COVER PAGE FOR PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Study Title: Ridge Preservation Following Tooth Extraction Using Mineralized Freeze-Dried Bone Allograft Compared to PUROS Mineralized Bone Allograft

NCT number: NCT02515058

IRB Approval Date: June 30, 2015

Unique Protocol ID: 15-0552H

Protocol Template Form

Item 1 UTHSCSA Tracking Number	HSC20150552H

Item 2 Abstract / Project Summary	Provide a succinct and accurate description of the proposed research. State the purpose/aims. Describe concisely the research design and methods for achieving the stated goals. This section should be understandable to all members of the IRB, scientific and non-scientific. **DO NOT EXCEED THE SPACE PROVIDED**.

Purpose/Objectives: The purpose of the proposed study is to examine histologic wound healing following ridge preservation using FDBA compared to PUROS bone allograft.

Research Design/Plan: The study is a two-arm, parallel-design, randomized, prospective clinical trial. This entire protocol involves procedures that are standard care. The protocol is similar in design to the protocol our study group has used repeatedly and published in multiple papers.[4-6,9]

Methods: Subjects will have a non-molar tooth extracted and the socket grafted with one of two different bone graft materials (FDBA or PUROS). At the time of implant placement 12 weeks after tooth extraction a small core biopsy specimen will be removed from the implant site. Clinical measurements of the ridge dimensions will be taken at the time of tooth extraction and again at the time of implant placement. The core biopsy will then be evaluated for the primary outcomes: % vital bone formation, % residual graft material, and % "CT/other" (fibrous tissue and marrow space). We will also be evaluating the following secondary outcomes using a custom measuring stent for each subject: change in ridge width; change in buccal ridge height, and change in lingual ridge height.

Clinical Relevance: Having a high percentage of new vital bone after tooth extraction and ridge preservation may be beneficial in maintaining ridge dimensions for implant placement. This study will examine differences in vital bone formation with FDBA VS. PUROS

Item 3 Background	
Describe past experimental and/or clinical findings leading to the formulation of your study. *For research involving unapproved drugs, describe animal and human studies.* *For research that involves approved drugs or devices, describe the FDA approved uses of this drug/device in relation to your protocol.*	Insert background: Bone grafting following tooth extraction is commonly performed to preserve bony ridge dimensions adequate to support subsequent implant placement. Alveolar ridge resorption often occurs following tooth extraction, and the decrease in bone volume has the potential to make dental implant therapy impossible without surgery to reconstruct the ridge.[1] The aim of ridge preservation grafting is to prevent or minimize this resorptive process, thereby preserving an adequate volume of bone for implant placement. Ridge preservation generally involves placement of a particulate bone graft material in the tooth socket, followed by use of a membrane or similar substance over the socket entrance to contain the bone graft. Various grafting materials have been recommended for these ridge preservation procedures, including cortical and cancellous freeze-dried bone allograft (FDBA), demineralized freeze-dried bone allograft (DFDBA), allografts processed without freeze-drying, various xenografts, alloplasts and autografts. Our research group has published several studies evaluating a variety of materials for ridge preservation.[2-9] Beck & Mealey examined healing using a non-freeze-dried bone allograft prepared using the proprietary Tutoplast process (PUROS) and found similar new vital bone formation following a healing period of 3 months versus 6 months after ridge preservation.[2] In both groups, newly formed vital bone constituted approximately 45% of the total volume of the histologic specimens. In Wood & Mealey the use of cortical DFDBA provided a higher percentage of new vital bone formation (38.4%) than did cortical mineralized FDBA (24.6%).[4] A major strength of this study was that the comparison graft materials for both study groups

came from the same tissue donor, eliminating any issues that might arise from using different donor sources. Similarly, two additional studies done by our research group used a single source of donor bone for both study treatment groups.[6,9]

Our research group examined the effect on new bone formation of ridge preservation procedures performed using either cortical or cancellous FDBA.[6] There was no significant difference in new vital bone formation between the cortical (median 16.1%) and cancellous (median 13.0%) FDBA groups. A significantly greater percentage of residual graft material was detected in the cortical FDBA group as compared to the cancellous FDBA group, and a significantly greater percentage of nonmineralized CT/other material was found in the cancellous FDBA group as compared to the cortical FDBA group. Finally, we have recently completed a study comparing 100% cortical FDBA to a combination of 70% cortical/30% cancellous FDBA.[9] The combination allograft resulted in increased vital bone percentage, (36.2%) compared to the 100% FDBA group (24.7%).

In reviewing the vital bone formation we have seen across multiple studies when FDBA was used in one or more study arms[4,6,9], it is noteworthy that the amount of new bone formation seen with the mineralized non-freeze-dried PUROS allograft used in Beck & Mealey[2] (approximately 45% new bone) was higher than that seen when mineralized freeze-dried bone allograft was used (approximately 13-25%). However, there are no published direct comparison data evaluating FDBA versus bone allograft processed via the Tutoplast process (PUROS) when used for ridge preservation after tooth extraction.

References:

1. Avila-Ortiz G, Elangovan S, Kramer KWO, Blanchette D, Dawson DV. Effect of alveolar ridge preservation after tooth extraction: a systematic review and meta-analysis. *J Dent Res* 2014; 93: 950-958.
2. Beck TM, Mealey BL. Histological Analysis of Healing After Tooth Extraction with Ridge Preservation Using Mineralized Human Bone Allograft. *Journal of Periodontology* 2010; 81: 1765-1772.
3. Hoang TN, Mealey BL. Histological comparison of healing following ridge preservation using human demineralized bone matrix putty with one versus two different sized bone particles. *Journal of Periodontology* 2012; 83: 174-181.
4. Wood RA, Mealey BL. Histological comparison of healing following tooth extraction with ridge preservation using mineralized vs. demineralized freeze dried bone allograft. *Journal of Periodontology* 2012; 83: 329-336.
5. Cook DC, Mealey BL. Histological comparison of healing following tooth extraction with ridge preservation using two xenograft products. *Journal of Periodontology* 2013; 84: 585-594.
6. Eskow AJ, Mealey BL. Histological evaluation of healing following tooth extraction with ridge preservation using cortical versus cancellous freeze dried bone allograft. *Journal of Periodontology* 2014; 85: 514-524.
7. Coomes AM, Mealey BL, Huynh-Ba G, Barboza-Arguello C, Moore WJ, Cochran DL. Buccal bone formation after flapless extraction: A randomized controlled clinical trial comparing recombinant human bone morphogenetic protein-2/absorbable collagen carrier and collagen sponge alone. *Journal of Periodontology* 2014; 85: 525-535.
8. Frost NA, Banjar AA, Galloway PB, Huynh-Ba G, Mealey BL. The decision-making process for ridge preservation procedures following tooth extraction. *Clin Adv Periodont* 2014; 4:56-63.
9. Borg T, Mealey BL. Histological comparison of healing following tooth extraction with ridge preservation using mineralized freeze dried bone allograft alone versus a combined mineralized-demineralized freeze dried bone allograft. *J Periodontology* 2015; 86: 348-355.

Item 4 Purpose and rationale *Insert purpose, objectives and research questions/hypotheses here. If you cut and paste from another document, make sure the excerpted material answers the question*	Insert purpose: The study is designed to examine histologic wound healing following ridge preservation using with mineralized freeze-dried bone allograft (FDBA) or mineralized bone allograft prepared via the Tutoplast process which does not involved freeze-drying. This entire protocol involves procedures that are standard care. The study is a 2-arm, parallel-design, randomized, prospective clinical trial. The test group subjects will have extraction sockets grafted with a PUROSE. This test group will be compared to an active control group using FDBA as the graft material. The null hypothesis is that there will be no significant difference in formation of new vital bone between treatment groups (primary outcome). In keeping with the protocol our study group has used several times before, the plan will be to extract non-molar teeth and graft with the test/control materials. Each subject will provide a single non-molar tooth site for study treatment. The graft material will be covered with a resorbable bovine pericardium membrane (called CopiOs). Following 12 weeks of healing, we will place the dental implant. To place a dental implant, an osteotomy (hole in the bone) is prepared into which the implant is placed. This osteotomy can be prepared with either a solid drill, in which case the bone that is removed is suctioned into the suction system, or with a hollow trephine drill into which a core of bone can be collected. The only "research procedure" being done in the current study is the collection of this bone core biopsy for histologic evaluation. The core biopsy will then be evaluated for the primary outcomes: % vital bone formation, % residual graft material, and % "CT/other" (fibrous tissue and marrow space). We will also be evaluating the following secondary outcomes using a custom measuring stent for each subject: change in ridge width; change in buccal ridge height, and change in lingual ridge height.

Item 5 Study Population(s) Being Recruited In your recruitment plan, how many different populations of prospective subjects do you plan to target? Provide number: 1		
e.g., a population can be individuals with type 2 diabetes controlled with diet and/or a population of healthy controls. Or a population can be individuals attending an education program, etc. <u>List each different population</u> on a separate row and provide a short descriptive **label**: *(e.g., normal-healthy, diabetics, parents, children, etc.)* *To add rows use copy & paste*	Identify the criteria for **inclusion**:	Identify the criteria for **exclusion**:
Patients seen in Periodontics	• A single rooted tooth that has	• who will not cooperate with the

	been identified by dental faculty as requiring extraction • Desire a dental implant to replace the missing tooth • Have adequate restorative space for a dental implant-retained restoration • Have at least 10mm of alveolar bone height, without impinging on the maxillary sinus or inferior alveolar canal. • Have a dehiscence of the buccal or lingual bony plate of the tooth socket extending no more than 50% of the total depth of the socket. • Female patients who have undergone a hysterectomy, tubal ligation, or menopause, and non-pregnant women of child-bearing potential. • Are nonsmokers or former smokers. Current smokers may be included if they smoke ≤10 cigarettes per day	follow-up schedule. • Patients will are mentally incompetent, prisoners, or pregnant. • Pregnant women or women intending to become pregnant during the study period. Prior to dental surgery, females of child-bearing age are asked verbally if there is any possibility that they are pregnant. If not, we proceed with surgery and no pregnancy test is done. If the woman states that there is a possibility that she is pregnant, we do a urine pregnancy test to rule pregnancy in or out. So use of the urine pregnancy test is only done if she says she may be pregnant. [If needed: An over-the-counter urine pregnancy test will be provided to female subjects in the graduate periodontics clinic of UTHSCSA. Subjects will be allowed access to a private restroom and the results of the tests will be read by one of the named investigators. Only those with a negative pre-operative pregnancy test will continue in the study.] Grafted patients who become pregnant during the study will be withdrawn, but will be followed until completion of pregnancy. • Smokers who smoke >10 cigarettes per day • Clinical and/or radiographic determinations which will preclude inclusion in this study are: Active infection other than periodontitis; Inadequate bone dimensions or restorative space for a dental implant; Presence of a disease entity, condition or therapeutic regimen which decreases probability of soft tissue and bony healing, e.g., poorly controlled diabetes, chemotherapeutic and immunosuppressive agents, autoimmune diseases, history of bisphosphonate use or long-term steroid therapy; Positive medical history of endocarditis following oral or dental surgery. Sensitivity or allergy to Bacitracin, Gentamicin, Polymyxin B Sulfate, alcohol and/or surfactants (per package insert)
Insert response here	Insert response here	Insert response here

Item 6
Research Plan / Description of the Research Methods *a.* *Provide a **comprehensive narrative** describing the **research methods**. Provide the **plan for data analysis** (include as applicable the **sample size calculation**).*

Step-by-Step Methods: The purpose of the proposed study is to examine histologic wound healing following ridge preservation using FDBA compared to PUROS bone allograft. The study is a two-arm, parallel-design, randomized, prospective clinical trial. This entire protocol involves procedures that are standard care. The protocol is similar in design to the protocol our study group has used repeatedly and published in multiple papers.[4-6,9]

There will be two subject groups in this study. All subjects will require extraction of at least one non-molar tooth, followed by replacement of the missing tooth with a dental implant. If a given subject requires more than one tooth extraction, only one tooth site will be included in the study (the site with the best bony walls present after extraction; if two or more sites have equally good bony walls, the study site will be selected randomly by coin toss if there are two teeth to be extracted, or by drawing a number from a hat if more than 2 teeth require extraction). Based on power analysis and drop-out experience from previous studies, a total of 22 subjects will be recruited per group, with a maximum enrollment of 44 subjects for the 2 groups combined.

Test group subjects will have extraction sockets grafted with cancellous PUROS mineralized bone allograft (prepared using the Tutoplast process). This test group will be compared to an active **control group** in which extraction sockets are grafted using cancellous mineralized freeze-dried bone allograft (FDBA). Allocation of subjects into test or control group will based on numbers drawn from a stack of sealed envelopes. At the beginning of the study there will be 22 envelopes containing a piece of paper inscribed with "Group 1" and 22 envelopes with "Group 2" (Group 1= cancellous PUROS; Group 2 = cancellous mineralized FDBA). The subjects will not be randomized until after the tooth to be extracted has been removed (see below).

At the time of subject enrollment, the following standard procedures will be performed: impressions will be made for fabrication of diagnostic casts. The tooth or teeth to be extracted will be noted on the casts. Radiographs will be taken of the tooth or teeth to be extracted. A clear resin measuring stent will be fabricated in the laboratory to allow standardization of the location of clinical measurements of ridge width and height. A small flap will be reflected to an extent about 3mm beyond the bony walls of the socket. The measuring stent will be placed and measurements of ridge width and ridge height will be taken and recorded to the nearest 0.5mm. Ridge width will be measured using a ridge caliper at a point approximately 4mm apical to the facial and lingual bony crest through small holes created in the stent at those locations. Ridge height will be measured through two holes in the occlusal aspect of the stent – one hole directly above the facial bony crest and another hole directly above the lingual bony crest. This stent will be retained for use during the subsequent implant placement surgery.

The tooth will be extracted, and the number of bony walls in each socket will be recorded along with the presence of any bony dehiscences or fenestrations. Buccal and lingual flap elevation will be minimized and will extend only approximately 3mm beyond the bony crest on the facial and lingual surfaces. The buccal plate thickness will be measured using an Iwanson gauge positioned perpendicular to the inner wall of the extraction socket. One beak of the gauge will be placed within the extraction socket 1.0mm below the alveolar bone crest. The other beak will be placed on the external bone surface. This buccal plate thickness measurement will be recorded to the nearest tenth of a millimeter. **The subject will then be randomized by drawing a sealed envelope from the stack**. The socket will be thoroughly debrided, and either cancellous PUROS or cancellous FDBA will be placed in the socket to restore the ridge to appropriate contour. A CopiOs bovine pericardium resorbable collagen membrane will then be placed over the socket orifice extending about 3mm beyond the bony socket walls. Sutures will be placed over the membrane to secure it in place. Primary closure will not be attempted. Dehiscences of the bony walls of the socket may be found during the extraction procedure, as they are relatively common. **If the dehiscence is deeper than 50% of the total socket depth, the subject will be withdrawn from the study.** If the dehiscence is less than 50% of the depth of the socket, a larger flap will be reflected so that the CopiOs membrane can extend 3mm beyond the bony walls of the dehiscence. The patient

will be seen 7-10 days after extraction/ridge preservation to assess healing, and again 1 month after the procedure.

At the time of implant placement approximately 12 weeks after extraction and ridge preservation[2], the individual subject's measuring stent and caliper will be used to determine the ridge width and ridge height at the same locations as done in the tooth extraction/grafting surgery visit. The first osteotomy will be prepared using a hollow trephine with internal diameter of 2.0mm instead of a solid drill. The trephine drill will be used to a depth of approximately 8mm, ensuring that the bone within the trephine comes from the former socket area and not from surrounding native bone. The bone removed from the osteotomy site remaining in the trephine will be prepared for histologic examination and analyzed for new bone growth. The histologic cores will be processed for demineralized sections and will be stained. The following histologic parameters will be measured: percent new bone formation, percent residual graft material, and percent connective tissue/other. A surgical guide stent will be fabricated on the diagnostic casts and will be used to ensure proper positioning of the trephine at the site of the grafted ridge preservation procedure. Following initial preparation of the implant site with the trephine, the osteotomy will be completed and an implant of the appropriate length and diameter will be placed. The size of the implant will be such that it will engage not only newly formed bone in the socket itself, but at least 2-3mm of bone apical to the former socket site. A healing abutment will then be placed. The study itself will end at the time of the bone core removal. All subjects will be examined at 7-10 days following implant placement and at several visits between implant placement and final determination of implant stability for restoration, usually about 3-5 months after implant placement. The patient will then be referred to his/her restorative dentist for final restoration.

At the 12 week post-extraction/ridge preservation visit, in cases where the bone width or bone density is insufficient for implant placement at the time of osteotomy preparation with the trephine drill and subsequent implant drills, the implant will not be placed. Instead, guided bone regeneration will be performed in the usual manner and the site will be allowed to heal for 4-5 months before implant placement. As for all subjects, the study itself will end at the time of bone core removal.

Data Analysis Plan: The primary hypothesis to be tested is whether the mean percentage of new bone observed histologically for patients receiving a mineralized cancellous PUROS graft for ridge preservation in an extraction site prior to implant placement is different than those receiving a mineralized cancellous FDBA graft at a time point approximately 12 weeks after grafting. The distribution of new bone percentages is expected to be positively skewed, conforming to a double exponential distribution. The hypothesis will be tested using a Mann-Whitney U test. A total of 44 patients will receive treatment in a non-molar tooth site, with a minimum of 70% of patients expected to be fully compliant under the study protocol, so that the sample size available for histologic examination will be at least 14 patients per treatment group. This proposed sample size is sufficient to detect a mean difference of one standard deviation or more by Mann-Whitney U test at the 0.05 level with power of 88.5%. For example, if the population standard deviation of new bone percentage within patients is 10% or less, then the proposed sample is sufficient to detect a clinically significant mean difference of 10% or more new bone for patients receiving PUROS compared to FDBA.

Item 7 Risks Section:

Complete the following table to describe the risks of all **research procedures** listed in Step 2, Institutional Form (items 28-34). *Do not list risks of Routine care procedures here.*

☐ N/A, Risks are described in the informed consent document – do not complete this table.

Research procedures *example:* • History and physical • Questionnaire • Laboratory tests *Add or delete rows as needed*	**Risks** List the reasonably expected risks under the following categories as appropriate:
Randomization - Ridge preservation following tooth extraction using one of 2 graft materials in preparation for dental implant 12 weeks post-grafting	Serious and likely; ○ Npne Serious and less likely; ○ None Serious and rare; This is a standard of care risk FDBA may contain traces of the processing reagents Bacitracin, Gentamicin, Polymyxin B Sulfate, alcohol, and/or surfactants. Caution should be exercised if the patient has a known sensitivity or allergy to any of these antibiotics or reagents. PUROS may contain trace amounts of residual chemicals used in processing: acetic acid, acetone, hydrogen peroxide and sodium hydroxide. Not serious and likely; ○ None Not serious and less likely ○ There is no additional research related risk involved in the use of the FDBA or PUROS materials for ridge preservation, as there is no alteration to the clinical course or standard care treatment for this study. Each of these materials is commonly used in clinical practice. While we may indeed find that there is more new vital bone formation in one group vs. another, there is no reason to think that will impact the success of the implant that is placed. Thus, the study is definitely minimal risk. There is, of course, a hypothetical risk that disease transmission could occur with the bone graft materials but it is not quantifiable, as it has not occurred. Both materials are terminally sterilized with gamma radiation to achieve a sterility assurance level (SAL) of 10^{-6}.
Insert procedure here	Serious and likely; ○ Insert risk here or enter "none" Serious and less likely; ○ Insert risk here or enter "none" Serious and rare; ○ Insert risk here or enter "none" Not serious and likely; ○ Insert risk here or enter "none" Not serious and less likely ○ Insert risk here or enter "none"
Insert procedure here	Serious and likely; ○ Insert risk here or enter "none" Serious and less likely; ○ Insert risk here or enter "none" Serious and rare; ○ Insert risk here or enter "none" Not serious and likely; ○ Insert risk here or enter "none" Not serious and less likely ○ Insert risk here or enter "none"