Title
Osteogenic potential of Platelet Rich Fibrin for ridge preservation: A comparative micro-CT evaluation

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Osteogenic Potential of Platelet Rich Fibrin for Ridge Preservation: A Comparative Micro-CT Evaluation

by

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THESIS

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Osteogenic potential of Platelet Rich Fibrin for ridge preservation: A comparative micro-CT evaluation

Abstract

Objective: Platelet-Rich Fibrin (PRF) is an autogenous blood product with clinical applications in dento-alveolar surgery. However, there is minimal information regarding its clinical efficacy. This randomized controlled clinical trial aims to evaluate the efficacy of PRF alone or with freeze-dried bone allograft (FDBA) as compared to FDBA alone or no graft (blood clot) in improving *de novo* bone formation during ridge preservation as determined by Micro X-ray computed tomography (Micro-CT) analysis.

Methods: Forty patients requiring extraction of non-molar teeth and replacement with dental implants were enrolled and randomized into one of four ridge preservation approaches: 1:PRF, 2:PRF+FDBA, 3:FDBA, or 4:blood clot. Non-traumatic extractions were performed without the elevation of a mucoperiosteal flap and the ridge preservation procedure was performed. After three months healing, bone core samples were harvested at the time of implant placement for micro-CT analysis.

Results: Analysis was performed for thirty three subjects that completed the study to date. The attrition rate was 12%. Implant success rate was 97%. All treatment groups allowed for the successful placement of implants after three months of healing. All treatment groups demonstrated evidence of new bone formation as measured by micro-CT analysis. Trabecular formation was noted in the blood clot, PRF, and PRF+FDBA groups, while minimal trabecular structure could be noted in the FDBA group. NO significant differences were noted between groups for bone volume fraction or bone mineral density. There was a trend for highest bone volume fraction with the PRF group and highest bone mineral density for the PRF+FDBA group.

Conclusion: This study demonstrates the use of PRF with or without FDBA for ridge preservation. A trend for increased bone formation at extraction sockets supports its application where *de novo* bone formation is desired. This study represents the first randomized controlled clinical trial evaluating the efficacy of PRF for ridge preservation by histological, micro-CT, and clinical measures.

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Introduction

The use of dental implants has become a common and popular treatment modality for the rehabilitation of the dentition. Since its first introduction, dental implantology has continued to evolve to meet the functional and esthetic demands of patients. One challenge consistently faced by clinicians is inadequate alveolar bone volume to support the placement of a dental implant of appropriate length and width in the ideal position. To overcome this challenge many surgical techniques and materials have been utilized to preserve or regenerate tissues to provide an optimal environment for successful implant placement. For this paper, the preservation and regeneration of bone with biomaterials will be the focus as the main tissue in supporting dental implants. Biomaterials used for this purpose generally consists of matrix scaffolding materials and biologic agents applied locally to the surgical site. Matrix scaffolding materials are typically osteoconductive and able to provide cell scaffolding and dimensional stability of the wound through space maintenance. These materials can be allogeneic, xenogeneic, or autogenic in nature. Biologic agents are molecular mediators with typical osteoinductive properties. Matrix scaffolding materials and biologic agents can be used separately or together to achieve the desired surgical outcome. Of the available biomaterials available, platelet-rich fibrin (PRF) has been increasingly popular since its first introduction in 2000 [1]. PRF is a platelet concentrate made of an autologous bioscaffold of mature fibrin matrix with naturally integrated growth factors capable of sustained release to promote healing of hard and soft tissues [2,3,4] . In the processing of PRF, the fibrin clot is allowed to polymerize, creating a natural scaffold with bound platelets and leukocytes [1]. PRF has been shown to be a source of transforming growth factor β-1 (TGFβ-1), vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF) and a coagulation matrix glycoprotein, thrombospondin-1 (TSP-1) [3,4,5]. The vast

majority of the growth factors have been shown to be bound within the fibrin matrix, resulting in a slow, sustained release through the natural maturation and reorganization of the clot [3,4,6]. The potential clinic applications of PRF are numerous, but to date the clinical performance has been evaluated only sparsely with heterogeneous design and inconstant results.

This randomized controlled clinical trial aims to evaluate the efficacy of PRF alone or with freeze-dried bone allograft (FDBA) as compared to FDBA alone or no graft (blood clot) in improving *de novo* bone formation during ridge preservation. Bone core biopsies were taken from healed extraction sockets and evaluated using micro-CT analysis to determine bone quantity and quality at the site developed to support a dental implant.

Background

Alveolar Ridge Preservation

There is a clinical need to maintain sufficient alveolar dimensions and encourage ample bone growth after tooth extraction to support dental implants in the ideal restorative position. However, normal healing of the alveolar ridge after tooth extraction follows a resorptive process where ridge width and height are reduced [7]. Horizontal ridge loss is more extensive than vertical and tends to be greatest from the buccal. Vertical loss also is seen to be most extensive on the buccal aspect [7,8]. The resorption of the alveolus is greatest during the first six months after tooth extraction but will continue throughout the life of the patient [9]. Studies have reported losses of 35% to 50% of ridge dimensions following tooth extraction [10, 11]. A systematic review showed an average horizontal loss of 3.87mm and average vertical loss 1.67mm of the alveolar ridge following tooth extraction [8]. The net loss of alveolar ridge resorption is the movement of a shorter ridge in a more lingual, or palatal position. The change in position limits placing the

implant in ideal restorative and esthetic positions as well as a closer approximation to vital structures such as the inferior alveolar canal and maxillary sinuses.

To limit the effects of the resorptive healing process, ridge preservation techniques have been employed at the time of surgery. For this procedure a grafting material is placed within the socket with the goal of limiting the dimensional change and providing adequate bone to support the implant for optimum esthetics and function. A systematic review has shown a reduced loss of dimensions with ridge preservation techniques with an average horizontal loss of 1.2mm and no vertical change [12]. Different materials are available to use for graft material within the socket. These materials must try to balance the necessary characteristics of providing adequate space maintenance during healing while simultaneously allowing the ingrowth of new tissues for the formation of quality bone. A discussion of available biomaterials follows.

Biomaterials

Biomaterials will be discussed here broadly as matrix scaffolding material or biologic agents. Matrix scaffolding materials are employed with the purpose of providing cell scaffolding and dimensional stability of the wound through space maintenance [13]. These materials demonstrate lower resorption and are able to maintain the space during healing of a grafted site. They are described as a biocompatible material with osteoconductive, and possible osteoinductive, properties. Ideally, these materials maintain the space as new tissues begin to occupy the space via angiogenesis and osteogenesis and eventually completely replace the material. Different grafts are available for matrix scaffolding.

Allografts are bone grafts, either freeze dried bone allograft (FDBA) or demineralized freeze dried bone allograft (DFDBA), sourced from a human cadaver. Xenografts are bone grafts

sourced from an animal, and alloplasts are synthetic materials designed to mimic the chemical and physical structure of bone. Finally, autogenous grafts are bone grafts from the same individual transferred from one location to another [14].

The processing of allografts minimizes the risk of disease transmission and complications of immunogenicity. Many studies have shown their efficacy as a bone substitute and have demonstrated osteoconductive and osteoinductive properties [15,16,17,18,19]. The osteoinductive properties of DFDBA have been purported to be greater compared to FDBA due to higher amounts of available growth factors (importantly bone morphogenic protein) sequestered within the boney matrix and able to be released upon implantation at the surgical site [14]. However, findings have been inconsistent and the inductive properties seem to be widely variable and affected by donor age, commercial preparation methods, and particle size [13,20,21,22]. Xenograft can be processed from a variety of sources including bovine, porcine, equine, and coralline [14]. Bovine is the most used source and has shown to have a similar hydroxyapatite content as human bone. It has also demonstrated osteoconductive properties but has not been shown to be osteoinductive [23,24]. Xenografts have been shown to have a slower substitution rate and allow for longer space maintenance with more residual particles present over time [14,39]. Alloplastic grafts vary in the material and can include synthetic hydroxyapatite, tricalcium phosphate, calcium sulfate, and bioactive glass [14]. They are completely synthetic which gives the manufacturer the advantage to control size and shape of the particles. There is also no risk of disease transmission and they are non-immunogenic. They have been shown to be biocompatible and some studies have shown them to be osteoconductive [16,25]. Finally, autogenous grafts are bone grafts taken from a distant donor surgical site and implanted at the recipient surgical site. The advantages of autogenous grafts are the biocompatible, osteoconductive and osteoinductive properties [15,16,26].

Disease transmission and immunogenicity are not a concern with autogenous grafts. Therefore, no processing of the graft material is required and results in preservation of the sequestered growth factors that are able to encourage osteogenesis. This has resulted in faster revascularization and integration of the graft [15]. However, autogenous grafts require a second surgical site which can add to patient morbidity and are limited in quantity at a single donor site. While the ideal properties of a matrix scaffolding material are understood, it can be difficult to obtain them all in a single material. A slowly resorbing material may be able to provide excellent space preservation throughout the early healing process; however, at the same time the slow resorption can prevent the ingrowth of *de novo* tissue disrupting angiogenesis and osteogenesis. These material have been described as being osteobstructive [27]. Multiple studies have shown histological samples from healed bone graft procedures that demonstrated extensive connective tissue sequestration of graft material without true evidence of bone formation at the graft particulate [28-31]. These observations call in to questions the true osteoconductive properties of the materials and instead may simply show biocompatibility of the material. In attempt to maximize the properties of these materials, the combination of different materials or the addition of biologic agents have been developed.

Biologic agents

Biologic agents are typically used alone or in combination with a matrix scaffolding materials to improve healing at the surgical site. Their goal is to maximize the quality and quantity of the specific tissue type of interest and to increase the rate of healing. The healing process is under close temporal and spatial control. The adequate matrix needs to be presents to support the early migration of progenitor cells followed by the appropriate signaling for further migration and

proliferation towards the final desired tissue type. Biologic agents act at these different steps to further enhance this complex healing process [32,33]. Growth factors and signaling agents generally make up biological agents, each with a different origin and mode of action with a specific cell and tissue type as the target.

Of the many commercially available biologic agents available, bone morphogenic protein (BMP-2), platelet derived growth factor (PDGF), and fibroblast growth factor (FGF-2) will be discussed here. Bone morphogenic protein is a strong osteoinductive member of the transforming growth factor-β family [33]. It has demonstrated increased osteoblast differentiation and upregulated mineralized tissue markers [34]. It is available commercially as Infuse (Medtronic, Minneapolis, USA) and is FDA approved for extraction sockets and sinus augmentation procedures with the main goal to enhance bone formation for implant site development. Animal and limited human studies have shown BMP-2 to be successful in preserving ridge dimensions and enhancing bone quality and rate of healing when applied to the surgical site with and without bone graft material [35-39].

Platelet derived growth factor is involved naturally in wound healing. As the name implies, it is released from platelets at the site of tissue injury and resulted in the promotion of fibroblast, cementoblast, and osteoblast migration and proliferation at the surgical site [33,40]. It is commercially available as Gem 21 (Osteohealth, New York, USA) and is FDA approved for the treatment of periodontal bone defects and related gingival recession. Beyond the specific FDA approval, studies have tested PDGF with bone graft material for ride augmentation for implant site development. These studies have shown successful bone formation with faster rates of healing and higher quality of bone [41,42,43].

Fibroblast growth factor-2 is a protein involved largely with fibroblast proliferation. *In vivo* studies have demonstrated increased osteoblast proliferation and a strong angiogenic potential [44,45]. While some animal studies have shown its potential for use within periodontology, no human studies have been completed to date. Work is still needed on deciphering the correct dosage and an adequate vehicle before its implementation into humans.

Theses biologic agents, and other similar commercially available ones have demonstrated clinical success in attempting to enhance the biologic process of wound healing. However, these processes are complicated and not fully understood. Natural growth factor secretion and cell signaling is under strict temporal and sequential control and this has proven difficult to mimic with current biomaterials. Attempts are made to maximize the substantivity of the agent at the surgical site. Different carriers are used as a material to deliver the protein or molecule to the site and retain it there for a period of time. If the substantivity is too short, the agent will be cleared from the site and not be available at the necessary time point of the healing process. However, if it is present for too long of a time it can have a negative effect and can dampen the intended result. Being able to control the release of the agent at the surgical site at a specific time point when it can best be utilized and not interfere with other processes is a characteristic of an ideal biologic agent that has yet to be achieved. For maximization of clinical results using biologic agents, a better understanding of the healing processes must be understood.

Platelet Rich Fibrin

Platelet rich fibrin is considered a second generation blood concentrate first introduced by Choukroun in 2006 [1,2]. The production of PRF requires collection of whole blood from the patient which is then immediately centrifuged at approximately 400g in a glass tube without the

addition of anticoagulants or additives. During the centrifuge process, the fibrin is allowed to slowly polymerize while the blood separates into three distinct layers; an acellular platelet poor plasma on top, a concentrated hematocrit at the base, and the fibrin clot in the middle [2]. The fibrin clot is the desired product which can be easily isolated and applied surgically as required. In the processing of PRF, the fibrin clot is allowed to polymerize, creating a natural scaffold with bound platelets and leukocytes [2]. Concentration of platelets within PRF have been shown to be more than 15 times higher than that of whole blood of the same volume [46]. This high concentration of platelets has been shown to be a significant source of transforming growth factor β-1 (TGFβ-1), vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF) and a coagulation matrix glycoprotein, thrombospondin-1 (TSP-1) [3,4,5]. The vast majority of the growth factors have been shown to be bound within the fibrin matrix acting as an ideal carrier of growth factors to be applied to a surgical site [3,5]. The fibrin matrix is naturally remodeled as part of the healing process. As the remodeling occurs, platelets are activated and release their associated growth factors to promote the continued healing process [6]. Therefore, PRF allows for the slow, sustained release of growth factors at the surgical site through the natural maturation and reorganization of the clot thus mimicking the temporal and sequential control of growth delivery and activation [3,5,6]. Additionally, cytokines demonstrated to be present in PRF (Interluekin-1β,4, and 6) have the ability to further recruit key growth factors to the site for the promotion of wound healing [3].

It is important to differentiate PRF from the earlier generation of blood concentrates known at platelet rich plasma (PRP). Platelet rich plasma was introduced as an autologous modification of fibrin glue, a hemostatic and adhesive agent used in the surgical field [47,48]. Generally, the preparation protocol begins with the initial introduction of anticoagulant to the collected blood

sample, followed by multiple centrifuge and separation cycles that result in a concentrated platelet product. Topical bovine thrombin is then added to activate the platelets and the clotting cascade in order to obtain an active product with gel-like handling properties conducive for to the dentoalveolar surgical field. However, the production protocol for PRF offers multiple reported advantages over PRP. Notably, there is no addition of anticoagulant and the clotting cascade is allowed to occur naturally with PRF. This results in a more robust and resistant fibrin scaffold that conveys two significant benefits to PRF over PRP. First, the fibrin entraps the concentrated platelets. This allows for substantivity and a slow release of the platelets and their associated growth factors at the surgical site as the fibrin clot is reorganized during normal wound healing. Second, the fibrin clot in PRF is more resilient than the gel-like consistency of the PRP. This allows for easies handling characteristics and use in a wider variety of applications. PRF clots can be compressed into a thin sheet and be applied in the surgical field as a membrane would. Additionally, it can be mixed into bone graft particulate or the whole fibrin clot can be applied to offer a stabilized clot at the surgical site with putative osteoconductive and osteoinductive properties.

Evidence of PRF as an effective bioactive scaffold has been readily demonstrated *in vitro*. In prior studies, osteoblastic proliferation and differentiation was promoted with PRF scaffolds over collagen or platelet rich plasma substrates [49,50]. Osteoblast-like behavior of progenitor cells grown on PRF substrates was evident with the detection of mineralization deposition at a rate comparable to cells grown in pro-osteogenic medium [51]. This increased mineralization coincided with a nearly fourfold increase in pro-osteoblast differentiation transcription factor RunX-2 [51]. PRF also demonstrated a significant enhancement of fibroblastic proliferation [52].

The *in vitro* literature supports PRF's potential efficacy as a bioscaffold to be used clinically. Some studies have used PRF augmented with particulate grafting material, as proposed in this study. Platelet rich fibrin has a natural fibrin matrix that imparts some scaffolding matrix characteristics; however, it is thought the addition of graft particulate to PRF will enhance the space preservation qualities desired for surgical application but could be used at a lower concentration to minimize that osteobstructive effects sometimes seen with graft particulate. In this effort, one study demonstrated that in the treatment of intrabony defects, PRF augmented with bovine porous bone mineral showed greater PD reduction and CAL gain compared to PRF alone [53]. Similarly, sinus grafts were performed using PRF augmented with FDBA or deproteinized bovine bone [54,55]. These studies demonstrated a faster histological healing rate and greater new bone formation with the experimental treatments. However, subject numbers were small and significant differences were not found. Additional clinical applications for PRF alone have included treatment of intrabony defects, furcation defects and root coverage procedures [56,57,58]. Success of these applications have been inconsistent, and minimal analysis beyond clinical parameters cannot demonstrate the true effect of PRF in vivo.

It is evident that the inconsistency of results associated with the current clinical applications of PRF have resulted in an inability to understand the specific and best clinical utility of PRF. The proposed study aims to reduce the heterogeneity by using a clinical model which negates many of the clinical variables and allows the assessment of the true osteogenic potential of PRF. By analyzing bone healing and monitoring dimensional stability, this study will demonstrate the osteogenic potential of PRF and show its utility as an effective autologous biomaterial for ridge preservation.

Materials and Methods

The Institutional Review Board at the University of California of San Francisco approved the study design. Subjects were recruited from the Dental Center at the University of California at San Francisco patient pool between December 2015 and May 2016. All patients were screened for inclusion criteria and eligible subject were enrolled into the study and written consent was obtained. Eligible subjects presented with a single rooted tooth requiring extraction and replacement with a dental implant supported restoration. Included teeth had root position and angulation that was consistent with planned implant placement. Teeth were excluded if they demonstrated a buccal dehiscence more than 25% of the length of the tooth or presence of acute infection of endodontic origin. Subjects were excluded from the study if they exhibited poor oral hygiene, pregnant woman or patients who intend to become pregnant, those who use tobacco; if they had any medical condition that would be a contraindication to dental surgery or could alter healing such as autoimmune disorders, immunosuppressed, or uncontrolled diabetes.

Forty subject were enrolled into the study. Subjects were randomized via random sequence generation into one of four ridge preservation treatment protocols to be performed immediately after the extraction. The four treatment groups included PRF, PRF+FDBA, FDBA, and blood clot.

Extractions and ridge preservation procedures were completed by three different residents at the UCSF Graduate Periodontology clinic. All subject were given 600mg ibuprofen and chlorhexidine gluconate 0.12% mouth rinse (Peridex, 3M, Minneapolis, USA) at the beginning of the appointment. Local anesthetic was administered at the site and non-traumatic tooth extraction was completed without the elevation of a mucoperiosteal flap. The socket was

thoroughly curetted, irrigated with sterile saline, and inspected for presence of perforation, fenestration, or dehiscence.

Subject enrolled in the PRF or PRF+FDBA group had blood drawn and PRF prepared according to the following protocol outlined by Choukroun[2]. Venous blood was collected via venipuncture of the forearm with a butterfly needle into one 10 ml sterile glass vacuum tube. The blood sample was immediately centrifuged at 3000 rpm for 10 minutes. The PRF was separated from the three distinct layers that had formed within the tube.

Ridge preservation Protocols

Subjects enrolled in the PRF group had PRF prepared as described above. The entire PRF clot was removed from the tube and placed into the socket. Gauze were used to compress the clot lightly within the socket so it was at the level of the boney crest. If the PRF required modification to fit within the socket, the end of the PRF clot closest to the top of the tube, away from the red blood cell layer, was trimmed off. A Collaplug (Integra Life Science, New Jersey, USA) was placed over the PRF from the boney crest to the gingival margin. Vicryl sutures were placed across the socket and cyanoacrylate (PeriAcryl90, GluStitch Inc, British Columbia, Canada) was applied to seal the margins.

Subject enrolled in the PRF+FDBA group had PRF prepared as described above. The clot was removed and cut into small pieces with a scissors starting from the end closest to the red blood cell layer. Freeze dried bone allograft (AlloOss, Ace Surgical, MA, USA) was added to the PRF pieces and mixed to achieve a final volume with at 1:1 ratio of graft particulate to PRF. This was approximately 0.5cc FDBA to a full PRF clot obtained from a single tube. The mixture was

added to the socket up to the boney crest with light compression. A Collaplug was applied in the same manner as described above.

Subjects enrolled in the FDBA group had the same graft particulate as the group above hydrated and added to the socket with light compression up to the boney crest. A Collaplug was applied in the same manner as described above.

Subjects enrolled in the blood clot group had no additional materials added within the socket. Further curettage of the socket walls was performed to allow the socket to fill up to the boney crest with blood. The Collaplug was applied in the same manner as described above.

All subjects for all groups received the same post-operative instructions. Chlorhexidine mouth rinse was prescribed to each subject for use twice daily for two weeks. For pain control, NSAIDs were recommended and prescription narcotics were prescribed as needed. Subject returned after two weeks for suture removal and again after one month for further evaluation of healing.

Bone Core Harvest

Subjects returned to clinic three months after the extraction for placement of the implant. Necessary radiographs were taken to plan appropriately for implant placement. A surgical guide was fabricated from the original cast to direct the trephine into the socket. Local anesthetic was delivered and an incision was made over the edentulous crest and a minimal mucoperiosteal flap was elevated for access. A trephine was used first with a 2mm internal diameter to obtain a core sample of the bone to the measured depth of the original socket. Harvested bone cores were immediately placed in 10% neutral buffered formalin. Osteotomies were continued and implants were placed according to standard protocol. One or two stage implant protocols were used based

on the clinician's preference and patients were followed according to standard clinical protocol to completion of the implant restoration.

Micro-CT Analysis

Bone core samples obtained from the subjects of all groups were fixed in 10% neutral buffered formaldehyde for a minimum of five days. A high-resolution micro-CT system (Sky- Scan 1172; Bruker-micro-CT, Kontich, Belgium) was operated at 100 kV and 100 mA using a 0.5-mm Al + Cu filter with a resolution of 13.68532-mm pixels. Data obtained during micro- CT scanning of the samples were transformed into images with NRecon v.1.6.3 software (Bruker-micro-CT) and analyzed with CTAn v.1.12 software (Bruker-micro-CT). The software was used to separate mineralized tissues (new bone and graft particulate) from non-mineralized tissues (connective tissue, vascular tissue) based on the differences of the materials' x-ray absorbance coefficient. Analysis was performed to define bone volume fraction and bone mineral density of the different materials within the same sample and allow for comparisons across groups. Finally, the software was used to generate three-dimensional images of the samples to allow for qualitative comparisons of bone quality across groups.

Results

Thirty three of the 40 subjects enrolled completed the study to date. There were 17 males and 16 females with an average age of 58 who completed the study. The majority of the teeth were extracted due to fractures or non-restorable carious lesions. Table 1 presents the demographic data of the subjects enrolled in the study.

One implant failure was noted in the PRF treated group during an early loading period with a total implant success rate of 97%. No other major surgical complications were noted. Thirteen percent of sites required additional hard tissue grafting at the time of implant placement. This consisted of sites generally in the maxillary anterior and was not significant for any one ridge preservation protocol. Average healing time for each group was 104 days for blood clot group, 104 days for PRF group, 105 days for PRF+FDBA group, and 103 days for FDBA group.

Micro-CT analysis

Qualitative differences were evident between the four treatment groups based on comparisons of the rendered cross sections (Figure 1). Blood clot, PRF, and PRF+FDBA groups were noted for having smooth trabecular formation throughout the samples. The FDBA group had very limited bone morphology that resembled trabecular formation and instead was dominated by disorganized and disrupted mineralized structures resembling graft particulate. Differences in the amount of bone present was evident as there was a trend for higher bone volume in PRF and PRF+FDBA groups compared to blood clot group.

Bone volume fraction was performed for all samples across all treatment groups (Figure 2). Total bone volume for the blood clot group was 33.6%, PRF group 41.1%, PRF+FDBA group 38.4% and FDBA group 29.5%. No significant differences between groups was noted.

Bone mineral density was performed for all samples across all treatment groups (Figure 3). Bone mineral density for the blood clot group was 484.7 g/cm^3, PRF group 500.9 g/cm^3, PRF+FDBA group 519.9 g/cm^3, and FDBA group 517.2 g/cm^3. No significant differences between groups was noted.

Discussion

In comparisons of bone volume fraction and bone mineral density, no significant differences were seen between groups using micro-CT analysis. The PRF group had the highest bone volume fraction and the PRF+FDBA group had the highest bone mineral density. A trend for better bone quality and quantity is seen with the addition of PRF but at this time point no significant differences can be detected.

The percent of new bone formation in extraction sockets shown in this analysis ranged from 29.5% to 41.1%. This range is consistent with what has been shown previously in the literature [59,60,61]. These studies generally used longer healing times, had different surgical protocols, and used histological analysis instead of micro-CT; however, the findings remained within the same range of new bone volume as this study. Interestingly, in this study the FDBA group showed the lowest percentage of new bone volume while the PRF group demonstrated the highest. The PRF group may have an advantage compared to the FDBA group in that the scaffolding provided by PRF is a dense fibrin matrix that may undergo faster resorption compared to FDBA. By resorbing in a timelier manner, there is more room for *de novo* tissue in growth. This higher bone volume could be further enhanced by the associated concentrated growth factors found in PRF and not FDBA. Osteobstructive characteristics of bone graft material have been described before and it appears this study supports FDBA as being more osteobstructive at 3 months healing time[27-31]. In addition to the lower bone volume, micro-CT also demonstrated a qualitative difference in bone formation of the FDBA group compared to the others. Trabecular formation and structure was quite evident in the blood clot, PRF, and PRF+FDBA groups, while little trabecular structure could be noted in the FDBA group (Figure 1). The mineralized tissues in the FDBA group were disorganized and disconnected, resembling graft particulate generally. Again,

these qualitative findings support an osteobstructive characteristic of FDBA. It is important to note that this study allowed for three months healing time which is fairly short in comparison to other ridge reservation studies [59-61]. With longer healing times, FDBA may be present at lesser amounts as it is resorbed and more trabecular formation may be noted. There may also be a benefit in better space maintenance for certain clinical scenarios where a more slowly resorbing material is desired.

Bone mineral density was also analyzed via micro-CT and no significant differences were found between groups. In this scenario of new bone formation at extraction sites, bone mineral density describes the maturity of bone that has formed. A higher bone mineral density can be associated with faster healing and more mature bone. Comparisons of bone mineral density across all groups in this study is limited. The PRF+FDBA and FDBA groups use a mineralized material with a high mineral density added as part of the treatment protocol. By performing a micro-CT on the FDBA material alone it was found to have a bone mineral density of 564.6 g/cm^3. This was higher than the averages found for all other treatment groups. Therefore, it could be expected that groups with FDBA used for treatment would have elevated bone mineral density due to the presence of residual graft material and not necessarily due to a higher rate of healing. In addition to the limitation above, micro-CT is also limited by the ability to differentiate materials with too similar x-ray absorbance coefficients. For this study, this limited the ability to separate new bone and graft particulate in a high throughput manner. Therefore, it was likely residual graft particulate was included with new bone volume and incorrectly elevated the percentage of new bone formation. Also, as stated above, residual graft was likely included with new bone volume and incorrectly elevated the bone mineral density as well. Other methods, such as histological analysis, exist to allow for better visualization and separation of graft

particulate from new bone for quantification. In future studies it may be best use both methods to more accurately analyze bone cores.

There is a limited number of studies who have used micro-CT for analysis of bone healing at extractions sockets [62,63]. The majority of other studies employ histological methods for analysis of new bone volume. The benefits of micro-CT include a wider volume of material being analyzed. In this study the bone cores were 2mm in diameter and the entire volume was analyzed and quantified. Histological methods use a single or multiple representative slices less than 0.5mm thick for analysis. Findings from these slices are then generalized as being representative of the entire specimen. However, it has not been shown how much more accurate micro-CT is compared to histology for bone volume quantification. Further research on this topic is required.

Conclusion

The present study demonstrated that ridge preservation using PRF with or without FDBA produced no statistically significant differences in bone volume fraction and bone mineral density compared to blood clot or FDBA alone. PRF was shown to be an acceptable autologous biomaterial to encourage bone healing and allow for successful implant placement at the treated site. No significant differences were seen in the PRF group compared to established ridge preservation methods and trends were seen for better performance of PRF compared to blood clot and FDBA. To the best of our knowledge, this is the first randomized controlled clinical trial to evaluate PRF as a material for ridge preservation.

Tables and Figures

	Group A N=9	Group B N=8	Group C N=8	Group D N=8
Age -yr	56.8±13.1	62.3±14.2	58.1±12.7	57.4±15.7
Gender				
Male	5	5	4	3
Female	4	3	4	5
Tooth position				
Incisor	1	1	2	3
Canine	0	2	0	0
Premolar	8	5	6	5

Table 1. Demographic data of subjects enrolled in study and tooth position of teeth extracted. Group A. Blood clot, Group B. PRF, Group C. PRD+FDBA, Group D. FDBA

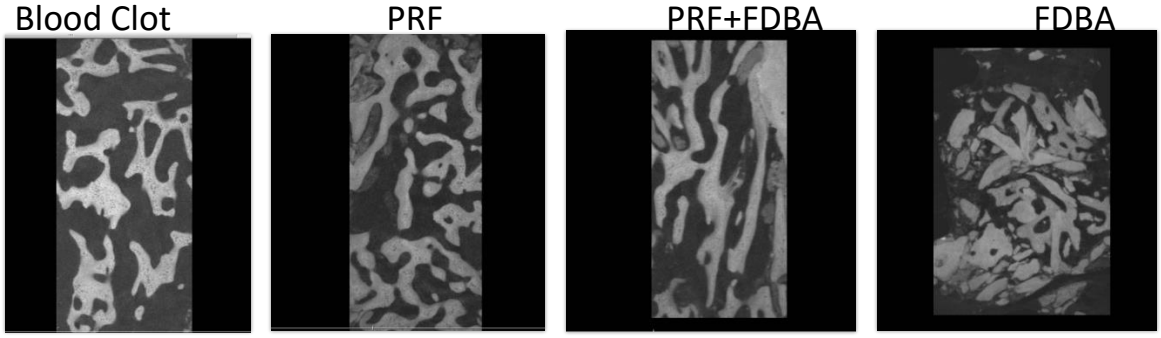

Figure 1. Representative micro-CT sections of bone cores from the four different treatment protocols.

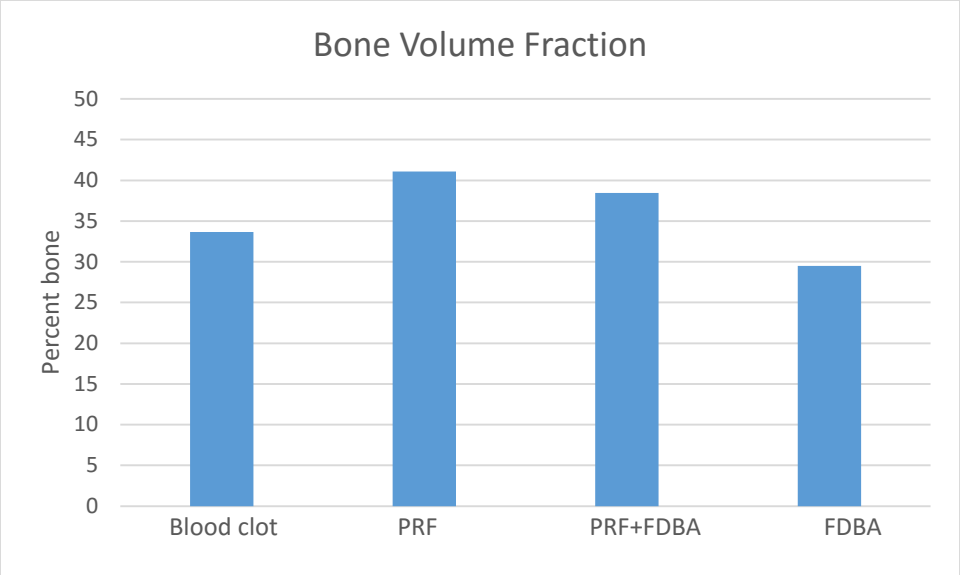

Figure 2. Bone volume fraction of the four ridge preservation protocols as analyzed via micro-CT

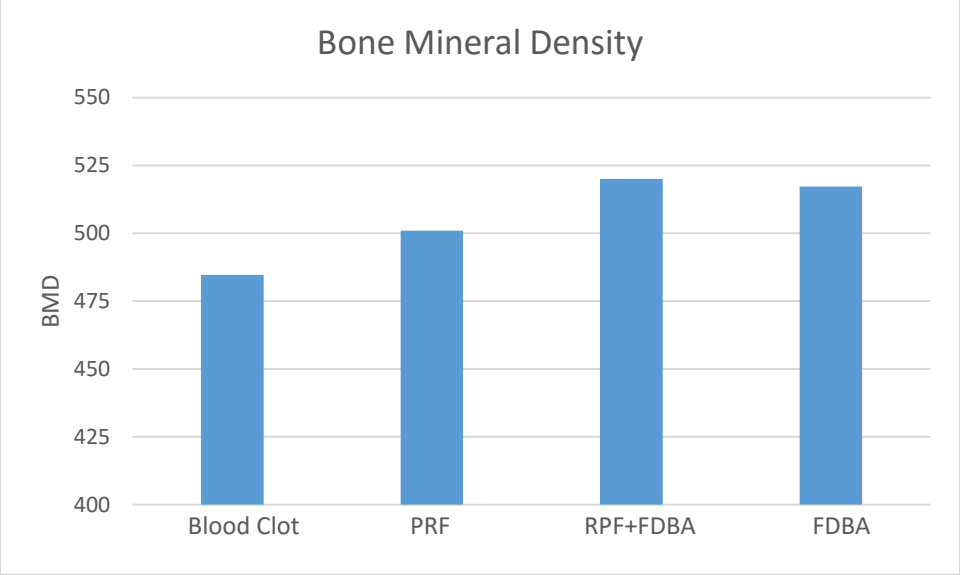

Figure 3. Bone mineral density of the four ridge preservation protocols as analyzed via micro-CT

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