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The Role of Leukocyte-Platelet Rich Fibrin in Human Alveolar Ridge Preservation: A Randomized Clinical Trial

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THE ROLE OF LEUKOCYTE-PLATELET RICH FIBRIN IN HUMAN ALVEOLAR RIDGE PRESERVATION: A RANDOMIZED CLINICAL TRIAL

A thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science in Dentistry at Virginia Commonwealth University

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Abstract

THE ROLE OF LEUKOCYTE-PLATELET RICH FIBRIN IN HUMAN ALVEOLAR RIDGE PRESERVATION: A RANDOMIZED CLINICAL TRIAL

By Thomas Foster Glazier, D.D.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science in Dentistry at Virginia Commonwealth University.

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PURPOSE: The aim of this study is to examine the healing of intact extraction sockets grafted with leukocyte-platelet rich fibrin (L-PRF) as compared to sockets grafted with freeze-dried bone allograft (FDBA) and a resorbable collagen barrier membrane (RCM).

METHODS: This prospective randomized, examiner blinded pilot study included 17 subjects randomized to two treatment groups. Serum total cholesterol, low density lipoprotein (LDL), 25-hydroxyvitamin-D_3, and platelet counts were determined preoperatively in all subjects. The experimental arm consisted of 8 posterior tooth-bounded intact extraction sites receiving L-PRF plugs. The control group consisted of 9 posterior tooth-bounded intact extraction sites receiving FDBA and RCM. An acrylic stent was fabricated to take duplicate clinical and CBCT measurements immediately post-extraction and at time of implant placement. A repeat-measures analysis of variance was utilized for statistical analysi

RESULTS: The study failed to detect a clinical or radiographic difference between treatment groups in horizontal or vertical ridge dimension changes. Serum cholesterol, LDL, 25-hydroxyvitamin-D_3, and buccal plate thickness had a non-significant effect on outcome measurements, although there was a high prevalence of hyperlidpidemia and hypovitaminosis in the study population.

CONCLUSIONS: The alveolar ridge dimension changes in intact posterior extraction sockets may be similar when either L-PRF or FDBA and RCM are utilized as socket grafting material. Although there was a high prevalence of high cholesterol and low 25-hydroxyvitamin-D_3 levels in the population, this study failed to detect a significant correlation between preoperative serum levels and postoperative ridge dimension changes.

Keywords: Platelet, bone, graft, cone beam computed tomography, fibrin

Introduction

Alveolar ridge deformation can result from the extraction of a tooth due to both hard and soft tissue loss. This deformation, or resorption, is a functional and esthetic concern, especially in the area of restorative implant and prosthetic dentistry. [1, 2] this deformation occurs with resorption of the buccal and lingual external socket walls.[2] Pietrokovski[3] found that following extractions in both the maxillary and mandibular arches, more resorption occurs from the buccal ridge. Schropp[4] found clinically and radiographically, at twelve months post-extraction, a fifty percent reduction in alveolar ridge width (from 12mm to 5.9mm), with two-thirds of the reduction occurring in the first three months. Ridge height, however, only slightly decreased (< 1mm). Ridge width resorption may increase in severity when the buccal plate of bone is thin or absent.[5, 6] McCall reported forty to sixty percent loss of original bone height and width within two years following multiple extractions.[7]

In order to minimize ridge resorption and soft tissue recession, as well as maximize formation of bone, many hard and soft tissue regenerative options are available for preserving and correcting ridge defects, including ridge preservation. Ridge preservation, with the use of grafted material, provides space maintenance in order to prevent tissue collapse and maintain a healthy architecture for future restorative options.[8] Grafting an extraction socket can result in preservation of eighty five percent of the initial alveolar ridge dimensions.[9]

Multiple ridge preservation techniques are available, and no one technique is considered superior to another.[8] Grafting materials include autogenous bone, demineralized freeze-dried bone allografts (DFDBA), freeze-dried bone allografts (FDBA), xenografts, bioactive glass,

hydroxyapatite and calcium sulphate.[2] In a split mouth prospective study, Lekovic,[10] found 1.50mm loss of alveolar height and 4.56mm loss of ridge width in extraction sites alone versus only 0.38mm of vertical loss and 1.31mm loss of ridge width in sites preserved using a bioabsorbable membrane after six months. Iasella et al[11] found that non-molar extraction and ridge preservation with FDBA and a type I resorbable collagen membrane resulted in a loss of 1.2±0.9mm compared to 2.6±2.3mm in extraction sites alone. Aimetti et al[12] compared ridge preservation with calcium sulfate to extractions alone and found a loss of 2.0 ±1.1mm versus 3.2±1.8mm in extraction sites alone. Kotsakis et al.[13] randomized extraction sites to receive nothing, an intrasocket bovine xenograft, or an intrasocket bone putty. Sites receiving nothing lost a mean of 2.53mm. Sites receiving bovine xenograft lost a mean of 1.39mm, and those receiving allograft bone putty lost a mean of 1.26mm.

Some variation in the literature following extraction with or without ridge preservation has been explained by surgical technique, [10, 14] presence and thickness of buccal plate, [6] and location within the arch.[4, 15] However, there still appears to be a variable response in terms of horizontal ridge resorption.

Recent animal studies suggest that insufficient serum levels of 25-hydroxyvitamin-D_3 negatively impact peri-implant bone formation, osseointegration, implant stability, and bone to implant contact. [16, 17] Furthermore, repletion of vitamin D following deficiency improves peri-implant bone formation [17] and improves the strength of post-fracture calluses in elderly animals [18] with a biologically active metabolite of 25-hydroxyvitamin-D_3 accumulating around fracture sites.[19] Epidemiological data suggests that the incidence and prevalence of insufficiency and deficiency have increased with current prevalence estimates of seventy-four to eighty percent of the United States population having some vitamin D abnormality.[20] Cross-sectional data from

orthopedic literature suggests that vitamin D abnormalities may lead to complications in osseous healing.[21, 22]

Low-density lipoprotein (LDL) can be cytotoxic to osteoblasts.[23] Animals given a high fat diet demonstrated increased mandibular alveolar bone porosity, less trabecular thickness, and impaired osteoblasts associated with an average plasma cholesterol level of 136±7.36mg/dl as compared to animals given a normal diet whose plasma cholesterol levels averaged 90.80 ± 4.33mg/dl.[24] Cholesterol has been shown to influence the fate of mesenchymal stem cells capable of differentiating into osteoblastic or adipocytic lineages that reside within bone marrow stromal cell populations. It is believed that increases in dietary fat may alter marrow lipid composition and subsequently influence marrow stromal cell phenotypes. [25]

Leukocyte-Platelet Rich Fibrin (L-PRF) is a three-dimensional heterogenous fibrin "clot" that contains greater than ninety-seven percent of platelets and greater than fifty percent of leukocytes present in a nine milliliter whole venous blood draw.[26] L-PRF has been shown to stimulate cell growth and replication of human osteoblast-derived-osteoscarcoma cells, human kertinocyte-derived carcinoma cells, and human fibroblast lung carcinoma cells, and to upregulate osteocalcin and ostepontin (bone sialoprotein-1) in human osteoblast-derived osteosarcoma cells in vitro.[27] The natural fibrin polymerization method combined with the centrifugal force enables entrapment of growth factors from platelets and cytokines from leukocytes into the fibrin matrix.[28] L-PRF has been shown to release the growth factors TGFβ-1, PDGF-αβ, VEG-F, and thrombospondin-1, an important coagulation glycoprotein of the cellular matrix, for 7 days after its preparation.[29] In vivo, the effect had mixed success in hard tissue regeneration. Gürbüzer et al[30] found no difference of osteoblastic activity in third molar extraction sites using scintographic evaluation at 4 weeks postoperatively, yet several authors

reported a positive effect in maxillary sinuses augmentations,[31, 32] mandibular class II furcations,[33, 34] and intrabony defects.[35, 36, 37]

Cone beam computed tomography (CBCT) provides a three-dimensional image of the dental and maxillofacial areas. CBCT is a useful tool to locate anatomical structures, support diagnostic implant planning, and as a guide for dental surgery. From a CBCT image, reliable linear measurements of dentomaxillofacial structures can be made. Therefore, CBCT has the potential to evaluate the healing of alveolar ridge preservation procedures.[38, 39]

Several studies have evaluated the accuracy and reproducibility of linear measurements made on cone beam computed tomographic images. Loubele et al[40] compared the accuracy of linear measurements of alveolar bone taken on Accuitomo CBCT images, multislice CT images, and direct measurements on an ex vivo model and found no statistical differences between any of the measurement methods. This point was later confirmed by Lund et al[41] who also reported a high level of agreement between measurements made by multiple observers.

Tomasi et al[42]reported a high correlation between linear measurements made with a caliper and measurements made on a CBCT image. Although,Torres et al[43] found that typical CBCT images underestimate the true linear distance by sixteen to seventeen percent in the horizontal plane and seven to eight percent in the vertical plane. Hashem et al[44] also found no differences between two different methods of capturing a CBCT image as compared to direct measurements made on porcine hemimandibles. Shokri et al[45] also found a high level of agreement between actual measurements with a digital caliper and measurements made on CBCT. They felt the most accurate horizontal measurements were made with slice thicknesses of

four to five millimeters. They concluded that measurements made on slices less than four millimeters may underestimate the actual distance.

Intraoral films, such as bite-wings and periapicals, provide a two-dimensional image while computed tomography produces a three-dimensional image.[46] CBCT obtains this image by using a two-dimensional detector to scan the entire head, rather than stacking multiple slices as in conventional computed tomography allowing for a more efficient, less expensive, and lower energy output image.[47] CBCT does not expend high radiation doses. The average radiation dose of 0.585mSv is well below that of a conventional medical-grade CT scan but above that of conventional dental radiographs. Cortical integrity and thickness, enlarged bone marrow spaces, postextraction irregularities, and trabecular bone density have all been identified clearly in the cross-sectional images produced by the CBCT.[48]

The aim of this prospective blinded pilot study is to clinically and radiographically evaluate the healing of extraction sites grafted with either human freeze-dried mineralized cortical allograft and a resorbable barrier membrane or L-PRF. A secondary aim is to evaluate the impact of serum cholesterol and vitamin D_3 on alveolar healing.

Methods and Materials

Study Population

The Institutional Review Board of Virginia Commonwealth University reviewed and approved this research protocol. Recruited patients were referred to Virginia Commonwealth University School of Dentistry Graduate Periodontics Clinic for dental extraction and alveolar ridge preservation of a premolar or molar between July 2013 and December 2014.

Inclusion Criteria

Inclusion criteria were systemically healthy (ASA1 or 2) adults over the age of 18 with a normal blood concentration of thrombocytes (150,000-450,000/μL) as determined by a preoperative platelet count. Subjects enrolled required one or two premolar or molar extractions and alveolar ridge preservation in preparation to receive a dental implant. Study teeth were bordered by intact teeth with intact periodontal ligaments on both the mesial and distal.

Exclusion Criteria

Exclusion criteria include uncontrolled systemic illness that may affect healing or clotting such as diabetes mellitus, immunocompromisations, ongoing chemo- or radiotherapy; active smokers; patients with known allergies to freeze dried bone allograft or collagen membranes; pregnancy, and any detectable loss of buccal or lingual cortex immediately following extraction.[6]

Following informed consent, whole venous blood was drawn to determine platelet count, total cholesterol, LDL, and 25-Hydroxyvitamin-D_3 (Virginia Commonwealth University Health System, Clinical Pathology Lab). Alginate impressions were also taken of the arch containing the study site to fabricate an acrylic radiographic/clinical measurement sent.

Surgical Intervention

All surgeries were performed by a resident surgeon under the direct supervision of a board-certified periodontist. Teeth were extracted utilizing a minimally traumatic technique. Subjects had one or two teeth extracted in a minimally traumatic manner utilizing periotomes and sectioning of molars. No mucoperiosteal flaps were elevated at any time during the extraction procedure. Following confirmation of intact buccal and lingual cortical plates, the surgeon completed the assigned graft protocol. Three to eight months post-operatively, dental implants were placed utilizing full thickness flaps. During implant osteotomy preparation, a 2x8mm core of bone was harvested utilizing a trephine bur. Samples were immediately placed in 10% neutral buffered formalin and stored for histologic analysis.

Control Group

Mineralized freeze-dried cortical bone allograft (Oragraft, Lifenet Health) with particle sizes ranging from 250 to 750µm was hydrated in sterile saline and gently condensed to the level of the pre-existing alveolar bone height taking care not to over fill. A trimmed resorbable cross-linked type I porcine collagen membrane (Renovix, Salvin Dental Specialties, Inc.) was used to cover the socket. Sutures were utilized to secure the membrane beneath the supracrestal gingiva on the buccal and lingual.

Test Group

54mL of venous whole blood was collected with a 21-gauge butterfly needle from the antecubital fossa into 6 red-top vacutainers containing no anticoagulant or biomodifiers (A-PRF, Process for

PRF). Each 9mL sample was immediately centrifuged at 2700RPM for 12 minutes taking care to begin centrifugation within one minute of the blood draw (Intra-Spin, Intra-Lock, Inc). Upon completion of the spin, forceps were used to transfer the L-PRF to a sterile kidney dish. The red blood corpuscle end was gently removed with a scraping action with the back of tissue scissors. The L-PRF was then transferred to a specialized metal box designed to fabricate plugs and membranes. L-PRF was processed into plugs by placing the L-PRF into the designated wells and compressed with a weighted piston for 2 minutes. [31-37] L-PRF plugs were condensed into the extraction site using sterile gauze and a bone condenser. The plugs were condensed to the level of the surrounding free-gingival margin. Sites were sutured in a similar manner to those used in the control group mainly utilized to retain the L-PRF. In all sites there was no coronal advancement of the buccal flap and absolutely no attempt at primary closure.

All patients received normal verbal and written post-operative instructions. Only subjects from the control groups received a seven day course of systemic post-operative antibiotics. All subjects were prescribed analgesics on an individual basis. Subjects were instructed not to brush the surgical area for the first 2 weeks post-operatively. Supragingival scaling of adjacent teeth and reinstruction in oral hygiene was completed at two and four weeks post-operatively.

Measurements

All measurements were obtained by a single blinded examiner not involved in patient treatment. All clinical and radiographic measurements were made utilizing an acrylic stent with radiographic markers at each measurement location.

Clinical Measurements

Immediately post-extraction (T_1) and prior to flap reflection at implant placement (T_2), clinical bone sounding measurements were made under local anesthesia. Using a Weiss Modified Castroviejo caliper, alveolar buccal-lingual ridge width was recorded at three different points along the ridge---mesial (M), center (C), and distal (D) at 3mm apical to the buccal crest of bone. The buccal plate thickness was recorded at baseline in the same location as the horizontal measurements. Utilizing a UNC-15 periodontal probe, relative vertical measurements were taken of the alveolar crest at the four line angles of the extraction socket---mesiobuccal (MB), distobuccal (DB), mesiolingual (ML), and distolingual (DL). All clinical measurements were made to the nearest half millimeter.

Radiographic Measurements

CBCT images were captured at two time points: immediately post-extraction and three to eight months later when implants were placed (Carestream CS9300, Carestream Health). Horizontal and vertical measurements were recorded in the sagittal plane at the locations described above. All radiographic measurements were made following the conclusion of the trial, however, the image taken immediately post-extraction was reviewed to confirm no loss of cortical plate. All CBCT measurements were made to the nearest one tenth of a millimeter.

Statistical Analysis

The primary outcome variable of this prospective pilot study is change in alveolar ridge width measured 3mm apical to the buccal alveolar crest. An *a priori* power analysis was computed

based on the mean (±0.68) of the inter- and intragroup standard deviations of three recent prospective trials on ridge preservation.[13, 14, 49] With an alpha value of 0.050 and a proposed sample size of n=15 per treatment arm, the study has a power of 79.9% to yield a statistically significant result. This effect was selected as the smallest effect that would be important to detect, in the sense that any smaller effect would not be of clinical or substantive significance. It is assumed that this effect size is reasonable, in the sense that an effect of this magnitude could be anticipated in this field of research.

Randomization was performed with a computer-generated randomization table that was only available to the surgeon residents and never the measuring examiner.

This was a two-group pre-post experimental design using two measurement methods—clinical and CBCT. The pre-post change in width and height was compared between the two groups using Analysis of Co-Variance (ANCOVA).

Results

A total of 19 subjects who met inclusion criteria supplying 21 study sites were enrolled in this prospective randomized examiner blinded pilot study. Throughout the course of the trial three patients with one study site each were lost to follow up, and one site was excluded due to cortical plate loss following extraction. 16 subjects (8 males, 8 females) with an average age of 54.13±16.96 (years; mean±SD) supplying 17 extraction sites successfully completed the trial—Table 1. There were no adverse events that occurred during the treatment. Eight subjects with nine sites were randomized to the control group and received a socket graft of human mineralized cortical bone allograft covered with a cross-linked type I porcine collagen membrane left intentionally exposed. Eight patients with eight sites were randomized to the test group and received a socket graft of L-PRF plugs condensed to the level of the free gingival margin.

Sixteen subjects with seventeen graft sites consisting of one maxillary molar, four mandibular molar, and twelve maxillary premolar intact extraction sockets were analyzed for changes in horizontal and vertical hard tissue changes following one of two surgical interventions. Seven of these teeth were extracted due to caries, eight due to fracture and non-restorability, and two due to endodontic failure.

Horizontal Change – Primary Outcome Variable

Baseline and re-entry measurements were taken of buccal-lingual ridge width 3mm apical to the facial osseous crest at the mesial, center, and distal of each study site using two methods: clinical bone sounding with calipers and radiographic measurements made on sagittal slices of a limited

field of view cone beam computerized tomographic scan utilizing an acrylic 0.040 vacuform stent with gutta markers marking each measurement location.

Pairwise correlations revealed a high correlation among horizontal ridge changes at the mesial, center, and distal clinical measurements of each site (Center-Mesial – 0.87; Distal-Mesial – 0.75; Distal-Center – 0.82), thus the composite mean of each site was used for comparison between treatment groups—Table 2. The study failed to detect a difference clinically between treatment groups (control – 1.88mm± 1.51; test – 2.02mm±2.17; mean±SD) —Table 3. The study also failed to detect a difference radiographically between treatment groups (control – 1.40mm± 0.72; test – 0.87mm±0.64; mean±SD) —Table 4. Using the mean clinical horizontal change as the response variable, the effects of mean buccal plate, surgical intervention, total cholesterol, vitamin D, and time between surgeries on the response variable were evaluated and no statistical significant effects were found. After adjusting for the above variables, the mean horizontal changes between treatment groups were again, non-significant (control – 1.95± 0.66; test – 1.94± 0.71; least square mean ± SE) —Table 5. This process was also conducted with the radiographic horizontal measurements and compared between treatment groups. Again, no statistical differences were observed between groups in mean radiographic horizontal changes.

Vertical Changes – Secondary Outcome Variable

Clinical bone sounding measurements with a UNC-15 periodontal probe and radiographic measurements on sagittal slices of a limited field-of-view cone beam computerized tomographic image were made at the mesiobuccal, distobuccal, mesiolingual, and distolingual line angles of each study site and baseline and re-entry utilizing the pre-described acylic stent. Relative clinical

measurements were made to the top of the acrylic stent. Relative radiographic measurements were made utilizing radiographic gutta percha makers within the acrylic stent.

Pairwise correlations revealed significant correlation between vertical changes at the mesio- and disto-lingual line angles only (Distolingual-Mesiolingual-0.80) —Table 6. Therefore a mean of these two variables was used for the compare lingual vertical change. Mesiobuccal (MB) and distobuccal (DB) measurements were analyzed separately. No statistical difference due to treatment was detected clinically between groups on the lingual (control – -0.11±2.11; test – -0.88±1.36; mean±SD) or MB (control – -0.44±2.11; test – -0.31± ±1.39; mean±SD) or DB (control –- 0.33±0.90; test – -0.38±2.20; mean±SD) line angles—Table 7. No differences were seen radiographically as well (control – -1.49±0.95; test – -0.99±0.57; mean±SD) or MB (control – -0.58±1.06; test – -0.41±0.85± ±1.39; mean±SD) or DB (control –-0.70±0.92; test – -1.01±0.85; mean±SD)—Table 8. No significant effects of intervention, total cholesterol, vitamin D, mean buccal plate, or time between surgeries were detected when using the mean lingual vertical change as the response variable. After adjusting for the above variables the least square means were non-significant between treatment groups (control – 0.12±0.66; test - -1.14±0.70; mean±SD) —Table 9. Analysis of the radiographic vertical changes also failed to detect a difference due to intervention at the four line angles measured.

Discussion

In 1975 Pietrokovski[3], in an attempt to determine the direction of buccal lingual ridge loss after dental extraction, reported post-extraction ridge width losses of 8.37mm and 7.48mm for maxillary and mandibular first molars, 4.20mm and 4.03mm for maxillary and mandibular second premolars, and 5.37 and 4.85 for maxillary and mandibular first premolars. These measurements were obtained on soft tissue casts made of patients who had a single missing tooth and were compared to the intact contralateral site using graph paper. He determined that greater resorption occurred on the buccal side of the socket although the absolute amounts and differences vary widely.

Several variables that influence post-extraction ridge dimension changes have been identified since Jaime Pietrokovski's work that were unaccounted for in his report, although he was aware of the first: tooth location. Schropp et al.[4] performed forty-six minimally traumatic single tooth extractions on premolars and molars. The widest point of the ridge was measured on soft tissue models at baseline, three, six and twelve months. At three months there was an overall loss of 3.8mm with premolars losing 3.1mm and molar sites losing 4.5mm. These figures are lower than that of Pietrokovski most likely due to a controlled, minimally-traumatic method of extraction that was not controlled for previously. This variable difference in ridge dimension change due to location has also been reported in alveolar ridge preservation studies by the University of Louisville[49]. They report a mean loss of 1.4±1.1mm (n=99) in maxillary premolar sites and a loss of 0.4±1.0mm (n=24) in mandibular premolar sites.

In the present study seven of eight sites in the L-PRF group were maxillary premolars along with one mandibular molar. Five sites in the FDBA group were maxillary premolars along with one maxillary molar and three mandibular molars.

Even within a single tooth location, there appears to be a varying response post-extraction, whether preservation was employed or not. One of the potentially largest variables influencing the degree of resorption is the status and thickness of the buccal plate. The work by Schropp et al[4] may have checked for buccal plate integrity but there is no mention of this within the materials and methods. Sites that had a very thin or absent cortical plate may have skewed the data, something the mean would mask. This is evident by one quarter of the sites losing 5.2mm and three quarters of the sites losing 2.3mm. Most of this variation was seen in the molar sites where minimally traumatic extraction is more difficult. More resorption was seen in mandibular molars than maxillary molars but both sites had great variation.

Spinato et al[6] examined the effect of thin (<1mm) versus thick (≥1mm) buccal plates on resorption, with or without a socket graft of FDBA covered with a rapidly resorbing type I collagen dressing and no primary closure. Thirty one single rooted sites with intact buccal plates post-extraction were examined. At four months the authors noted that all the grafted sites resorbed less, regardless of buccal plate thickness. Sites with thin buccal plates resorbed more than sites with thick buccal plates in both the grafted and extraction alone sites. The most resorption, therefore, was seen in ungrafted sites with thin buccal plates (2.67±0.52mm, mean±SD) and the least in thick grafted sites (0.125±0.35mm, mean±SD).

In the present study there were more molar sites enrolled into the FDBA group, but the mean buccal plate thickness was slightly larger in the L-PRF group although this difference was not significant. There was, however, a wide range of buccal plate thicknesses encountered in

both groups with values ranging from 0.69mm to 2.98mm in the FBDA group and 0.60mm to 3.40mm in the L-PRF group. The high buccal plate measurements typically occurred due to the presence of buccal exostoses at 3mm apical to the facial osseous crest. The impact of buccal plate thickness was examined in the present study. No significant effect of buccal plate thickness could be detected utilizing ANOVA. This is most likely due to the low power and sample size of the study.

The surgical technique can greatly influence the amount of resorption encountered post-operatively. This was another variable that was not controlled for in the Pietrovoski work. Surgical technique can jeopardize the integrity of the buccal and or lingual cortical plates as well as disrupt vascular supply to the healing socket. Lekovic et al[10] reported a drastic reduction in ridge width post extraction when a polygycolide/polylactide barrier membrane was employed (1.31±0.24mm vs. 4.56±0.33mm; mean±SD). Full thickness mucoperiosteal flaps were elevated with four vertical incisions. Primary closure was achieved in all sites. To make repeat measurements, titanium pins were punctured through the buccal plate two to five millimeters below the facial crest. The flap technique combined with pins through the facial plate may have compromised the blood supply to the sites thus inducing a high amount of resorption in the extraction alone sites.

A similar study design was employed by Barone.[50] Full thickness flaps with vertical incisions were made on non-molar teeth. Sites received extractions alone or extraction and socket grafting with a porcine xenograft and a collagen membrane. Primary closure was achieved in all sites. The extraction alone group lost 4.5±0.8mm, and the grafted group lost 2.5±1.2mm. The concept of flap reflection and consequently primary or secondary closure of ridge preserved sites was investigated by the same author in 2014[14]. Sixty four premolar or molar sites with intact

adjacent teeth and intact buccal plates received a porcine xenograft covered with a collagen membrane. Thirty-two sites had no mucoperiosteal flap reflection and were left open to heal secondarily. The other thirty-two sites were flapped on the facial with vertical incisions and closed primarily. Sites that were closed primarily lost 3.5±0.9mm compared to 1.7±0.6mm lost in the group left open.

Several clinical studies have been performed on single rooted teeth with a variety of techniques. Iasella et al[11] studied twenty-four single rooted extraction sockets that received an intrasocket graft of FDBA and a bioresorbable collagen membrane or extraction alone. Full thickness flaps were reflected in all sites and presence of buccal plate was confirmed clinically. The authors report a horizontal loss of 2.6±2.3mm when no graft was performed compared to a loss of 1.2±0.9mm with ridge preservation. Aimetti et al.[12] recruited twenty-two single rooted teeth to receive calcium sulfate hemihydrate as an intrasocket graft compared to eighteen extractions alone. At three months the negative control sites lost 3.2±1.8mm compared to the grafted group losing 2.0±1.1mm.

Beck and Mealey[51] tested for histologic differences in non-molar ridges preserved with FDBA and type I collagen at four and six months post-operatively. Although the primary outcome of this study was histologic differences at two time points, the authors report horizontal losses of 1.47±1.81mm in the early group and 1.43±1.89mm in the delayed group. Seeking to determine histologic differences between FDBA and demineralized FDBA (DFDBA), Wood and Mealey[52] cite mean horizontal ridge losses of 2.09±1.71mm in the FDBA group and 2.18±1.62mm for the DFDBA group at eighteen to twenty weeks post-operatively.

Kotsakis et al.[13] studied thirty two extractions randomized to receive nothing, an intrasocket graft of bovine xenograft, or an intrasocket graft with a bone putty. No

mucoperiosteal flaps were reflected and teeth were extracted in a minimally traumatic fashion. Sites receiving nothing lost a mean of 2.53mm. Sites receiving bovine xenograft lost a mean of 1.39mm, and those receiving allograft bone putty lost a mean of 1.26mm. And finally, data presented from Master's Theses from the University of Louisville report an average loss of 1.41±1.1mm in premolar sites that were preserved. [49]

Due to the varied responses following extractions and ridge preservation procedures in the literature, the authors of the current pilot trial employed very narrow inclusion/exclusion criteria in an attempt to control for these noted variables. We also explored potential emerging systemic variables in an attempt to explain the variable post-operative responses. All sites recruited for the present study were premolars or molars. All sites were bordered on both sides by intact teeth with intact periodontal ligaments. Only one consecutive tooth was extracted. On the one patient that received two extractions, they were on the contralateral sides of the arch. Immediately following extraction, the buccal plate was checked for integrity not only clinically but radiographically as well. All extractions were performed in a minimally traumatic manner with absolutely no mucoperiosteal flap reflection and no attempt at primary closure. In addition, serum cholesterol and 25-hydroxyvitamin-D_3 levels were assessed in all subjects.

25-hydroxyvitamin-D_3 is a hepatic prehormone whose serum concentrations can be used to detect how much vitamin D is in the body with normal values between thirty and one hundered nanograms per milliliter. Preclinical animal data suggests that 25-hydroxyvitamin-D_3 plays an active role in bone healing and metabolism. Jinguishi et al.[19] showed that 1,25$(OH)_2$-Vitamin D_3, a biologically active metabolite of serum 25(OH)Vitamin D_3 that is produced in the kidneys, localizes from the plasma to the site of fractured femurs in rats as measured by administering H^3-1,25$(OH)_2$-Vitamin D_3 and measuring via autoradiography and high-

performance liquid chromatography. The administration of 25-OH Vitamin D is capable of improving the mechanical strength of femoral post-fracture calluses in elderly rats as measured by maximum torque of induced fractures at 5 weeks post-op, when the rats were anesthetized. Serum 25-OH Vitamin D levels were measured in both a control and test group preoperatively and at death. The test group was administered 250IU/100g weight of 25-OH Vitamin D subcutaneously at time of fracture and 150IU/100g weight at day 15 and day 30 to bring their average levels to 16.7±5.3ng/mL at death. The test group showed statistically significant greater maximum torque values as measured by a Student's t test with an accepted level of significance set at $p<0.05$ with 95% CI. The authors state that the differences were so great between groups, statistical significance was achieved with a relatively small number of animals. There was a positive correlation between values of the maximum torque of the healing femora and blood levels of 25-OH Vitamin D at death ($p<0.01$; $r=0.55$ via Pearsons correlation for linear regression 95% reliability).[18]

Kelly et al.[16] used a rat implant osseointegration model to show that 25-hydroxyvitamin-D_3 insufficiency negatively impacts peri-implant bone formation, osseointegration, implant stability, and bone to implant contact. The authors placed 28 implants with two different surfaces in 10 rats that were divided into control rats and test rats. The test rats went 4 weeks with a complete lack of sunlight and a Vitamin-D absent diet. Serum levels of 1,25-hydroxyvitamin-D_3 and 25-hydroxyvitamin-D_3 were measured at sacrifice which was fourteen days after implant placement. 1,25-hydroxyvitamin-D_3 levels were not significantly different between groups, however, 25-hydroxyvitamin-D_3 were (control = 19.1±9.7ng/mL; test = 1.9±0.6ng/mL; mean±SD). Implant osseointegration was measured via a push in test in which an appliance applied a unidirectional force up to 2000N onto the implant head that is embedded in the en-

block sectioned femur that was embedded in acrylic. A raw value was determined by measuring the peak of the load-displacement curve. This was followed by scanning electron microscopic (SEM) observation and bone to implant contact (BIC) measured at the cortical region of the implant. The push-in values were as follows: Control Acid Etched - 24.99±7.92N, Test-Acid Etched – 15.94±8.20N; Control HA+AE-37.48±17.58N, Test HA+AE-15.63±3.96. The bone to implant contact values were as follows: Control AE-69.06±11.79%, Test-AE-45.89±13.49%; Control HA+AE-70.31±6.95%, Test HA+AE-38.13±5.25. The differences between test and control implants were statistically significant for bone to implant contact and push-in values. The authors note that under SEM, there were no differences in marrow space but the Vitamin-D insufficient group showed an unusual soft tissue interface at the implant surface in the cortical area that was not seen in the control group. It has also been shown that repleting vitamin-D levels following induced deficiency improves peri-implant bone formation in rats.

Dvorak et al.[17] induced vitamin-D deficiency in a group of rats and compared BIC values and fluorescence labeling to a group of controls and a depletion-repletion group. They showed that vitamin-D depletion is associated with significant decreases in bone to implant contact in the cortical area (59.68%) compared to controls (76.30%) but not compared to their depletion-repletion group (64.88%). There were no significant differences detected for fluorescence labeling among groups, although vitamin-D receptors were localized to the PDL, cementoblasts, gingiva, and bone-associated cells.

Orthopedic epidemiological data support an association between serum 25-hydroxyvitamin-D_3 levels and the need for particular orthopedic procedures. Stoker et al[21] found that eighty-four percent of patients undergoing spinal fusion surgery from 2010 to 2013 had some vitamin-D abnormality. Fifty-seven percent of subjects were defined as having 25-

hydroxyvitamin-D_3 inadequacy (<30ng/mL) and twenty-seven percent having deficiency (<30ng/mL). The authors concluded that they revealed a substantially high prevalence of vitamin D abnormality in adults undergoing spinal fusion, and although age is a well-established risk factor for hypovitaminosis, young adults undergoing spinal fusion should not be overlooked with regard to vitamin D screening.

Another report from the orthopedic literature supports the same conclusion. Brinker et al[22] report that of six hundred eighty three patients treated for non-unions of fractured long bones from 1998 to 2005, thirty-seven with unexplainable etiology were referred to endocrinologists for evaluation. Of the thirty seven patients referred, thirty one had undiagnosed endocrine or metabolic abnormalities. The most common diagnosis was vitamin-D deficiency. In addition, non-unions spontaneously healed by vitamin-D supplementation in eight of the twenty five patients diagnosed with vitamin-D deficiency.

NHANES data gathered from 1988 to 1994 compared to data gathered from 2001-2004 support that 25-hydroxyvitamin-D_3 insufficiency and deficiency are prevalent in the United States. The mean serum 25-hydroxyvitamin-D_3 level was thirty nanograms per milliliter during NHANES III and decreased to twenty-four nanograms per milliliter during NHANES 2001-2004. The prevalence of 25-hydroxyvitamin-D_3 levels of less than ten nanograms per milliliter (frank deficiency) increased from two percent to six percent. The prevalence of levels thirty nanograms per milliliter or more (normal) decreased from fourty-five percent to twenty-three percent. The current prevalence of Vitamin D abnormalities in the United States population is estimated at seventy-seven percent (74-80%, 95%CI).[20]

In the present study, serum levels of 25-hydroxyvitamin-D_3 were non-significant between groups at baseline and seemed to have a limited effect on the post-extraction ridge dimension

changes. This may have been due a low sample size. The mean serum level of 25-hydroxyvitamin-D_3 in the present study was 31.12±10.58ng/mL with a range of 15.7-50.2 ng/mL. Vitamin-D abnormality was found in ten of the sixteen subjects with a prevalence of sixty-five percent. These levels were non-significant at baseline between treatment groups.

Bone marrow stromal cell populations contain mesenchymal stem cells capable of differentiating into osteoblastic or adipocytic lineages. Cholesterol can determine the pathway. In addition, diet that increases cholesterol can alter marrow lipid composition and determine marrow cell phenotypes. Parhami et al.[25] investigated the ability of minimally oxidized low density lipoprotein (MM-LDL) to inhibit osteoblastic activity as well as the ability of lipid to change marrow stromal cell phenotype. MM-LDL inhibits osteoblastic differentiation of the murine marrow stromal cell line. This inhibition is mediated through a MAP kinase signaling pathway. MM-LDL enhances adipogenic differentiation of marrow stromal cells in the presence of free fatty acids, thiazolidinediones, and other peroxisome proliferator-activated receptor-gamma activators. The peroxisome proliferator-activated receptor-gamma is the "master controller" of adipocytic differentiation and triggers an intracellular cascade leading to fat droplet accumulation in the cytoplasm. Mice that were fed a high fat, atherogenic diet displayed increased proliferation and inhibited osteogenic potential of their marrow stromal cells in vitro compared to controls as determined by reductions in alkaline phosphatase, collgen I expression, and mineralization assays. The authors suspect that changes in dietary fat may alter marrow lipid composition and subsequently influence marrow stromal cell phenotypes. They conclude that inhibition of osteogenesis and promotion of adipogenesis by lipids and oxidized lipids may contribute to the paucity of mature osteoblasts and excess adipocytes in osteoporotic bone. LDL has also been shown to be cytotoxic to osteoblasts in vitro.[23]

The clinical effect of hyperlipidemia has been demonstrated in rats.[24] Fifty rats were divided into two groups: normal (ND) and high fat diet (HFD) (19.77% vs. 59.28% fat). Rats were sacrificed at twelve weeks. Two and three-dimensional images were taken of the mandibles and compared between groups. The high-fat diet group demonstrated a decline in bone formation and an increase in mandibular porosity in both 2D and 3D micro-CT images compared to rats on a normal diet. The 3D micro-CT analysis revealed a significantly greater ratio of bone volume to total volume and trabecular thickness and significantly less percent porosity in the ND group, whose plasma cholesterol was (90.80 ± 4.33mg/dl; n=25) compared to the HFD group (136±7.36mg/dl). For note, the normal diet rats weighed 1.01lbs and the HFD rats 1.20lbs. LDL and total cholesterol can be used as markers of a high fat diet that has been demonstrated to cause not only clinically detectable bone abnormalities but also impaired osteoblastic function in vitro.

Medications that are presently used in humans to lower serum cholesterol levels have been shown to inhibit bone loss and RANKL expression as well as increase bone-morphogenetic protein-2 in rates with experimental ligature-induced periodontitis.[53] Although less significant, this effect has also been demonstrated in humans.[54] Atorvastatin, an inhibitor of hepatic cholesterol synthesis, was administered to hyperlipidemic patients at twenty milligrams per day for three months. This was compared to a matched placebo group. The test group demonstrated lowered LDL and total cholesterol levels, increased alveolar bone height, and decreased CEJ to alveolar bone distance compared with measurements from the negative control group. Bone mineral density (BMD) of the calcaneus bone (heel bone) in the test group increased from 21.5±7.7mm^2 to 26.3±6.9mm^2. Although not statistically significant, the study was not designed

to test for differences in this parameter and was most likely underpowered to detect a difference. There was no increase in BMD for the control group.

Similar to the findings with 25-hydroxyvitamin-D_3, serum LDL and total cholesterol levels had minimal significance on the outcomes measured in the present study. This again was most likely due to the low sample size and power. Pairwise correlations revealed a high correlation between LDL and total cholesterol levels, therefore, only total cholesterol was used in the statistical analysis. The mean prevalence of hyperlipidemia in the present study was thirty-eight percent.

Leukocyte-Platelet Rich Fibrin (L-PRF) is a three-dimensional heterogenous fibrin "clot" that contains >97% of platelets and >50% of leukocytes present in a nine milliliter whole venous blood draw.[26] It is obtained by drawing whole venous blood into a glass or plastic vacutainer containing no anticoagulants or biomodifiers. The nine milliliter blood sample is immediately centrifuged table-top for twelve minutes at twenty-seven hundred revolutions per minute. This produces three layers within the vacutainer: a red corpuscle base at the bottom, the L-PRF layer in the middle, and platelet poor plasma at the top. The natural fibrin polymerization method combined with the centrifugal force enables entrapment of growth factors from platelets and cytokines from leukocytes into the fibrin matrix.[28] L-PRF has been shown to release the growth factors TGFβ-1, PDGF-αβ, VEG-F, and thrombospondin-1, an important coagulation glycoprotein of the cellular matrix, for 7 days after its preparation.[29] L-PRF provides a suitable substrate for cell growth and division. It has been shown to stimulate cell growth and replication of human osteoblast-derived-osteoscarcoma cells, human kertinocyte-derived carcinoma cells, and human fibroblast lung carcinoma cells, and to upregulate osteocalcin and ostepoontin (bone sialoprotein-1) in human osteoblast-derived osteosarcoma cells in vitro. [27]

One of the first clinical uses of L-PRF studied in the literature was its combination with FDBA in maxillary sinus augmentations.[31] Six cases of maxillary sinus augmentation with L-PRF and FDBA were performed and compared to three sinus augmentations with FDBA alone. The sites with L-PRF and FDBA were re-entered at four months to place implants and harvest bone core biopsies. These were compared to cores taken at eight months in the FDBA group. The authors found no differences between treatment groups. Both groups showed a mean of twenty to twenty one percent vital bone and between nine and eleven percent non-vital bone.

Three years later a similar study was completed using only L-PRF as the sole graft material in maxillary sinus augmentations. Mazor et al[32] completed twenty five lateral window sinus elevations on twenty patients. Radiographic analysis was made on CBCT images taken immediately preoperatively and six months post-operatively to determine changes in bone height. Nine bone cores were retrieved six months postoperatively for histologic analysis. Forty one implants were placed uneventfully. All implants survived and were clinically stable during abutment tightening at twenty-five newton centimeters. All biopsies showed well organized and vital bone (33±5%, mean±SD). Because only L-PRF was used, all biopsies showed one hundred percent vital bone. Bone height gained was between seven and thirteen millimeters (10.1±0.9mm; mean±SD).

The effect of L-PRF was studied in third molar extraction sites. Gurbuzer et al.[30] performed a split mouth prospective trial comparing osteoblastic activity in vertical soft tissue impacted third molar extraction sites receiving either L-PRF or nothing. Primary closure was achieved in all sites. Four weeks post-operatively all patients received intravenous injection of 555MBq technetium-99m methylene diphosphonate and static oblique images were taken of the left and right extraction sockets. No differences were detected scintigraphically. The average

uptake of technetium-99m was 4.54±1.03 for the L-PRF group and 4.61±1.02 in the negative control group. The authors conclude that L-PRF does not seem to increase bone healing within extraction sockets of soft tissue impacted mandibular third molars 4 weeks after surgery.

The effect of L-PRF on guided tissue regeneration (GTR) has been documented by at least five recent controlled prospective clinical trials, three of which examine intrabony defects[35, 36, 37] and two examining mandibular class II furcation defects.[33, 34] The first study compared open flap debridement (OFD) to OFD plus a graft consisting of L-PRF covered with an L-PRF membrane.[36] Sites enrolled were first or second mandibular molars with an intrabony defect depth of at least three millimeters and a probing depth of at least five millimeters. Differences between treatment groups were compared radiographically and clinically at nine months. For all the parameters, the test group performed better than the negative control group. These differences were found to be statistically significant. The mean bone fill was greater in the L-PRF group (46.92% vs. 28.66%). Mean probing depth reduction was greater (4.56±0.37mm vs 3.56±0.27mm; mean±SD), and clinical attachment loss gain was greater (3.69±0.44mm vs. 2.13±0.43mm; mean±SD) in the L-PRF group. The authors conclude that within the limitations of their study design, there was a greater reduction in probing depth, more clinical attachment gain, and greater intra-bony defect fill at sites treated with L-PRF than for OFD alone.

Sharma et al[35] studied three walled intrabony defects measuring at least three millimeters deep radiographically and probing at least five millimeters clinically. Seventeen patients supplying twenty eight sites were randomized to the control, open flap debridement (OFD) group. Eighteen patients supplying twenty-eight study sites received OFD plus the placement of L-PRF as described above. At nine months, differences between the treatment groups were compared. Statistically significant differences in probing depth reduction, gingival margin level,

and defect fill were detected, with the L-PRF group outperforming the OFD alone group. The greatest of these differences was for percent bone fill. The L-PRF group showed a 48.26±5.72% bone fill at nine months compared to 1.80±1.56% in the OFD alone group. The authors conclude that there was greater probing depth reduction, attachment level gain, and bone fill at sites treated with L-PRF compared to those treated with conventional open flap debridement.

The same group later completed a similar study comparing L-PRF to platelet rich plasma (PRP) in intrabony defects.[37] The authors observed similar probing depth reduction, clinical attachment level gain, and bone fill at sites treated with L-PRF or PRP. These results were statistically significantly better than the negative control group of open flap debridement alone. The authors conclude that because L-PRF is less time consuming, less technique sensitive, and less expensive, it may seem to be a better treatment option than PRP.

The effect of L-PRF has been studied in the treatment of mandibular class II furcation defects in two trials. Sharma and Pradeep[33] used a split mouth design to enroll thirty six mandibular class II furcation defects. Sites were randomly assigned to test or control groups. The authors found that all clinical and radiographic parameters were statistically significantly improved in sites treated with PRF and open flap debridment (OFD) compared to OFD alone. Bajaj et al[34] confirmed these findings in a subsequent trial. Seventy-two mandibular class II furcation defects were enrolled to be treated with open flap debridement (OFD), PRP with OFD, or L-PRF with OFD. Sites were examined clinically and radiographically. At nine months, all clinical and radiographic parameters showed statistically significant improvement over OFD alone.

L-PRF itself has minimal space maintaining properties although it has been shown to have drastic biological effects in vitro.[26-29] Therefore we designed the present study to utilize

intact posterior extraction sites as a clinical model for a four-walled defect to test a space-maintenance method against a biological method for preventing ridge collapse. It was important in our pilot study to minimize noted variables that influence post-extraction resorption, namely, surgical technique, presence of an intact buccal plate, and intact adjacent periodontal ligaments. At no point during our statistical analysis did we find any significant differences due to treatment. The clinical and radiographic means of horizontal ridge changes are well within the expected ranges as reported in the literature for ridge preservation procedures. The vertical mean changes, although less focused on in the current study, were also well within reported means.

The main limitation of the current pilot study is low sample size. Poulias et al[49] performed an *a priori* power analysis based on ridge width changes in non-molars and calculated they needed a sample size of eleven per group to give an eighty percent statistical power to detect a difference of 1mm between treatment groups. Even with adequate power, they observed a wide range of results. The reported change over four months was 1.6±0.8mm ranging from a loss of 3.4mm to 0.5mm. The same situation was seen in the current study. There appeared to be similar means between groups but a wide variation of responses that appeared to be independent of any of the predictor variables we measured. Similar standard deviations are seen as the sample size is increased. Barone et al[14] enrolled sixty four premolar and molar sites randomized to two groups: flapless with secondary closure or flapped with primary closure following extractions. Both groups received an intrasocket graft of porcine xenograft and a collagen barrier. Flapless sites resorbed 1.7±0.6mm and the flapped sites resorbed 3.5±0.9mm, again demonstrating the wide variability of responses.

Measurements in the current study were made clinically with a caliper and radiographically on sagittal slices of a limited field of view CBCT image. Although the same

conclusion can be made by using the clinical or radiographic data alone, the actual measurements were not well correlated with the exception of buccal plate thickness. One potential source of error may be the use of gutta percha as radiographic markers. According to Lund et al[41] the use of gutta percha is controversial due to low opacity and dimensional instability. The authors of the present study agree with this statement.

Several authors have examined the correlation between physical measurements and measurements made on CBCT images. Loubele et al[40] compared gold standard physical measurements of an ex vivo human maxilla to measurements made on both CBCT and multislice computed tomographic images. The accuracy of the linear measurements made on CBCT was -0/09±1.64mm. Tomasi et al[42] discuss the ability to get different linear measurements on CBCT images simply by changing the angulation of the object being scanned. They made linear measurements on a dry human skull and then imaged this skull parallel to the horizontal plane and at forty five degrees. The accuracy of the CBCT measurements was evaluated with comparisons of standard deviations and estimations of intraclass correlation coefficients. They report an intraclass correlation coefficient between CBCT measurements and physical measurements with a caliper of 0.992mm. The absolute mean measurement error for CBCT was 0.40±0.39 (mean ± SD). The percent of error exceeding one millimeter was 6.7 percent.

Hashem et al[44] compared measurements made on CBCT images taken at 360 degrees and 180 degrees to direct measurements on twelve sectioned porcine hemimandibles. They found good to excellent intra- and interobserver agreement. A mixed regression analysis could not detect a statistical significant difference between either scan acquisition protocol or the direct measurements.

Some authors report that variation involved with linear measurements on CBCT images may be due to voxel size.[45]To examine this effect, Torres et al[43] evaluated four image acquisition protocols that varied the voxel size and scan time. They compared measurement error between each protocol and direct measurements. They express their results as a percent difference of direct measurements. They found that typical voxel sizes underestimate the true distance. They found that in horizontal measurements there was a 16.22-17.22% difference and a 7.19-8.20% difference in vertical measurements. This equates to around 0.68-0.72mm less than the real distance. Although the authors hypothesized that changing the voxel size and scan time would influence linear measurements made on the scans, they did not find any difference between protocols.

Shokri et al [45] compared measurements made on CBCT images with slices of 0.5-, 1-, 2-, 3-, 5-, and 10-mm slice thicknesses with physical measurements made with digital calipers on eleven dry human mandibles. They found no significant differences in bone width in any area among any of the slice thicknesses. The only measurement they found that was not statistically significantly different from the actual direct measurement was width using 4mm slices and height using 5mm slices. They felt that slice thicknesses of less than 4mm may underestimate the actual measurement. In the present study, most CBCT image measurements were made with slice thicknesses around 0.5mm. This may explain the lack of correlation between direct measurements and radiographic measurements, as CBCT measurements tended to be less than clinical.

The ultimate goal of ridge grafting at time of extraction in the posterior mandible or maxilla is to prevent ridge collapse enough to adequately place and house a dental implant, ideally, without additional grafting at time of implant placement. Extraction sockets in the

posterior maxilla and mandible are not typically in an esthetic area of the mouth and thus more resorption may be tolerated. This is also true due to the fact that many posterior ridges can tolerate even a fifty percent reduction in ridge width and still be adequately wide to receive a dental implant. In the present study all implants were placed successfully with at least thirty newton centimeters of torque. Additional bone graft was added to one implant in each group at time of implant placement. All implants were successfully restored and are still in function at the time of publication.

Conclusions

It can be concluded that within the limitations of the current pilot study, leukocyte-platelet rich fibrin may function similarly to mineralized freeze dried human cortical bone allograft and a cross-linked type I porcine collagen membrane in intact posterior extraction sites when a minimally traumatic approach is employed. However, due to the low sample size in the current study, no definitive conclusions can be drawn. More research is needed with a larger enrollment and a negative control group.

VITA

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Table 1. Baseline Study Demographics

Demographic	Control FDBA + RCM	Test L-PRF	Overall
Sites (n)	9	8	17
Patients	8	8	16
Age (years) Mean ± SD Range	 45.50±64.71 27-74	 62.75±13.13 44-84	 54.13±16.96 27-84
Sex (n;males/females)	6 / 2	2 / 6	8 / 8
Tooth type (n;maxillary molar / maxillary premolar / mandibular molar / mandibular premolar)	1/5/3/0	0/7/1/0	1/12/4/0
Mean buccal plate thickness (mm) Clinical (mean± SD; range) Radiographic (mean± SD; range)	 1.21±0.75;0.69-2.98 1.06±0.97;0.50-3.50 1.36±0.61;0.63-2.47	 1.49±0.88;0.60-3.40 1.40±0.79;0.50-3.00 1.59±1.01;0.70-2.70	 1.32±0.78 1.27±0.91 1.45±0.78
Platelet count (10^9/L) Mean ± SD Range Ref. Range (179-373)	 255.13±65.07 172-374	 262.63±82.52 192-435	 258.88±71.90 172-435
Total Cholesterol (mg/dL) Mean ± SD Range Ref. Range (100-200)	 205.13±62.94 142-304	 190.25±42.49 116-248	 199.06±52.44 116-304
LDL (mg/dL) Mean ± SD Range Ref. Range (0-130)	 142.38±57.88 85-232	 111±27.20 64-144	 126.69±46.60 64-232
25-Hydroxyvitamin-D_3 (ng/mL) Mean ± SD Range	 28.43±9.75 15.7-40.9	 33.81±15.47 21.2-50.2	 31.12±10.58 15.7-50.2
Weeks between surgeries Mean ± SD Range	 14.78±1.09 13-16	 18.13±6.04 15-32.5	 16.35±4.42 13-32.5

Table 2. Pairwise correlations of Clinical Horizontal Measurements

Variable	by Variable	Correlation	Count	Significant Probability
CCH	CMH	0.87	17	<0.0001*
CDH	CMH	0.75	17	0.0005*
CDH	CCH	0.82	17	<0.0001*

*All values significantly correlated
(Clinical mesial horizontal, CMH; Clinical center horizontal, CCH; Clinical distal horizontal, CDH)

Table 3. Clinical Horizontal Change

	Mean Horizontal Change (mean ± SD)	CMH	CCH	CDH
FDBA + RCM n=9	1.88±1.51	1.50±1.58	2.25±1.78	1.89±1.80
L-PRF n=8	2.02±2.17	1.75±2.15	2.06±2.41	2.25±2.07

Table 4. Radiographic Horizontal Change

	Mean Horizontal Change (mean ± SD)	CMH	CCH	CDH
FDBA + RCM n=9	1.40±0.72	1.32±0.72	1.68±1.04	1.21±0.76
L-PRF n=8	0.87±0.64	1.01±0.92	1.00±0.91	0.60±0.40

Table 5. Analysis of Variance- Horizontal Ridge Width

Source	p
Mean buccal plate thickness	0.287
Treatment	0.994
Total Cholesterol	0.336
25-Hydroxyvitamin-D_3	0.271
Time Between Surgeries	0.165

Level	Least Square Mean	Standard Error	Mean
FBDA + RCM	1.950	0.662	1.880
L-PRF	1.941	0.711	2.020

Table 6. Pairwise Correlations of Clinical Vertical Measurements

Variable	by Variable	Correlation	Count	Significance Probability
CRVDB	CRVMB	0.26	17	0.03197
CRVML	CRVMB	0.24	17	0.3576
CRVDL	CRVDB	0.16	17	0.5402
CRVDL	CRVMB	0.41	17	0.1049
CRVDL	CRVDB	0.49	17	0.0450*
CRVDL	CRVML	0.80	17	0.0001*

*Significantly correlated
(Clinical relative vertical mesiobuccal, CRVMB, Clinical relative vertical distobuccal, CRVDB; Clinical relative vertical mesiolingual, CRVML; Clinical relative vertical distolingual, CRVDL)

Table 7. Clinical Vertical Change

	Mean Lingual Vertical Change (mean ± SD)	Mean Mesiobuccal Vertical Change	Mean Distobuccal Vertical Change
FDBA + RCM n=9	-0.11±2.11	-0.44±2.11	-0.33±0.90
L-PRF n=8	-0.88±1.36	-0.31±1.39	-0.38±2.20

Table 8. Radiographic Vertical Change

	Mean Lingual Vertical Change (mean ± SD)	Mean Mesiobuccal Vertical Change (mean ± SD)	Mean Distobuccal Vertical Change (mean ± SD)
FDBA + RCM n=9	-1.49±0.95	-0.58±1.06	-0.70±0.92
L-PRF n=8	-0.99±0.57	-0.41±0.85	-1.01±0.85

Table 9. Analysis of Variance- Clinical Lingual Mean Vertical

Source	Estimate	Standard Error	t Ratio	Prob > |t|
Total Cholesterol	0.14467	0.008851	1.63	0.1304
Treatment	0.629981	0.533606	1.18	0.2627
Mean Buccal Plate Thickness	0.618596	0.565694	1.09	0.2975
Time Between Surgeries	0.108618	0.112576	0.96	0.3554
25-Hydroxyvitamin-D_3	0.048706	0.051278	0.95	0.3626

Level	Least Square Means	Standard Error	Mean
FDBA + RCM	0.1223	0.6567	-0.1111
L-PRF	-1.1376	0.7059	-0.8750

Table 10. Outcome Variables by Group

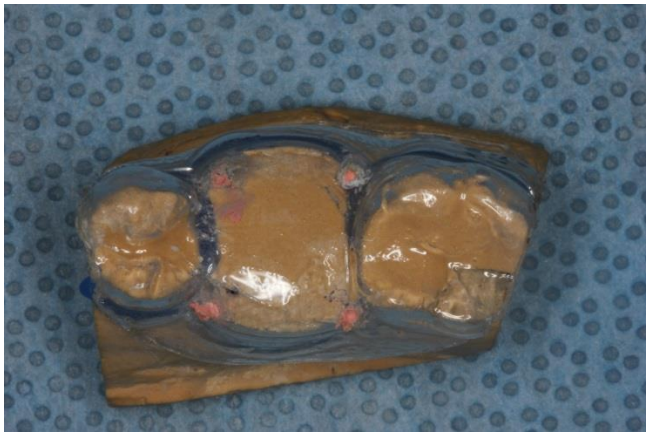
	FDBA + RCM n=9			L-PRF n=8		
Variables	**T_0**	**T_1**	**Δ**	**T_0**	**T_1**	**Δ**
Clinical Ridge Width	10.71±1.68	8.83±1.64	1.88±1.51	9.94±2.16	7.92±1.58	2.02±2.17
Radiographic Ridge Width	11.68±2.31	10.27±2.64	1.40±0.72	10.53±2.20	9.66±2.43	0.87±0.64
Relative Clinical Buccal Ridge Height	6.22±1.92	6.61±1.24	-0.39±1.43	7.53±3.12	7.88±3.07	-0.34±1.30
Relative Radiographic Buccal Ridge Height	5.12±1.45	5.68±1.58	-0.64±0.78	4.94±1.02	5.65±1.23	-0.72±0.57
Relative Clinical Lingual Ridge Height	6.75±2.64	6.86±1.35	-0.11±2.11	7.13±2.76	8.00±2.60	-0.88±1.36
Relative Radiographic Lingual Ridge Height	4.66±1.57	6.14±1.42	-1.49±0.95	4.94±1.41	5.93±1.30	-0.99±0.57

Table 11. Overall Mean Changes by Group

	Horizontal	Vertical
FDBA + RCM n=9	1.64±0.91	-0.657±0.832
L-PRF n=8	1.43±1.25	-0.731±0.458

Figure 1. Measurement Stent

a. Radiographic/Clinical measurement stent – Occlusal view; b. Radiographic/Clinical measurement stent – Buccal view



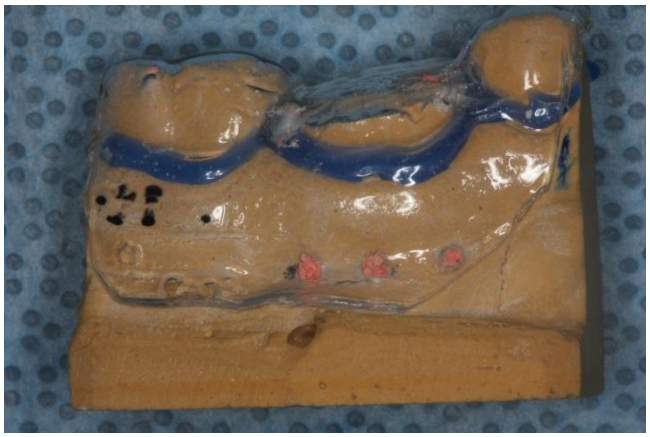
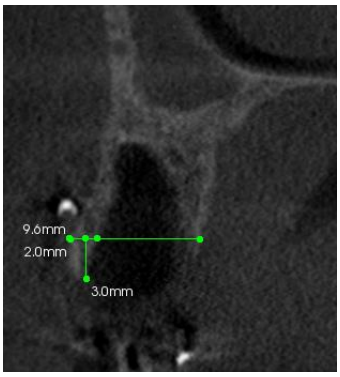


Figure 2. Radiographic Horizontal Measurements

a. Test Group – immediately post-extraction; b. Test Group – at implant placement; c. Control Group – immediately post-extraction; d. Control Group – at implant placement.

a.



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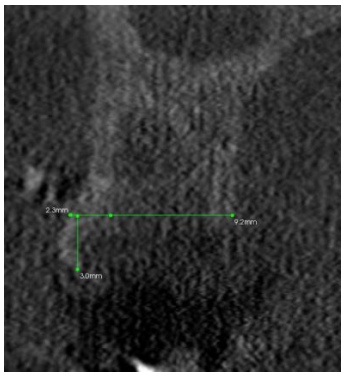

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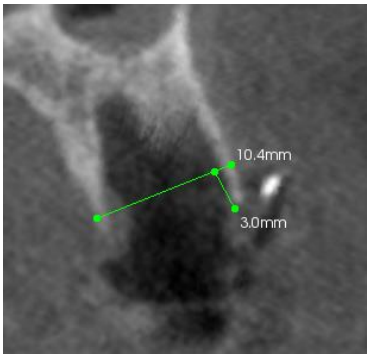

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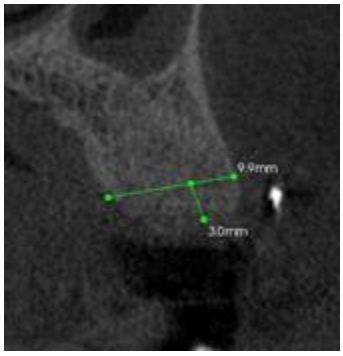

Figure 3. Radiographic Vertical Measurements

a. Test Group –immediately post-extraction; b. Test Group – at implant placement; c. Control Group –immediately post-extraction; d. Control Group –at implant placement.

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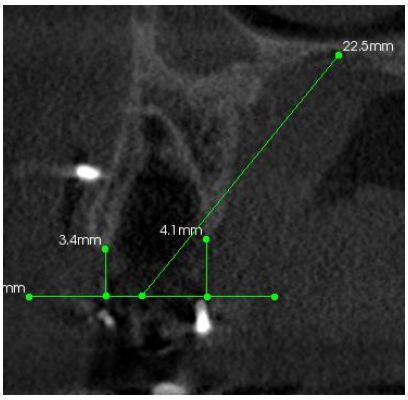

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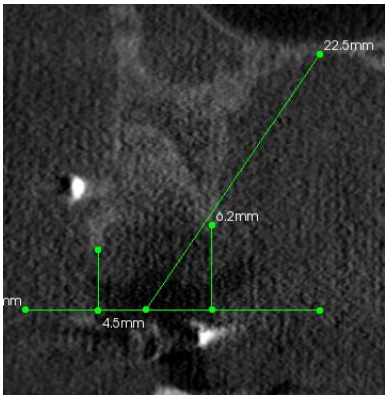

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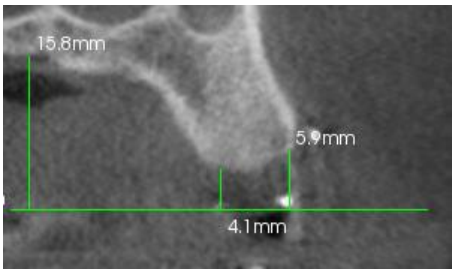

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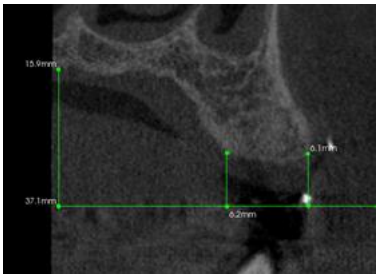

Figure 4. L-PRF Surgical Group

a. Extraction – Occlusal view; b. Completed graft – Occlusal view; c. Implant placement – Occlusal view; d. Implant placement – buccal view; e. Implant placement – flap reflected; f. Implant placement – implant in place; g. Table-top centrifuge; h. Whole venous blood draw; i. L-PRF armamentarium; j. Blood immediately after twelve minute spin; k. L-PRF layer being removed from vacutainer; l. Removal of red blood cell layer; m. PRF Box – fabrication of L-PRF plugs; n. L-PRF plug being transferred to socket

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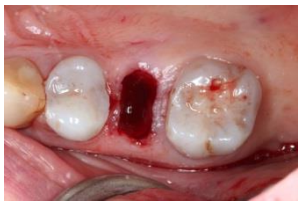

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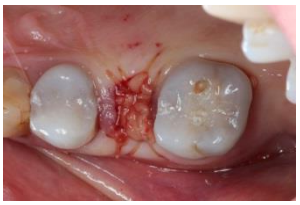

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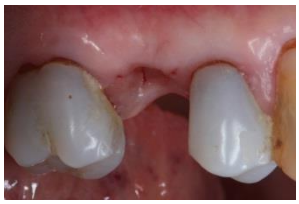

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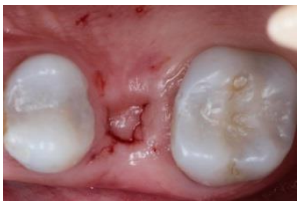

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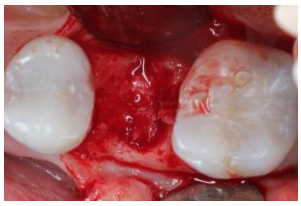

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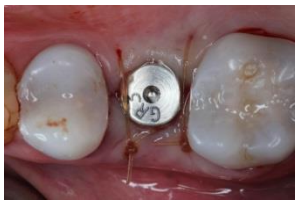

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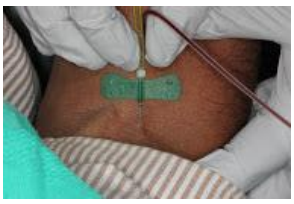

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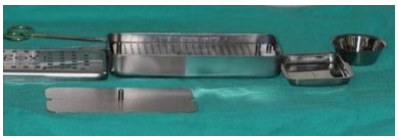

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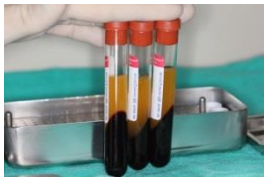

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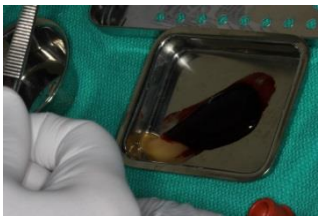

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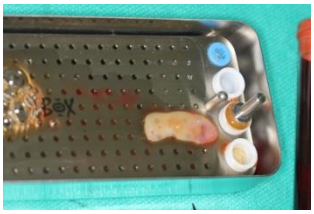

n.


Figure 5. FDBA + Resorbable Collagen Membrane Group

a. Extraction – Occlusal view; b. Extraction – Buccal view; c. Completed graft – Occlusal view; d. Completed graft – Buccal view; e. Two Week Postoperative – Occlusal view; f. Implant placement – Occlusal view; g. Implant placement – Occlusal view; h. Freeze-dried bone allograft; i. Resorbable collagen membrane

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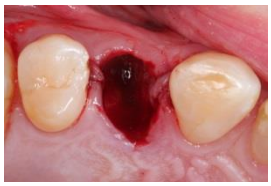

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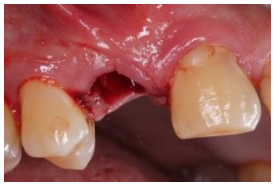

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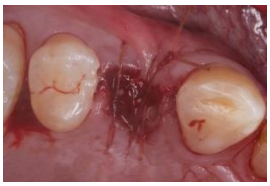

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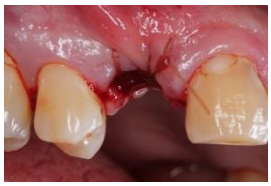

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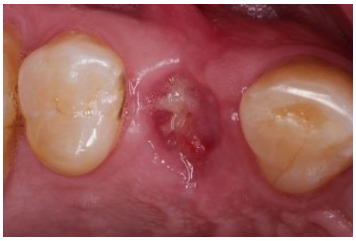

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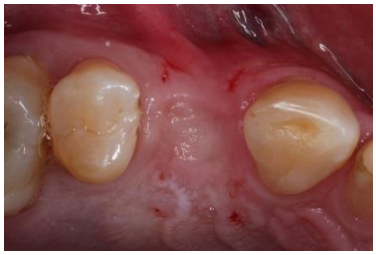

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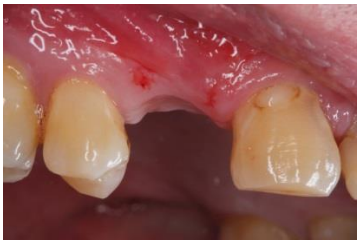

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