Leukocyte Platelet Rich Fibrin (L-PRF) and Freeze-dried Bone Allograft (FDBA) Layered Technique vs L-PRF/FDBA and L-PRF Alone in Influencing Quantity and Quality of New Bone Formation in Grafted Extraction Sockets

Study Protocol & Statistical Analysis Plan

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Clinical, Radiographic, and Histomorphometric Outcomes Following Ridge Preservation using Leukocyte Platelet Rich Fibrin (L-PRF) and Freeze-Dried Bone Allograft (FDBA) Layered Technique: A Prospective, Randomized Clinical Trial

Protocol

Ver. 1

1.0 Investigational Design

1.1.Study Design

This investigation is a randomized controlled trial of three techniques to evaluate clinically, radiographic, and histologically the efficacy of L-PRF with freeze-dried bone allograft (FDBA) layered technique in improving quantity and quality of new vital bone formation and alveolar ridge dimensional stability following ridge preservation in comparison to L-PRF alone and L-PRF/FDBA.

Control technique: Ridge preservation using L-PRF/FDBA and ridge preservation using L-PRF alone

Test technique: Ridge preservation using L-PRF/FDBA layered technique

1.2. Objectives:

- To evaluate clinical and radiographic dimensional changes at alveolar ridge 3 months after ridge preservation procedure using L-PRF alone, L-PRF/FDBA, and L-PRF/FDBA Layered technique.

- To evaluate quantity and quality of new vital bone in sockets 3 months after ridge preservation procedure using L-PRF alone, L-PRF/FDBA, and L-PRF/FDBA Layered technique.

1.3. Hypotheses and Specific Aims:

- **Hypothesis 1:** Ridge preservation using L-PRF/FDBA Layered technique will best preserve alveolar ridge dimensions in comparison to using L-PRF alone**.**
- **Hypothesis 2:** Ridge preservation using L-PRF/FDBA Layered technique will improve new vital bone formation at the middle and apical thirds of the socket in comparison to using L-PRF/FDBA.

- **Specific Aims:**

1. To compare quantitative clinical alveolar ridge dimensions (in mm) 3 months after ridge preservation between the three groups using an intraoral scan and direct clinical assessment.

2. To compare quantitative two- and three- dimensional radiographic alveolar ridge dimensional changes (in mm) 3 months after ridge preservation between the three groups using Cone Beam Computed Tomography (CBCT) and virtual implant planning software.
3. To compare qualitative and quantitative histologic characteristics of new bone between the three groups after 3 months of ridge preservation by quantifying the distribution (in %) of new vital bone, connective tissue, residual graft particles, and artifact at the healed socket site.

2.0. Study Population

2.1 Entrance Criteria

Patients presented to the postdoctoral periodontal clinic at UAB School of Dentistry with a treatment plan for tooth extraction and implant placement will be screened according to the criteria. Thirty healthy adult eligible patients (ten in each group) of any ethnicity or gender may be enrolled into the study.

Participants must be at least 18 years old with demonstrated ability to understand and consent to the proposed study procedures. Decisionally-impaired adults and/or minors who cannot consent for themselves will not be enrolled.

Smokers (>10 cigarettes/day) patients or women who report a current pregnancy or patients who are not systemically healthy enough to receive out-patient elective dental care at the time of enrollment will be excluded.

Participants must have one or more hopeless single-rooted tooth that require extraction and planned to be replaced with a dental implant, with healthy adjacent teeth not planned for extraction. The extraction socket should have residual 4 bone walls following atraumatic tooth extraction.

Patients must demonstrate high compliance, adequate oral hygiene, and denied history of alcohol or drug abuse.

3.0 Study procedures

3.1 Summary of study procedures

- **Screening/baseline visit**: Upon enrollment into the study the patient's medical history and electronic records will be reviewed. Patients will be treatment planned by an interdisciplinary team consisting of a surgeon and a prosthodontist or restorative dentist. Clinical examiners will conduct clinical and radiographic exams to determine eligibility according to the above inclusion criteria. An intraoral scan will be taken to perform clinical measurements. Study visits and objectives will be explained to all participants. IRB approved written informed consent will be obtained from all the participants.

- **Visit 1 (Surgical visit): Extraction and ridge preservation**

- **Randomization:** Participants will be stratified and randomized to one of three treatment groups: Table 1, by permuted block randomization approach to ensure same number of patients in each group, using computer-generated random number list.

Table 1. Groups of treatment.

Group	Treatment	Number of participants
Group 1	Atraumatic tooth extraction followed by ridge preservation using Leucocyte and Platelet Rich Fibrin (L-PRF) + Freeze-Dried Bone Allograft (FDBA) Layered technique	10
Group 2	Atraumatic tooth extraction followed by ridge preservation using Leucocyte and Platelet Rich Fibrin (L-PRF) + Freeze-Dried Bone Allograft (FDBA)	10
Group 3	Atraumatic tooth extraction followed by ridge preservation using L-PRF	10

- **Surgical procedure:** A loading dose of prophylactic antibiotic will be dispensed (Amoxicillin 2 grs or Clindamycin 600mg, 30 minutes to one hour prior to the surgery). Venous blood will be drawn (antecubital fossa) with a 25-butterfly needle to fill 4-8 collection tubes of 10ml each. The tubes will not contain any additive or anticoagulant. One tube will be centrifuged for 3 minutes and the other tubes will be centrifuged for 12 minutes. After centrifugation, L-PRF clots will be collected and stored in a closed sterile storage container that enables compression of clots into membranes/plugs. Additionally, the one tube that received less centrifugation (fibrinogen) will be used as an additive with the L-PRF membranes and bone allograft. The bone graft material utilized for patients in group 2 and 3 will be MinerOss cortico-cancellous freeze-dried bone allograft (FDBA).

L-PRF Preparation		
		
Venous blood collection	Centrifuge white-covered tube for 3 minutes and red-covered tubes for 12 minutes	White-covered tubes: Fibrinogen Red-covered tubes: L-PRF membranes

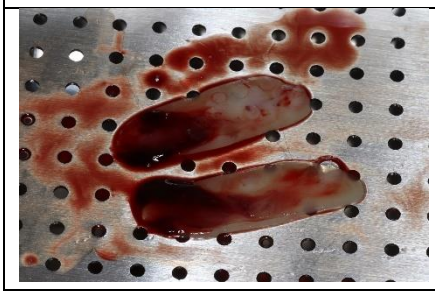
After preparation and isolation of the surgical area, local anesthesia will be accomplished by infiltration and/or intraoral nerve block. Intravenous moderate conscious sedation or inhalation sedation will be used in patients who requested it.

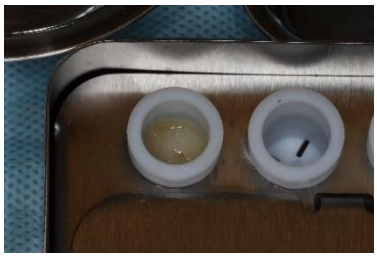
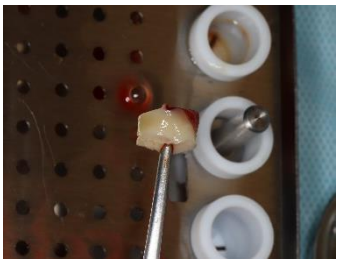
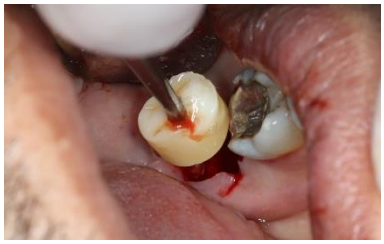
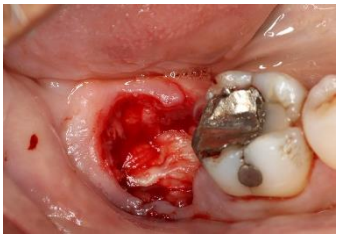
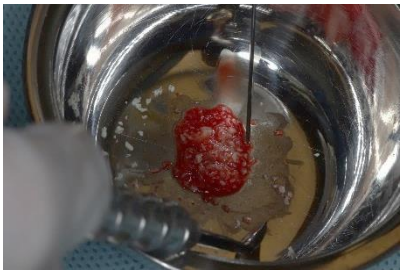
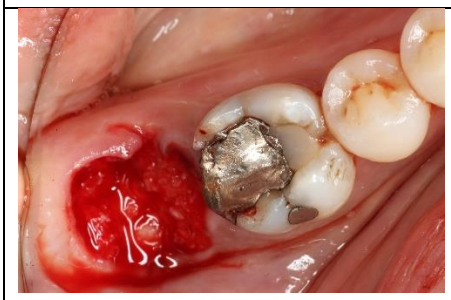
Tooth or teeth will be gently elevated using a minimally traumatic technique that prevent loss of supporting bone. Socket(s) will be curetted, irrigated with saline, and inspected following extraction and verified for the presence of intact walls.

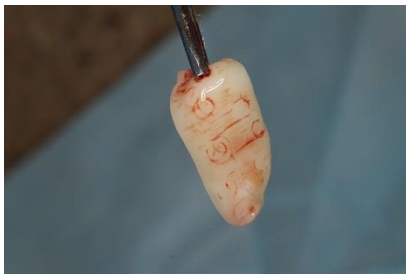
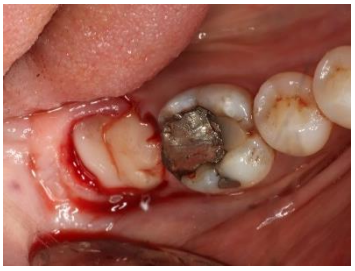
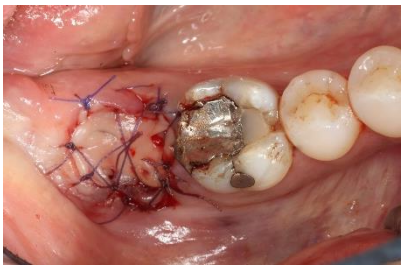
A Cone Beam Computed Tomography (CBCT) scan will be taken immediately after the tooth extraction, before ridge preservation procedure. It will serve for baseline radiographic measurements.

Group 1: L-PRF/FDBA Layered technique:

After socket is cleaned, a L-PRF plug will be formed, placed and compacted at the apical and middle third of the socket. Two membranes of L-PRF will be cut into small pieces and combined with 0.5 cc of cortico-cancellous bone allograft (FDBA), in a 50%-50% proportion, and fibrinogen forming sticky bone. The sticky bone will be placed and compacted at the coronal third of the socket (over the L-PRF plug already placed). A membrane of L-PRF will be placed at the coronal extent of the socket, flaps will be reapproximated and sutured with Monocryl 5-0 sutures.

Group 1: L-PRF/FDBA Layered technique	
	L--PRF membranes

   	L-PRF plug formed and placed in the apical and middle third of the socket
   	L-PRF membrane chopped into small pieces and combined with FDBA and fibrinogen forming sticky bone
	Sticky bone is placed at the coronal third of the socket (over the L-PRF plug)

  	A L-PRF membrane is placed covering the graft and sutured.

Group 2: L-PRF/FDBA:

After socket is cleaned, L-PRF membranes will be chopped into small pieces and combined with 0.5 cc of cortico-cancellous bone allograft (FDBA) and fibrinogen forming sticky bone. The sticky bone will be placed and compacted at the along the socket. A membrane of L-PRF will be placed at the coronal extent of the socket, flaps will be reapproximated and sutured with Monocryl 5-0 sutures.

Group 3: L-PRF

After the socket is cleaned, the socket will be completely filled with L-PRF plugs and a L-PRF membrane will be placed at the coronal extent of the socket, flaps will be reapproximated and sutured with Monocryl 5-0 sutures.

Post-surgical analgesics will be prescribed and/or dispensed as deemed necessary by the treating surgeon and a continued course of antibiotic will be prescribed for a 1-day time of period. Written and oral home care instructions will be provided.

- **Visit 2 (Follow-up visit):** Sutures will be removed after two weeks. Surgical site will be evaluated for healing status and post-operative instructions on resuming oral hygiene measures will be given to patients. Medical history will be reviewed, and patients will complete a questionnaire regarding their assessment of the post-operative discomfort they experienced during the initial healing phase, including number of days of altered activity/discomfort and number of tablets of pain medications taken.

- **Visit 3 (Follow-up visit):** After four weeks, surgical site will be evaluated for healing status and oral hygiene instructions will be reinforced. Medical history will be reviewed, and adverse events will be record.

- **Visit 4 (Surgical visit): Bone biopsy and implant placement**

  After three months, surgical site will be evaluated. A second intraoral scan will be taken to evaluate clinical ridge dimensions. A second CBCT scan will be taken to evaluate the dimensional changes of the healed socket and plan the optimal implant location using implant planning software. Medical history will be reviewed, and adverse events will be record.

  A surgical approach will be performed to take a bone biopsy for research purposes and implant placement at the same site. The procedure will be performed under local anesthesia and IV conscious sedation if patient requested it. After a full-thickness mucoperiosteal flap will be raised, the ridge dimensions will be measured at the crestal bone for dimensional changes in height and width using a calibrated surgical caliper and UNC-15 periodontal probe. Prior to implant placement, a bone biopsy will be taken from the center of the new bone using a 2mm internal diameter trephine bur. The dimensions of the harvested bone core will be 2mm in diameter and the length will be according to the implant dimension to be placed. The biopsy will be stored in 10% neutral buffered formalin and will be sent to the Histomorphometry and Molecular Analysis Core in the UAB Lyons Harrison Building. The Implant preparation osteotomy site will be completed following manufacturer protocol.

- **Visit 5 (Follow-up visit):** Sutures will be removed two weeks after the bone biopsy and implant placement. Surgical site will be evaluated for healing status and post-operative instructions on resuming oral hygiene measures will be given to patients. Medical history will be reviewed and patients will complete a questionnaire regarding their assessment of the post-operative discomfort they experience during the initial healing phase, including number of days of altered activity/discomfort and number of tablets of pain medications taken.

- **Visit 6 (Follow-up visit):** After four weeks, surgical site will be evaluated for healing status and oral hygiene instructions will be reinforced. Medical history will be reviewed, and adverse events will be record.

- **Visit 7 (Final study visit):** In six months, surgical site will be evaluated for healing status. This evaluation will include a clinical examination, periapical radiograph, intraoral scan, and assessment of overall oral health and hygiene. Patients will complete a questionnaire regarding their satisfaction with study participation. Patients will be offered a dental cleaning at no charge at this visit. Medical history will be reviewed, and adverse events will be record.

Table 2. Study schedule of events

Procedure Events	Screening Baseline	V1 Extraction & ridge preservation	V2 – V3 2-4 wks Follow-up	V4 3 months Bone biopsy and implant placement	V5-V6 2-4 wks Follow-up	V7 6 months
IRB-Consent	x					
Medical history review	x	x	x	x	x	x
Inclusion/exclusion criteria	x					
Oral exam	x	x	x	x	x	x
Randomization to either group		x				
Tooth extraction/Ridge preservation		x				
Bone core biopsy				x		
Implant placement				x		
X-ray	x			x		x
CBCT		x		x		
Intraoral Scan	x			x		x
Photograph	x	x	x	x	x	x
Clinical assessment of ridge dimensions	x	x		x		
Radiographic assessment of ridge dimensions		x		x		
Histologic evaluation				x		
Adverse events recorded			x		x	x
Pt. satisfaction survey						x
Statistical Analysis/Final Report						x

3.2. Visual Analog Scale (VAS) survey is a questionnaire regarding post-operative pain, swelling, bruising effect, and daily activities tolerance. For each question, patients are asked to mark on a 0 to 10 scale their perception of the level of corresponding pain, swelling, bruising, and alterations to daily activities with 0 being no symptoms and 10 being the worst symptom imaginable. It will be completed two weeks after every surgical procedure.

3.3.Outcomes measurements:

- **Clinical and Radiographic Measurements:**

Clinical measurements will be taken by one experienced blinded examiner to the randomization process before ridge preservation procedure and 3 months after ridge preservation, using intraoral scan and direct clinical assessment using a calibrated surgical caliper and UNC-15 periodontal probe (in millimeters). Another blinded examiner will perform the radiographic measurements at time of ridge preservation procedure and 3 months after ridge preservation, using a CBCT and implant planning software (in millimeters). Horizontal and vertical ridge dimensions will be evaluated clinically and radiographic. Intra-examiner calibration will be conducted to ensure reliability of measuring method.

- **Histomorphometric Analysis:**

Each specimen will be fixated with 10% neutral buffered formalin for 48 hours prior to be dehydrated and embedded in methylmethacrylate. It will be ground sectioned at the center of the biopsy in its long axis into 50-70 micron-thick sections (Exakt Technologies, Inc., Oklahoma City, OK), and polished with 4000 grit sandpaper and Novus Polish to create a surface as smooth as possible. All sections will be stained with Sanderson's Bone Stain and imaged for quantification of bone formation. Histomorphometry will be done using the Bioquant Image Analysis Software (R&M Biometrics, Nashville, TN) by measuring the total surface of vital bone, residual graft particles, organic matrix and artifact/air components. Corresponding percentages will be calculated for each of these tissues and compared between the three groups (L-PRF, L-PRF/FDBA, LPRF/FDBA layered technique). These experiments and measurements will be conducted by an experienced blinded lab technician.

3.4.Statistical Analysis:

A power analysis was conducted to determine the sufficient number of patients in each treatment group to obtain a power of 80% to detect a 1.0mm change in ridge dimensions using a type 1 (alpha) error rate of 0.05. The power analysis determined that a minimum of 10 patients would be required for each group, and 30 patients in total. An alpha of 0.05 was assumed after a Bonferroni correction for five relevant 2-way comparisons between the treatment groups.

Two-tailed *t* test will be used to compare clinical characteristics between patients with the L-PRF/FDBA layered technique, L-PRF/FDBA, and those with L-PRF alone for continuous variables with a normal distribution. Nonparametric (Wilcoxon) test will be used otherwise.

For categorical variables, inter-group comparisons will be conducted using chi-square test or Fisher's Extract 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 Mc Nemar'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 9.4 (SAS Institute, Inc, Cary, North Carolina).

4.0 Adverse Event Reporting

Participants will be interviewed and instructed to call if they experience any adverse events after study procedures. 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.

5.0 Cost/Benefits to participants

5.1 Cost/Benefits to participants

There is no additional cost for study participation and study participants will pay for a reduced fee for the surgical procedures including extraction, grafting, and implant placement ($1200). This represents a fee reduction of approximately $800. Additionally, participants will receive a dental cleaning at no charge at the 6-month follow-up visit.

Benefits of this project for individual participants could include enhanced bone healing, due to the potential benefit of L-PRF at extraction socket grafting sites. Overall benefits include the assessment of the practicality and utility of utilizing L-PRF either alone or in combination with a bone allograft in an extraction socket model to improve new bone formation and produce a more ideal environment for the installation of dental implant(s) at an edentulous site.

5.2 Additional Dental Procedures

Study visits are not designed to take the place of routine dental care. Costs for non-study related treatment will be charged to participant account or insurance provider. Implant restorative treatments including implant abutment(s), crown(s), and/or other restorative components are not included in the reduced surgical fee for study participants.