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Histological analysis of extraction sockets grafted with Platelet Rich Fibrin in comparison to Freeze Dried Bone Allograft

by

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Oral and Craniofacial Sciences

in the

GRADUATE DIVISION

Histological analysis of extraction sockets grafted with Platelet Rich Fibrin in comparison to Freeze Dried Bone Allograft

Abstract

Objective: The present study is a randomized controlled clinical trial that aims to evaluate histological bone healing of platelet rich fibrins (PRF) in comparison to natural healing, and freeze-dried bone allografts (FDBA).

Methods: Forty patients were recruited, randomized and treated in one of the following four groups: group A blood clot (control), group B PRF, group C PRF mixed with 1:1 ratio of FDBA and group D FDBA. Single rooted teeth were extracted and the sockets were treated in accordance to the protocol for each group. Extraction sockets were covered using Collaplug. After 12 weeks, bone core samples were removed prior to implant placement. Clinical dimensional changes, Micro-CT and histological analysis were completed.

Results: Histological studies of H&E slides demonstrated the percent area of vital bone was 38.59% in group A, 53.71% in group B, 41.31% in group C and 29.06% in group D. There was a statistical significance between vital bone percentage between the PRF group and FDBA group (student t-test).

Conclusion: This study provides the first histological evidence to our knowledge that PRF in extraction socket can significantly improve vital bone quality after 12 weeks of healing.

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Introduction

Advancements in biomaterial and the constant evolution of implant dentistry continue to raise the bar on treatment modalities available to patients. Despite a significant number of innovations and increasingly more rigorous research available, the strive for perfection is a reminder of unmet challenges. Notably, aesthetic zone and posterior mandible reconstruction present unique difficulties in restoring and reconstructing natural form and function and continue to be a focus of future studies.

To understand dentoalveolar anatomy and the alveolar remodeling, both animal and human studies have researched histological and anatomical changes that occur following extraction of teeth. In an effort to retain as much hard and soft tissue post surgery, investigators focused on regeneration and studied the histological changes that ensue various grafting material and cell exclusive membranes. More recent literature shows a second wave of studies that look to achieve this goal by utilizing biologics that can help direct cellular level healing. This project to our knowledge provides the first available histological evidence comparing the effect of Platelets Rich Fibrin (PRF) in comparison to Freeze Dried Bone Allograft (FDBA) in socket preservation of single rooted teeth.

Background

Examining normal healing stages of an extraction socket on a cellular level provides the understanding needed to direct cellular healing by modulating various biological mediators. The best model to observe these changes will be human studies. However, human histological studies face ethical limitations as removal of large bone blocks are required to visualize the stages and dimensional changes that occur in the dentoalveolar complex following extractions. Consequently, the existing human histological studies use biopsies of the marginal or central portion of the extraction socket. Our understanding of healing socket dimensions are

augmented by animal studies done on canines [1] . It is important to note that in canine bone remodeling and healing there is three to five times faster healing than in humans yet the principles are consistent [2] .

Araujo and Lindhe presented some of the highest quality histological evidence depicting the dimensional changes. They showed histological slides at one, two, four and eight weeks post extraction. One week healing showed a moderate level of inflammatory cells with rich vascularity. The lining of the socket, which is supported by the bundle bone, showed an increase level of osteoclasts activity. Lingual bone was consistently thicker while both facial and lingual plate contained bone marrow spaces. The coagulum or a provisional matrix filled the socket spaces.

Week two healing highlighted the formation of bone from the apical portion of the socket. By the forth week, the bundle bone was completely resorbed in the crestal area and furthermore there was significant resorption associated with the buccal crestal bone. The provisional matrix started to convert to lamellar bone at this time. At eight weeks, mineralized tissue started to bridge the buccal and lingual wall. The osteoclastic activity of the buccal wall on average resulted in a 2 mm loss of vertical height. The woven and lamellar bone formation and significant osteoblastic activity was noted in the socket space. The significant loss of vertical bone was attributed to loss of function for bundle bone caused by tooth extraction [1] .

The findings of changes in alveolar dimension, increased osteoclastic activity and loss of bundle bone due to loss of function have been confirmed in the human histology as well.

Trombelli showed through human histology that following tooth loss, at 3 months greater than 30% and at 12 months greater than 50% of buccal-lingual alveolar ridge volume was resorbed [3] . Further more, he looked at histological healing at: early (2-4 weeks), intermediate (6-8 weeks) and late phase (12-24 weeks) [3] . The woven bone percentage in human healing was 6.9% in early healing, 34% in intermediate and 41.5% in late phase. In late phase bone healing was a mixture of 9.1% lamellar bone and 32.4% woven bone. Osteoblastic activity peaked at 6 weeks

and remained stable. One important observation is the distribution of bone percentage seen in subjects. This finding ranged from 0% to 65% supporting that bone healing in humans is a slow process and it depends on complex cascades that are functions of aging, overall health, immune system efficiency and genetics [3].

Aside from histological studies, other groups have examined these changes through differences of pre and post extraction using cast models and radiographs. These studies can account for clinical changes of the soft tissue in addition to osseous changes. Schropp noted that 12 months following an extraction, there was a 50% change in clinical ridge width. Further more, two thirds of these changes occur in the first three months [4].

It should be noted that these studies used irreversible hydrocolloid for impression which is known to have inconsistent expansion and shrinkage specifically if not used with proper technique [5]. Additionally, utilizing periapical and bitewing radiography for measurements of bone height has been proven to have limitations due to inaccuracy and distortions [6].

While the aforementioned studies did not benefit from a large sample, as do many other histological studies, the findings have been combined in systematic reviews. Weijden reported in a systematic review with a 1B grade for quality of evidence the following [7]: An average of 3.87 mm reduction in ridge width and a mid buccal height loss of 1.67 mm was observed post tooth loss in natural healing [8].

A recent study by Chen investigated the 8 weeks healing of maxillary lateral and central incisors with intact buccal plate, fenestration and dehiscent type defects [9]. They report that the largest defect was seen in at 28.4% in sites with existing dehiscence. Sites with fenestration defects demonstrated the greatest vertical defect 2.9 mm (+/- 2.67 mm). Interestingly, sites with original buccal plate developed a dehiscence 56% of the time. Similarly, 55% of the time fenestration type defects converted to dehiscence. This study in particular shows how unpredictable the healing of socket with existing defect or even with intact buccal plates can be. Thin phenotype

patients were found to have thinner buccal plates and Chen reports that the gingival phenotype had significant influence on the outcome [9] .

The studies discussed up to this point provide clinical, radiographical, histological and high quality systematic review supporting the notion that there is a significant shrinkage in dentoalveolar complex following tooth loss. This remodeling creates serious aesthetic and functional limitations for future implant placement. Thus, the use of grafting material has been advocated to minimize the amount of shrinkage of the extraction socket.

The dogma of alveolar augmentation was inspired by regeneration studies in periodontal defects that at the time were shown both histologically and clinically [10] . The first wave of studies included a large variety of materials including autografts, allografts, xenografts, alloplasts and resorbable and non-resorbable membranes to find the ideal choice for maintaining both ridge dimension and new vital bone.

Allografts

While autogenous bone is considered the golden standard [11] , concerns for both quantity and morbidity of a second intraoral or extraoral surgical site drove research in search of alternative options. Periodontal regeneration studies identified advantages that osteoinductive material such as Demineralized Freeze Dried Bone Allograft (DFDBA) offer as compared to osteoconductive grafts [10,12] .

Becker investigated socket preservation using DFDBA in comparison to autogenous bone. He found histological evidence of residual nonvital bone and signs of resorption of DFDBA particulates [11] . While osteoinductive potential of DFDBA [13] through bone morphogenetic protein-2 and platelet derived growth factors was known, this was some of the first evidence that supported the lack of consistency of these mediators both in terms of quantity and efficacy in DFDBA [11,12,14] . This has been attributed to several reasons including differences in processing techniques amongst bone banks, age, genetics and bone type of the donors [14] .

Understanding the differences of FDBA and DFDBA in alveolar bone defects was studied in a Rhesus Monkey histological study. Yukna studied one month, two month, and three months healing in FDBA and DFDBA in human primates. The results were that FDBA produced a significantly more new bone at 1 month, 2 months and 3 months. DFDBA showed a significant amount of residual bone remaining at 1, 2 and 3 months in compare to FDBA [12] .

The evolution of evidence from animal study to human studies led to investigation of socket preservation with FDBA in human histology at 14 to 27 weeks of healing [15] .

The results of 14 weeks healing showed the presence of 45.8% new bone formation and 14.6% of residual bone. At 27 weeks of healing, a mean value of 45% new bone and 13.5% residual bone was observed. This study confirmed that the healing of FDBA beyond 6 months offers no advantages [15] .

Mealey further looked at the healing of FDBA when comparing cortical versus cancellous bone from the same donor. Although both are considered osteoconductive material there is a difference in healing patterns [16] . In cancellous bone, the healing is via a substitution pattern. That is, the coagulum surrounding the particulates will recruit osteoblasts from the host mesenchymal cells and as new bone is formed, the graft resorbes [16] . Whereas, in cortical bone healing the particulates resorbe right away. This process involves the release of residual bone morphogenetic proteins and growth factors, which then encourage new bone formation. The residual cortical particulates tend to remain for a longer period of time and not resorbe readily [16] . They found no significant difference in new bone formation for cortical and cancellous groups at 16.1% and 13% respectively [16] . Their result for percentage of new bone was considerably lower than other previously completed socket preserving studies. However, studies have reported a relatively large range for percentage of new bone including: 13%, 25%, 38%, 48% [15-18] . This finding perhaps further supports that results of socket preservation can have a significant variation that may depend on the host related factors as well as processing and

quality of the donor bone. This is a significant challenge to overcome as the increase in host and donor variability introduces confounding variables that are hard to standardize.

Since cortical and cancellous bone offered no particular advantages, studies aimed to compare DFDBA versus FDBA. Wood, discovered 24.63% new bone in FDBA group, compared to 38.42% in DFDBA [19] . This was a significant difference attributed to the osteoinductive potential of DEFDBA. Borg then explored the combination in search of an ideal mix of osteoconductive and osteoinductive potential [17] . He compared a mixture of 70% FDBA and 30% DFDBA and compared it to 100% FDBA. Study showed 36.16% vital bone in the combination mixture and 24.69% in FDBA group [17] . This difference was significant for vital bone formation while the changes in ridge width were not significant [17] .

Xenografts

In addition to allograft, investigators have also explored the use of xenograft and alloplasts as the material of choice for socket preservation.

Xenografts available include: bovine, porcine, and equine. However, The most well studied and used xenograft material is processed from bovine bone. The use of xenograft as bone augmentation has been studied most extensively in lateral wall sinus augmentation [20] . The xenograft particles have demonstrated the ability to remain in the grafted sites and resist resorption. Current histological evidence of lateral wall sinus augmentations utilizing xenografts has confirmed the presence of these particles as far as nine years post surgery [21] . Some of the best evidence for socket preserve using xenograft include animal studies that report particular advantage of xenograft in maintaining the ridge width due to their strong affinity to not resorbe [22] . Kim et al. study offered the same conclusion but failed to provide information on the quality of the bone, vital bone percentage or a comparison to controls such as other grafting materials [23] . Vital bone produced using xenograft was reported in other studies with the range of 32%-47%, however, it must be noted that the protocol in the study was not similar to others and the xenograft included a 10% mixture of collagen fibers and a non-cross linked resorabable

membrane which can influence the results through exclusion of epithelial cell growth [24] . The evidence in support of use of xenografts for socket preservation are low quality studies with high heterogeneity and one systematic review that cannot offer any meaningful conclusions [25] .

Alloplasts

Alloplast grafts are well documented in periodontal literature including their use as carrier for biologics such as Platelet Derived Growth Factor-21 shown in multi-center randomized clinical trials [26] . Given the synthetic nature of alloplasts, biocompatibility and osteoconductivity are areas of concerns. Verity of alloplasts have been studied of which beta-tricalcium phosphate and medical grade calcium sulfate have emerged from.

The use of alloplast in socket preservation has produced literature with conflicting data as well. Aimetti reported that in calcium sulfate grafted sites he observed 58.8% vital bone as compared to 47.2% for non-grafted sites [27] . This finding compared similarly to Kutkut who reported that mixture of calcium sulfate with PRP in comparison to non-grafted site showed 66.5% to 38.3% advantage for vital bone presence [28] . These are some of the highest reported percentage for vital bone formation across all reported data in the literature for any grafting material. It has been hypothesized that the faster resorption rate of calcium sulfate provides angiogenic advantages, which result in faster formation of osteoblasts and thus higher vital bone percentage. However, there are contradicting studies that report significantly lower vital bone percentages [27,29,30] . Faster resorption down effect on retaining ridge dimensions was compared in a study with FDBA. Comparing calcium sulfate directly to FDBA showed reduced ridge dimensions in both group but with no statistical significance amongst the two groups. While vital bone percentages reported were low for both groups, the advantage was to calcium sulfate with 32% to 16.7% edge [29] .

Aside from periodontal literature, Orthopedics and Neurosurgery currently utilize alloplasts for spinal fusions using similar properties and principles. The medical literature also faces similar

challenges with regards to available evidence [31] . Systematic reviews from both dental and medical literature conclude that the quality of evidence is low; there is significant heterogeneity among present studies and that future studies are indicated [31,32] .

Biologics

Biologic agents have brought on a second wave of research efforts. Investigators have focused on identifying biological agents that can help modify, modulate and accelerate healing. Several agents have been identified currently and have shown advantages in both soft tissue and hard tissue healing. These include bone morphogenic protein, platelet derived growth factors, enamel matrix derivatives and platelet rich fibrins.

Platelet rich fibrins were introduced by Choukroun in 2006 and have been subject of both medical and dental research [40, 41] . PRF is formed from autologous blood that is collected from patients. Blood samples are centrifuged to allow for separation of platelets from other components, which include acellular platelet poor plasma that is formed on top and concentrated hematocrit, which collects at the bottom [42, 43] . PRF itself consists of a large quantity of growth factors including: 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) [44.] These growth factors have been shown to play key roles in wound healing cascades; they are complex in their range of functions and redundancy in downstream effects is a common characteristic. As such, a large range of function for these growth factors has been identified. This includes but not limited to: cell growth recruitment, differentiation, proliferation, collagen synthesis and blood vessel growth. All of which are essential functions in wound healing cascades [44].

Additionally, PRF has shown a special ability to sustain a constant release of these mediators [44]. Longer substantivity is a desired feature as it can allow for continuous signaling and presumably a more profound downstream effect of these mediators [44].

These promising qualities have led to a broad use of PRF in the literature. PRF has been used in sinus augmentation along with FDBA, Soft tissue grafting and regeneration of periodontal defects. These studies report mixed results. To our knowledge there has been no study investigating histological healing of PRF in sockets for ridge preservation.

Material and Methods

A randomize single blind controlled clinical trial was designed to compare bone healing in single root sockets. The four groups studied include: group A blood clot (control), group B PRF, group C PRF mixed with FDBA and group D FDBA. Histomorphometric analysis utilized to evaluatate changes three months following extraction. All patients were followed for proper postoperative healing. This study was conducted as part of a larger study that evaluated clinical changes of the alveolar ridge as well as micro CT analysis of the bone cores. Those other observations are presented and discussed in separate papers.

The Institutional Review Board of the University of California San Francisco reviewed and approved this research protocol. All subjects were recruited from December 2015 to May 2016 at the University of California San Francisco Post Graduate Periodontics department. From the preliminary statistical power analysis it was determined that ten subjects were required for each of the four treatment groups for statistical significance. All surgeries performed by one of three periodontal residents at UCSF Post Graduate Periodontics.

Inclusion and Exclusion Criteria

Subjects were excluded if they were currently or had a history of: smoking, type I or II diabetes, were immunocompromised, on bisphosphonate therapy, pregnant or intending to become pregnant, had any blood disorders, uncontrolled systemic diseases, poor oral hygiene, failing endodontic treatment, detection of dehiscence, or a history or presence of sinus tract and significant infection.

Additionally, the following inclusion criteria were used: patients with single rooted teeth requiring extraction, intact buccal and lingual plate, and absence of clinical and radiographic signs of periapical infections.

Subjects were randomly assigned to one of four groups using computer generated randomization software. Informed consent for the study and surgical procedures were given. Subjects were informed of the four possible treatment groups involved in the protocol prior to consent. Subjects received free consultation, digital images, surgical extraction, socket graft preservation and follow up.

Thirty-three subjects between the ages of 24-74 were recruited and completed the study. The subject pool consisted of 17 males and 16 females. Extraction sites consisted of: 7 maxillary incisors, 2 maxillary canines, 18 maxillary premolars, and 6 mandibular premolars. Demographic characteristics including age and gender as well as numbers of tooth types for each treatement group are described in Table 1.

Surgical Protocol

All subjects received a consultation, and photographs, digital radiographs and alginate impressions were taken. A caliper and either a UNC-15 or Marquis-12 periodontal probe were used to record all measurements pre extraction and at 3 months post extraction prior to implant placement. A stent was fabricated using a Triad® VLC to replicate identical clinical measurement with maximum accuracy. The measurements included the position of the buccal crestal bone from the mid-root position to stent to evaluate for vertical bone loss using a periodontal probe (Figure 1). The socket width of the crestal 1/3, middle 1/3 and apical 1/3 were measured using a caliper which penetrated the soft tissue to bone contact to evaluate for horizontal bone loss

The preoperative protocol included 600 mg ibuprofen and chlorhexidine gluconate 0.12% mouth rinse (Peridex, 3M, Minneapolis, USA). All subjects underwent extraction with care to preserve the buccal and lingual plate by minimizing trauma and fracture (Figure 2).

The extraction sockets were then meticulously debrided. Subjects then received one of the following four treatments: Blood clot (control), PRF clot, PRF and 250-1000 μm FDBA mixture at a 1:1 ratio, or 250-1000 μm FDBA.

For PRF clot treatment group, the clot preparation from peripheral venous blood was in accordance with the protocol described by Choukroun (Douban 2006, Choukroun 2006). Venous puncture was obtained in most cases through antecubutal fossa. On Average, 10-20 ml of blood was collected in a sterile tube. The blood samples were centrifuged at 13,000 RPM for 8 minutes. PRF clot was separated from the tube and placed into the socket as a one whole clot. For the PRF+FDBA group, the PRF clot was separated into several pieces using an iris scissors and mixed with FDBA (AlloOss, Ace Surgical, MA, USA) in a metal bowl at a ratio of 1:1. For the FDBA alone treated group, the extraction sockets were augmented using 0.5 cc FDBA hydrated in saline solution.

All subjects received a Collaplug (Integra Life Sciences, New Jersey, USA) along with horizontal mattress 5.0 sutures and cyanoacrylate adhesive (PeriAcryl®, GluStitch Inc, British Columbia, Canada) to seal the margins. The post operative instructions included a 5 day regimen of Amoxicillin 500 mg three times daily, use of a chlorhexidine 0.12% mouth wash for 30 seconds two times daily and NSAID's for pain management.

After 3 months of healing, radiographs and CT scans were completed and the dental implants were placed. Before implant placement, bone cores from the implant site with a diameter of 2 mm internal diameter were trephined for histological analysis. Stents fabricated for the diagnostic casts were used to guide the trephine drills at the implant sites. The bone cores were harvested and stored in 10% neutral buffered formalin (Figure 3).

Histological Processing

All specimens were placed in a formalin solution and delivered to the Pathology lab at the University of Pacific for analysis. Processing for histology involved dehydration of the biopsies

and embedding in paraffin prior to sectioning the cores in an axial direction. Sectioned specimens stained with Hemoatoxylin and Eosin (H&E). Five sections per sample were analyzed under low power light microscopy to determine the most representative section for each core. The images were viewed under 40x magnification and a PDF image was created. The images were analyzed using ImageJ software (National Institute of Health, Bethesda, MD).

Histomorphometric Observations

Each sample was analyzed for: percent area of vital bone, residual bone, and connective tissue Following methods used in previous studies [15-17,33] . Total percentage of the area of connective tissue was determined as a function of the total area excluding bone area and artifacts. Vital bone was differentiated from residual bone by determining the presence of osteoblasts in the lacunae (See Figure 4A and 4B).

Results

The results showed that at 3 months post operatively the percent area of vital bone ranged from 29.06-53.7% across all groups with the lowest percentage seen in the FDBA group and the highest in the PRF group (Table 1). Overall, the highest percentage of vital bone was observed in the PRF and PRF+FDBA group, both of which had PRF introduced to the socket. The percent area of in the PRF group was 53.7%, and 41.31% in the PRF+FDBA group. The FDBA group had the least amount of percent area of vital bone at 29.06%. In comparison to the blood clot group (control group) both groups with PRF showed a higher percentage of vital bone.

A student T test was used to determine statistical significance between groups that had a P value of <0.05. There was a statistically significance between the percent area of vital bone in the PRF+FDBA group in comparison to the group FDBA group (Chart 1).

Residual bone graft material was observed in the PRF+FDBA (4.48%) and the FDBA group (12.41%). When combined the percent area for both residual bone and vital bone, the percent

area of bone was comparable for total bone area at 41.46% with the blood clot and PRF groups.

With regards to presence of connective tissue at the extraction sites, the PRF group had the least amount of connective tissue, whereas, the blood clot group had the highest amount at 52.44%. No statistically significant differences were noted for connective tissue or residual bone percentages between the four groups (Chart 2).

There were two trends noted: The higher the percentage of the area of FDBA, the smaller the percentage of vital bone. A second trend was that introduction of PRF into the socket was associated with a higher percentage of vital bone in healed sockets after three months.

Discussion

The primary goal of this paper was to evaluate the effect of PRF on bone healing through extraction sockets of single rooted teeth. The results from the present study with regards to percentage of vital bone present are similar with other studies reported in the literature. In the control group (blood clot group) of the present study, 38.59% of the area consisted of vital bone was observed. This was similar to Trombeli's work and others where they found 30-40% vital bone in non augmented sites at 12 weeks [3] .

In the present study, there was 29.06% vital bone observed in the FDBA group at 12 weeks. Wood reported a percentage of vital bone of 24.6-38.4% after 20 weeks when using FDBA and DFDBA respectively [33] . Isaella also observed 28% vital bone in healing sockets with FDBA [34] . These findings are consistent with our results despite the almost 2 months longer healing period in Wood study. By contrast, Beck showed that in 14 weeks there was 45.8% new bone and 14.6% residual bone in the FDBA group [35] . This percent of vital new bone is greater than the present study (29.06% in the present study). However, the residual graft is closely comparable between Beck's finding at 14.6% to the present study of 12.41% [35] .

The results presented in this study suggest that PRF has an osteogenic effect in bone formation. The presence of PRF mixed with FDBA from both clinical and histological perspective improved the quality and vital bone percentage from 29.06% to 41.31%. Vital bone percentage improved even more with removal of FDBA completely and presence of only PRF. Our data showed a statistical significance in percentage of vital bone present when using PRF as compared to FDBA, with an improvement from 29.06% to 53.71%. It should be noted that the reported 53.71% vital bone are some of the highest vital bone percentages reported among all grafting material in the literature including autograft, alloplast, allografts and xenografts [11,12,14,16,17,22,31,33,34,36]. Histological observations showed that FDBA particulates were typically not surrounded by vital bone, and most often FDBA socket cores were accompanied large artifacts likely associated with poor quality and density of FDBA healing. While the effect of FDBA in retaining ridge volume has been previously shown by several studies, it is reasonable to consider and evaluate the "osteo-obstructive" effects that FDBA may introduce in healing extraction sockets from both histological and clinical quality standpoint.

At present, the histological evaluation of vital bone among different materials is a standard evaluation approach. However, there are issues in the study design, case selection and analysis of data that may significantly influence the final results. Such considerations inherently introduce higher error rate. Case selection, root positioning and future implant placement position should strongly be considered with the position for bone core removal in mind. Ideal implants are placed in a prostheticaly driven position that may not be consistent with root positioning. This is a frequent occurrence in the anterior maxilla and particularly when a screw-retained prosthesis is desired. Currently, many of existing studies have controlled for single rooted teeth, and most studies that have contributed to our knowledge and literature have been limited to the premaxilla. This introduces a source of error in bone core collection and that data driven from such cores due to the differences in angulation from the root position and the implant body position. Other considerations such as presence of fenestrations and gingival phenotype have

been shown to influence healing and are typically not controlled for in ridge preservation studies.

Aside from histological evaluation, clinical assessment of bone quality should be considered as well. This can be a subjective parameter. Currently, there is a void in a recognized method for an objective assessment of grafting material from clinical viewpoint. Bone density classification may be considered, but caution should be taken due to limited stratification options [37] . In our experience, the quality of bone was in fact poorest in the FDBA group. Many times during the trephine bone core removal, residual particulates that were not well integrated would separate with poor vascularity. In comparison, group A (Blood clot) and group B (PRF clot) from a clinician perspective, offered a much more stable bone quality. In group A, B and C the trephine cores were removed successfully most often and bone appeared to be well vascularized and intact.

In evaluating the literature for socket grafting material of choice, the most important parameter to consider from histological perspective is the presence of vital bone as detected by osteoblasts in the lacunae. Based on biologic principles osteoinductive scaffolds should lend themselves to higher and perhaps faster vital bone formation. Some evidence from existing literature supports that DFDBA can produce higher amount of vital bone but that there is inconsistency with the amount of bone morphogenic protein-2 available within sample from each bone bank due to donor variations. Evidence suggests that that alloplasts such as calcium sulfate may in fact be quiet efficient in resorbing in opportune time where angiogenesis can occur without much compromise of clinical changes in ridge dimension.

However current systematic reviews fail to offer meaningful outcomes for clinicians to make decisions. This is due to the significant heterogeneity involved among existing literature. Some of the biggest confounding factors in comparing existing studies include the variation in healing time from one month to six months, lack of standardization in use of membranes (e.g. resorbable, PTFE, collagen plus), lack of negative or positive controls and difficulties in

standardizing donor bone. With regards to allografts, a current challenge is that the use of bone from various banks means no standardization among donor bone, and thus one subject can receive a donor that is of higher quality, thereby making comparisons difficult. If studies use bone from one donor, as some studies have done, then the disadvantage is that the result are not generalizable as they can change by donor factors such as age and genetics. Another contributing reason to the heterogeneity of existing literature is that the number of new studies on various products grows nearly as fast as new materials are introduced to the market. Thus, while a study may show promise of one particular material, the direction and follow up studies change direction by advances in our understanding of material and biologics.

The present study follows and suggests the following to help in said challenges: use of collagen to cover sockets for graft retention, use of FDBA and blood clot as controls, use of stents for consistent clinical measurements that can help compare and relate histological findings to clinical significance for clinicians. In the present study, we elected to use bone from bone banks to allow for generalizability of our data. Furthermore, we used FDBA as opposed to DFDBA in order to avoid the effect that bone morphogenetic proteins (BMP's) may have on different samples. Additionally, because of the large body of existing literature using FDBA, this can allow for increasing power of analysis for future systematic reviews and meta-analysis.

Some existing systematic reviews have made conclusions that socket preservation does not prevent against bone resorption [38] . Heggeller in a systematic review reports that without socket preservation, an average alveolar resorption of 2.6-4.6mm was observed, and among socket preserving materials, FDBA was the most successful though still resulted in an average width resorption of 1.2 mm. Contrarily Avila, in a systematic review stated that socket preservation can be an efficient technique in preventing horizontal and vertical physiological bone resorption [39] . In future systematic reviews, the outcomes measured should have relevance to both patients and clinicians. Therefore we suggest the consideration for following outcomes in interpretation of evidence: implant placement, implant placement without additional

augmentation and implant success. Future studies should aim to report this information and follow similar protocols in study design to allow for higher power systematic reviews.

Conclusion

The data from this study suggests that PRF can play an osteogenic role in bone healing as evident though histology from single rooted extraction sockets. Ability to modulate growth factors, substantivity, ease of access, safety with regards to transmitted-diseases all make PRF a promising material. Future studies will be needed to replicate and expand on our findings as well as exploring additional benefits that platelet rich fibrins may offer in regenerative surgery.

Tables, Charts 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. Demographics of study patients

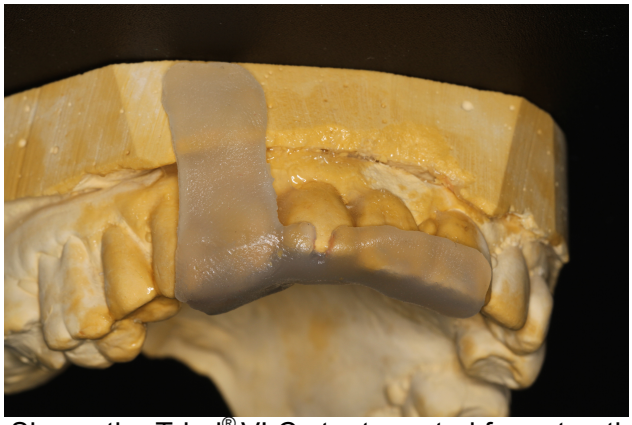

Figure 1- Shows the Triad® VLC stent created for extraction of tooth #7

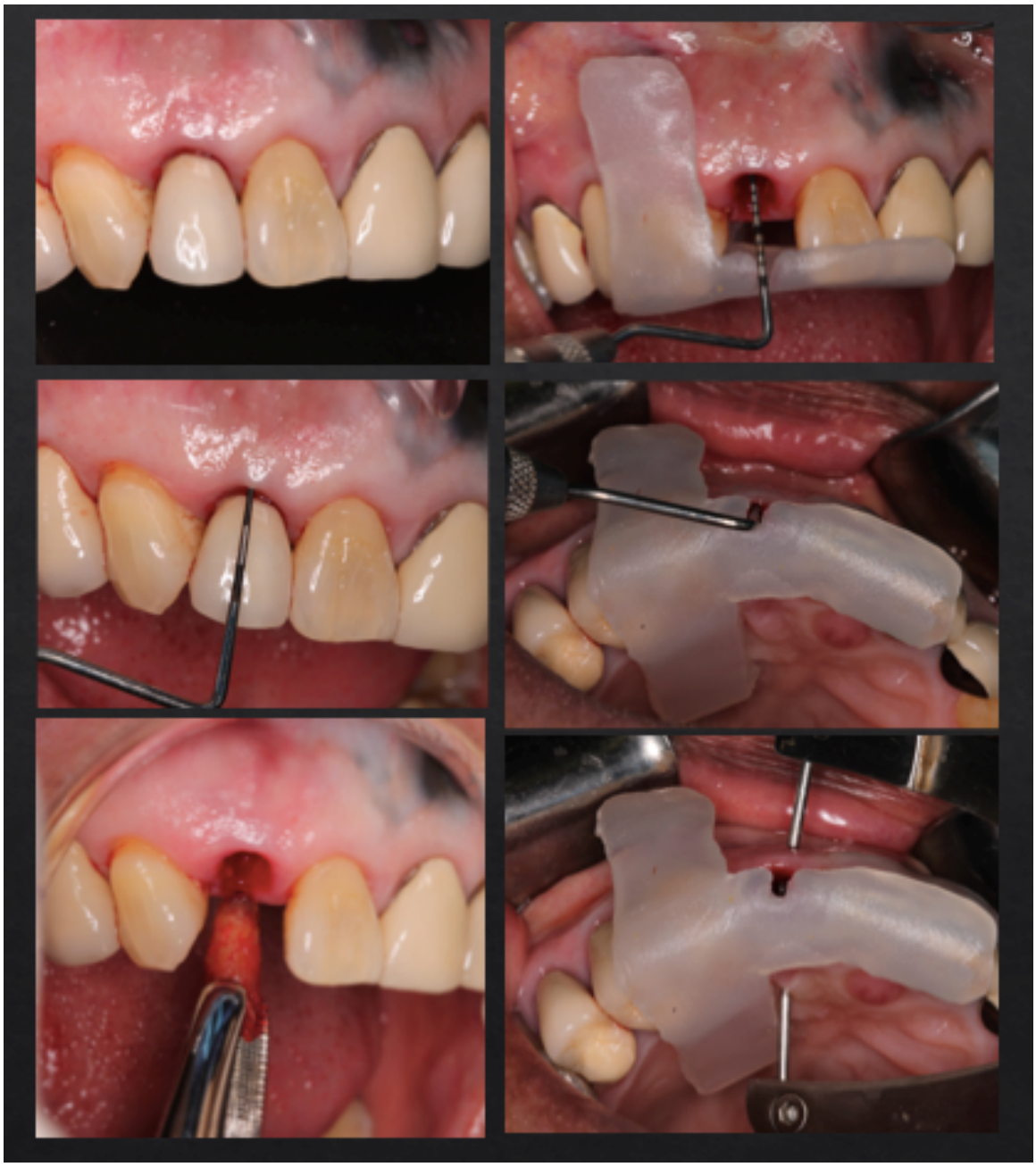

Figure 2. Illustrating presence of intact buccal bone, atraumatic extraction and ridge dimension measurements immediately post extraction

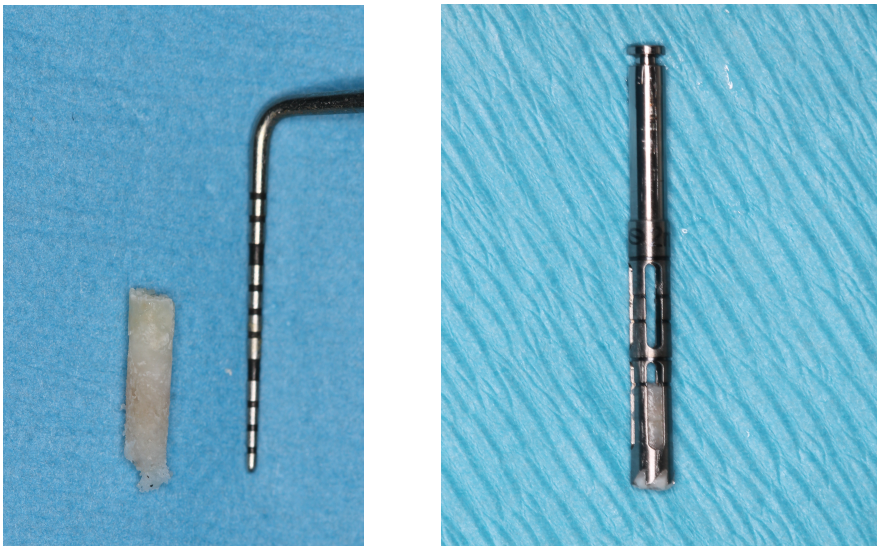

Figure 3: Bone core immediately at the harvest

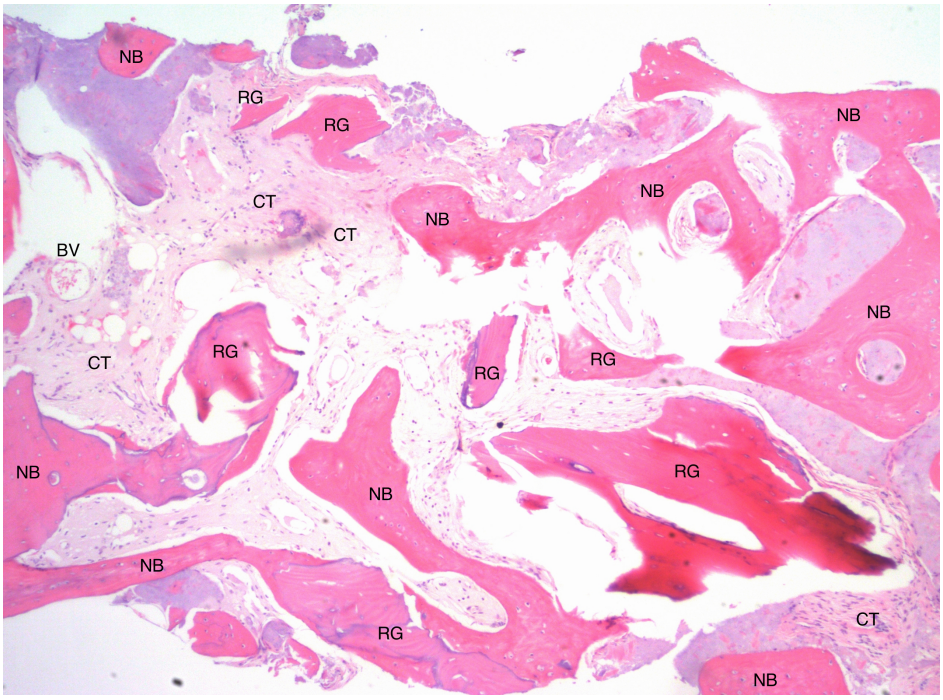

Figure 4A: H&E of group C specimen. New Bone: NB, Residual Graft: RG, Connective Tissue: CT

Figure 4B. H&E Samples of group A, B, C, D from left to right

Blood Clot PRF PRF+FDBA FDBA

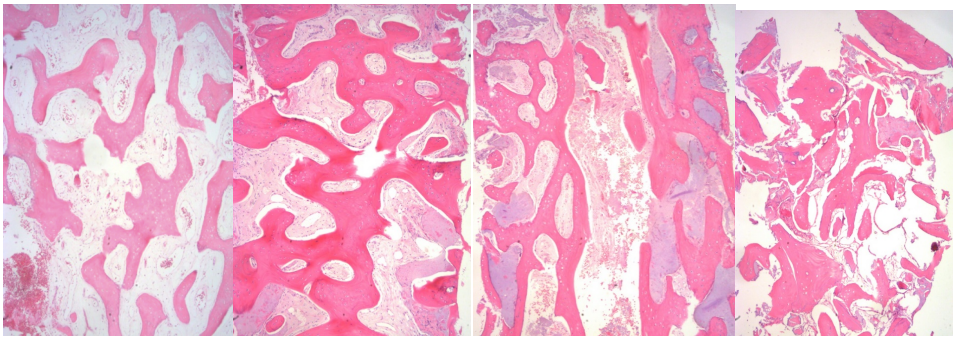

Table 2. Histological Observations

Core	% New Bone	% CT	%FDBA
A3	32.20%	63.00%	
A8	55.94%	38.50%	
A17	44.08%	48.05%	
A20	39.21%	51.01%	
A22	44.52%	47.25%	
A12	28.85%	66.42%	
A26	28.83%		
A42	13.56%	69.41%	
A45	60.13%	35.89%	
MEAN / SD	38.59 +/- 14.53	52.44 +/- 12.6	
B5	76.76%	18.65%	
B6	38.17%	54.82%	
B13	23.71%	65.13%	
B16	34.28%	64.17%	
B19	76.46%	9.43%	
B25	47.28%	33.61%	
B47	56.33%	40.39%	
MEAN / SD	53.71 +/- 21.2	38.63 +/- 21.2	
C2	52.88%	41.93%	
C9	52.07%	37.75%	
C18	52.04%	41.12%	3.49%
C28-1	40.01%		
C28-2	50.48%	33.10%	8.21%
C41	15.30%	49.42%	1.77%
C44	26.37%	43.44%	
MEAN / SD	41.31 +/- 15.00	41.13 +/- 5.49	4.48 +/- 3.33
D1A	37.50%	41.10%	2.89%
D1B	33.05%	43.54%	12.51%
D10	21.85%	45.73%	23.62%
D7	3.70%	72.97%	4.72%
D40	40.17%	50.28%	
D43	38.10%	27.21%	18.31%
MEAN / SD	29.06 +/- 14.05	46.81 +/- 15.00	12.41 +/- 8.81

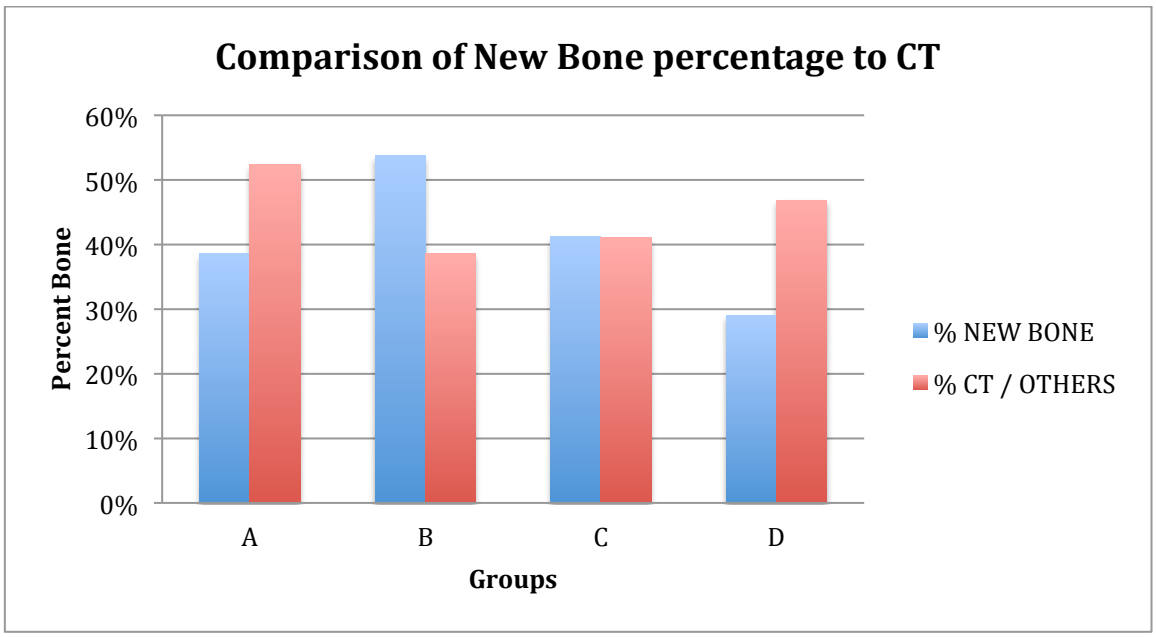

Chart 1. Comparison of New Bone and Connective Tissue among all groups

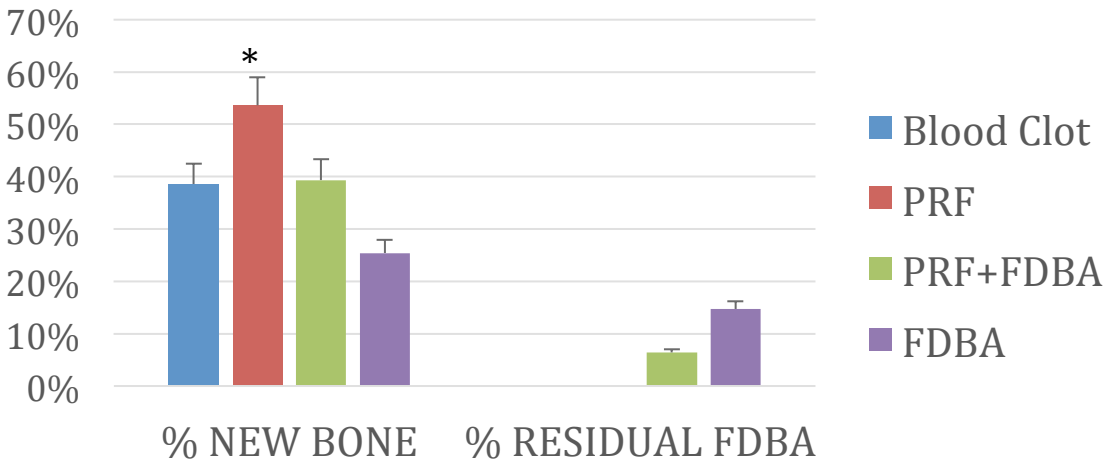

Chart 2. Comparison of New Bone and Residual Bone among all groups

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