction site.

Image analysis software will be used to capture images from the standardized radiographs taken at 12 and 24 months following implant placement. Analysis procedures will assess the implant/bone interface and measure changes in adjacent crestal alveolar bone levels over time.

13.2 Subjective data

Observations of bone quality (D1-D-4) recorded by surgeons at implant placement.
Clinical observations of post-extraction soft-tissue healing
Clinical observations of soft-tissue and osseous response to dental implants
Clinical observations of overall aesthetic appearance

13.3 Outcome Endpoints

The primary efficacy parameter for implant success will be the duration of implant survival from surgical placement to 24 months post-placement across all groups. Measurement of bone height along the implant mesial and distal surfaces at 12 and 24-month follow-up evaluations will be captured from radiographic images and compared to same measurements at insertion. These measurements will be used to derive changes from baseline values.

Efficacy endpoints will include implant and prosthesis function as assessed by gingival health, bone levels and participant subjective satisfaction with the study therapy. Differences between groups will

be calculated and used to determine if osseous changes and implant function are different between groups.

Safety will be evaluated by clinical signs and symptoms of dental and medical events.

13.4 Statistical Analysis Plan

Two-tailed *t* tests will be used to compare clinical characteristics between patients with the Bone Allograft (control therapy) and those with the Bone Allograft/Amnion Allograft (test therapy) for continuous variables with a normal distribution. Nonparametric (Wilcoxon) tests will be used otherwise.

For categorical variables, inter-group comparisons will be conducted using chi-square tests or Fisher's Exact Test, as deemed necessary. To determine whether the groups experienced equivalent changes in bone growth from baseline to follow-up, paired *t* (or Wilcoxon signed-rank) and McNemar's tests will be used for continuous and categorical variables, respectively.

The α level will be set at 0.05. Statistical analysis will be carried out using SAS software version 9.2 (SAS Institute, Inc, Cary, North Carolina).

## 14.0 CONFIDENTIALITY

14.1 Protection of Personal Health Information

Identifiable personal health information will be protected from public dissemination and information gathered during this study will be kept confidential to the extent permitted by law. Study staff with access privileges, as well as members of the IRB may access study-related records and information that will identify participants by name.

14.2 Electronic Files

Data that link an individual to identifying information (birth date, address, telephone numbers plus pregnancy status at enrollment) will be entered into a protected electronic master file created specifically for this study. A participant log will be created and used to track each participant's progress through the study.

Each participant will be assigned a unique study-specific identification number; except for screening, names will not be written on case report forms. Screening forms that include demographic and personal information such as birthdates, address and telephone numbers will be filed in the binder with the original consent documents.

All study-related dental data will be captured on paper case report forms crafted specifically for this study. These forms will be used to record all data point measurements and assessments for the duration of the study. Data collected on the case report forms will be coded and transcribed to an electronic spreadsheet created for this study and will be used by the study statisticians for statistical analyses.

A study-specific participant file will be created to house completed paper case report forms. These forms will not be stored in the patient's dental file; however, in the interest of maintaining an accurate

of record-of-dental-treatment, treatment notes will be transcribed and inserted into each participant's dental record.

The data-collection system will be maintained by the study coordinator and will be secured through entry into a password protected computer database in the coordinator's office. Only members of the research team granted access privileges will be able to log-on to the study files.

## 15.0 REGULATORY ISSUES

15.1 IRB Approval

This protocol will be evaluated and approved by the responsible Institutional Review Board prior to screening/entering the first participant into the study. In accordance with federal, state, and university regulations governing research on human subjects, required documents will be submitted for IRB review by the study coordinator. Initiation of any study-related procedure (including screening) will not commence until receipt of IRB approval.

15.2 Informed Consent

The study investigators, or designee, will participate in a private English language-witnessed consent discussion with all potential candidates prior to obtaining their consent to participate in this trial. Participants must demonstrate an adequate understanding of the spoken and written English language and must be able to complete the consenting process without the aid of an interpreter.

Participant's consent signature will not be obtained under the influence of strong persuasion or any effort that could be perceived as coercion; each participant will be given ample opportunity to have all their concerns addressed and all their questions answered prior to obtaining the consent signature.

Each participant must demonstrate an autonomous understanding or what it means to be a participant in a research study and must be made fully aware of the risk, benefits and costs of participation. In addition, all participants must be made aware that they can withdraw their consent at any time during the trial and for any reason without jeopardizing their future treatment at the dental school. Participants will be made aware that the consequences of early withdrawal including that the study treatment (teeth extraction, bone grafts and implant placement) are irreversible procedures.

Only the most current IRB-approved informed consent document will be used for consent signatures. The consenting process will be conducted in full compliance with University/IRB ethical policies and will follow departmental standard operating procedures. The consent form must be signed in the presence of a reliable witness, who must also sign the consent form, before any study therapy can be initiated. Each participant will be given a copy of his or her signed consent; the original signed consent form will remain with the investigator.

15.3 Regulatory Documents

All original study-related regulatory documents, associated correspondence and original consent documents will be housed in a study-specific regulatory binder. The binder will be secured in the coordinator's office and only study personnel will have access to the binder.

## 16.0 STUDY SPONSOR

The Study is funded by BioDlogics, LLC, 7740-A Trinity Road, Suite 106, Cordova Tennessee, USA. BioDlogics will supply the bone grafting materials used in this study. Other information related to the conduct of this trial is included in the Clinical Trials Agreement between the sponsor, BioDlogics, and the University of Alabama at Birmingham.

The PI will prepare and submit ongoing progress reports to the sponsor; a final report will be submitted following completion of the study.

With prior written notice to the PI, designated representatives from BioDlogics will visit UAB periodically to observe clinical procedures and to monitor study files.

Study graft material will be packaged and supplied by the sponsor in sealed, pre-labeled disposable vials:

Amnion Allograft (AA), from one donor, will be supplied in 0.25ml vials and stored on-site in a -70$^{o}$F freezer dedicated to research materials.

Bone Allograft (BA), from one donor, will be supplied in 0.5cc vials and stored in a designated, locked cabinet located within the research clinic.

Detailed instructions for handling and storage of study materials are included in Appendix-C

Tear-off or adhesive labels will include a unique ID/lot number; as each vial is used, the label will be removed and attached to the appropriate data collection form in the participant's study file.

An inventory of study materials will be maintained by the study manager, or designee; this individual will create and maintain a spreadsheet accounting for materials received for the study and those dispensed for study procedures, and will reconcile any materials remaining when the study is completed.

The PI will provide guidance and training to investigator/surgeons and to clinical personnel related to appropriate storage, administration, and accountability of the study materials.

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## 18.0 STUDY PERSONNEL

| Name | Title | Primary Role |
|---|---|---|
| Philip J. Vassilopoulos, DDS, DMD | Principal Investigator<br>Surgeon | Overall responsibility for conduct of the study<br><br>Obtaining consent, screening, treatment planning, dental surgery, follow-up care. Monitoring AEs. |
| Nicolaas C. Geurs, DDS, MS | Sub-Investigator<br>Surgeon | Obtaining consent, screening, treatment planning, dental surgery, follow-up care. Monitoring AEs. |
| Michael S. Reddy, DMD, DMSc | Sub-Investigator<br>Surgeon | Obtaining consent, screening, treatment planning, dental surgery, follow-up care. Monitoring AEs. |
| Maria L. Geisinger, DDS, MS | Sub-Investigator<br>Surgeon | Obtaining consent, screening, treatment planning, dental surgery, follow-up care. Monitoring AEs. |
| Ramzi Abou-Arraj, DDS, MS | Sub-Investigator<br>Surgeon | Obtaining consent, screening, treatment planning, dental surgery, follow-up care. Monitoring AEs. |
| S. Jean O'Neal, DMD, MS | Sub-Investigator<br>Prosthodontist | Obtaining consent, screening, treatment planning, implant restoration, patient care, monitoring AEs. |
| Perng-Ru Liu, DDS, MS, DMD | Sub-Investigator<br>Prosthodontist | Obtaining consent, screening, treatment planning, implant restoration, patient care, monitoring AEs. |
| Daniel A. Givan, DMD, PhD | Sub-Investigator<br>Prosthodontist | Obtaining consent, screening, treatment planning, implant restoration, patient care, monitoring AEs. |
| Wen-Chou Wu, DDS | Sub-Investigator<br>Prosthodontist | Obtaining consent, screening, treatment planning, implant restoration, patient care, monitoring AEs. |
| Chin-Chuan Fu, DDS | Sub-Investigator<br>Prosthodontist | Obtaining consent, screening, treatment planning, implant restoration, patient care, monitoring AEs. |

| | | |
|---|---|---|
| Jose V. Paiva, DDS | Sub-Investigator<br>Prosthodontist | Obtaining consent, screening, treatment planning, implant restoration, patient care, monitoring AEs. |
| Stephanie McLean, BS, MS | Project manager | Supervise daily clinical activities: patient care, scheduling, data collection, maintenance of study files, manage study progress and subject retention. Conduct consent discussion and obtain consent. Protect security and confidentiality of subject information |
| Abu Faisal M. Hasme BDS, MPH | Assistant Manager | Assist Ms. Stephanie McLean with daily clinical activities: data collection, maintenance of study files, study progress and subject retention. Conduct consent discussion and obtain consent. Protect security and confidentiality of subject information. Obtain radiographs and chair-side surgical assisting |
| Sandra J. Haigh, BS, MS | Research Coordinator | Regulatory Issues, maintenance of investigator's regulatory file; liaison between UAB, IRB,Sponsor |
| Sheila D. Akers, RDH | Research Hygienist | Obtaining consent, performing oral prophylaxis, patient care, obtain radiographs. |

## 19.0 PROTOCOL SIGNATURE PAGE

A Prospective Study of Bone Augmentation Techniques in Extraction Sockets
Protocol Number Amnio-12

Principal Investigator:

Philip J. Vassilopoulos, DDS, DMD
Printed name

_____
Signature

_____
Date