EFFICACY OF PLATELET-RICH FIBRIN ON WOUND HEALING AND PAIN FOLLOWING GUIDED BONE REGENERATION

by

Sohyun Park

Virginia Commonwealth University, 2006

State University of New York at Buffalo, School of Dental Medicine, 2010

University of Pittsburgh, School of Dental Medicine,

Department of Periodontics and Preventive Dentistry, 2017

Submitted to the Graduate Faculty of

University of Pittsburgh in partial fulfillment

of the requirements for the degree of

Masters of Dental Science

University of Pittsburgh

2017

UNIVERSITY OF PITTSBURGH

SCHOOL OF DENTAL MEDICINE

This thesis was presented

by

Sohyun Park

It was defended on

July 11, 2017

and approved by

Satish Kumar, DMD, MDSc, MS, Assistant Professor,

Department of Periodontics and Preventive Dentistry

Anitha Potluri, BDS, DMD, MDSc, Associate Professor,

Director of Oral Maxillofacial Radiology, Department of Diagnostic Sciences

Herbert Ray, DMD, Assistant Professor,

Director of Graduate Endodontics, Department of Endodontics

and

Thesis Director: Kelly Williams, DMD, Assistant Professor,

Director of Graduate Periodontics, Department of Periodontics and Preventive Dentistry

Efficacy of Platelet-Rich Fibrin on Wound Healing and Pain

Following Guided Bone Regeneration

Sohyun Park, DDS, MDS

University of Pittsburgh, 2017

Purpose: The purpose of this study was to evaluate the effect of platelet-rich fibrin (PRF) on the soft tissue wound healing and postoperative pain when it is used as an adjunct therapy for guided bone regeneration (GBR) procedures.

Materials and Methods: Seven patients were randomly assigned to test group (GBR + PRF; n=5) and control group (GBR only; n=2), and soft tissue healing and pain levels were evaluated. Routine GBR procedures using dense polytetrafluoroethylene (dPTFE) membranes were performed for control group. For the test group, the liquid-form PRF was mixed with bone graft materials and PRF membranes were placed on top of the dPTFE membrane. Following surgery, patients in both groups were given a visual analog scale (100 mm) to use for rating their pain level every night for the next week. Post-operative appointments were scheduled at weeks 1, 2, 4, 6, 8, 12, and 16. During the post-operative appointments, the soft tissue healings were evaluated. The present report follows patients for the first 6 weeks post-GBR surgery.

Results: Patients in the test group showed a more rapid decline in self-reported pain during the first week after the surgery, with ratings for the test group being substantially lower than those for the control group. Whereas no membrane exposure was noted for either control patient,

exposure was noted for two of the five test patients during follow-up visits. In contrast to membrane exposure, evidence of gingival inflammation was noted in all patients. Similar to the findings for membrane exposure, abnormality in soft tissue consistency was noted for participants in the test group only, mainly in the exposure patients.

Conclusion: The present study suggests that addition of PRF to GBR procedures may lower the postoperative pain level. But due to the small sample size, it was difficult to draw a conclusion on the effect of PRF on all of the outcomes especially the membrane exposure and other soft tissue parameters. After additional data are collected by the next investigator, we may be able to draw more definitive conclusions. The next report will also look at the bone quality and quantity in the histologic and radiographic analysis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

1.1 PLATELET-RICH FIBRIN

Platelets are cytoplasmic fragments of approximately 2 μm in diameter of megakaryocytes which is formed in the bone marrow and survive between 7 and 10 days.[1] They have more than 30 bioactive proteins, many of which are important for hemostasis or tissue healing through the inflammation process. The following fundamental protein growth factors are actively secreted by platelets and commence wound healing processes: platelet-rich fibrin (PRF) including platelet derived growth factor (PDGF), transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblastic growth factor (FGF), connective tissue growth factor (CTGF), and insulin-like growth factor (IGF-1).[2,3]

1.1.1 Platelet Concentrate Products

Platelet concentrates are a relatively new form of biomaterials for regenerative procedures, which are widely used in medicine and dentistry. It is known that the regenerative potential of these concentrates derives not only from the previously

mentioned growth factor contents, but also from associated leukocytes, circulating progenitor cells, and cell adhesion molecules such as fibrin, fibronectin and vitronectin.[4,5]

Two main types of autologous platelet concentrates are currently used in dentistry: platelet-rich plasma (PRP) and platelet-rich fibrin (PRF). As a first-generation platelet concentrate product, PRP was the first to be used as a therapeutic adjunct for oral and maxillofacial surgery in 1997 by Whitman et al.[6] and thus has been more widely studied than the second-generation product, PRF. Since Choukroun and colleagues first published their work on PRF—initially in French (2001)[7] and later in English (2006)[8-12]—several comparison studies have demonstrated the advantages of PRF over PRP, which can be summarized as follows:[2,13]

- simplified preparation;
- no biochemical manipulation of blood;
- greater number of platelets;
- greater number of growth factors (GF);
- about 65% leukocyte contents whereas PRP has 0-50% leukocytes;
- more gradual release of GF; and
- stronger and more durable effect on proliferation and differentiation of osteoblasts.

These characteristics of PRF will be compared to PRP in detail below for further understanding.

1.1.2 Superiority of PRF over PRP

Simpler Preparation and manipulation. Dhurat and Sukesh (2014)[3] described the PRP preparation protocol. Whole blood is drawn by venipuncture in acid citrate dextrose tubes and centrifuged using a soft spin for 10 minutes. The supernatant plasma layer on top of the tube is separated from the red blood cell layer and transferred to a new tube for second centrifugation at hard spin for another 10 minutes. The most platelet and leukocyte concentrate will settle in the bottom ⅓ of the tube. Therefore, by removing the upper ⅔, a provider gets the final product for PRP from the remaining bottom layer. (Figure 1). This layer is then activated by adding bovine thrombin or calcium before being applied to the surgical site.

Unlike PRP preparation described above, PRF preparation only needs one centrifugation and does not need any additives either in the blood collecting tube or on the final product after centrifugation.

According to the French group who invented PRF preparation procedure (Dohan et al., 2006),[8] a blood sample is collected in 10-mL glass tubes without any anticoagulant additives. Anticoagulants are not needed for PRF because natural silica on the glass tube walls initiates the coagulation cascade.[14] This natural silica does not represent a cytotoxic risk like the bovine thrombin used for PRP preparation.[15] Without anticoagulants, the activation of platelets happens as soon as it comes in contact with the natural silica on the glass tube and the coagulation cascade initiates, which transforms highly concentrated fibrinogen into fibrin by the circulating thrombin. Therefore, a quick blood collection and immediate centrifugation is a key strategy for PRF preparation.[8]

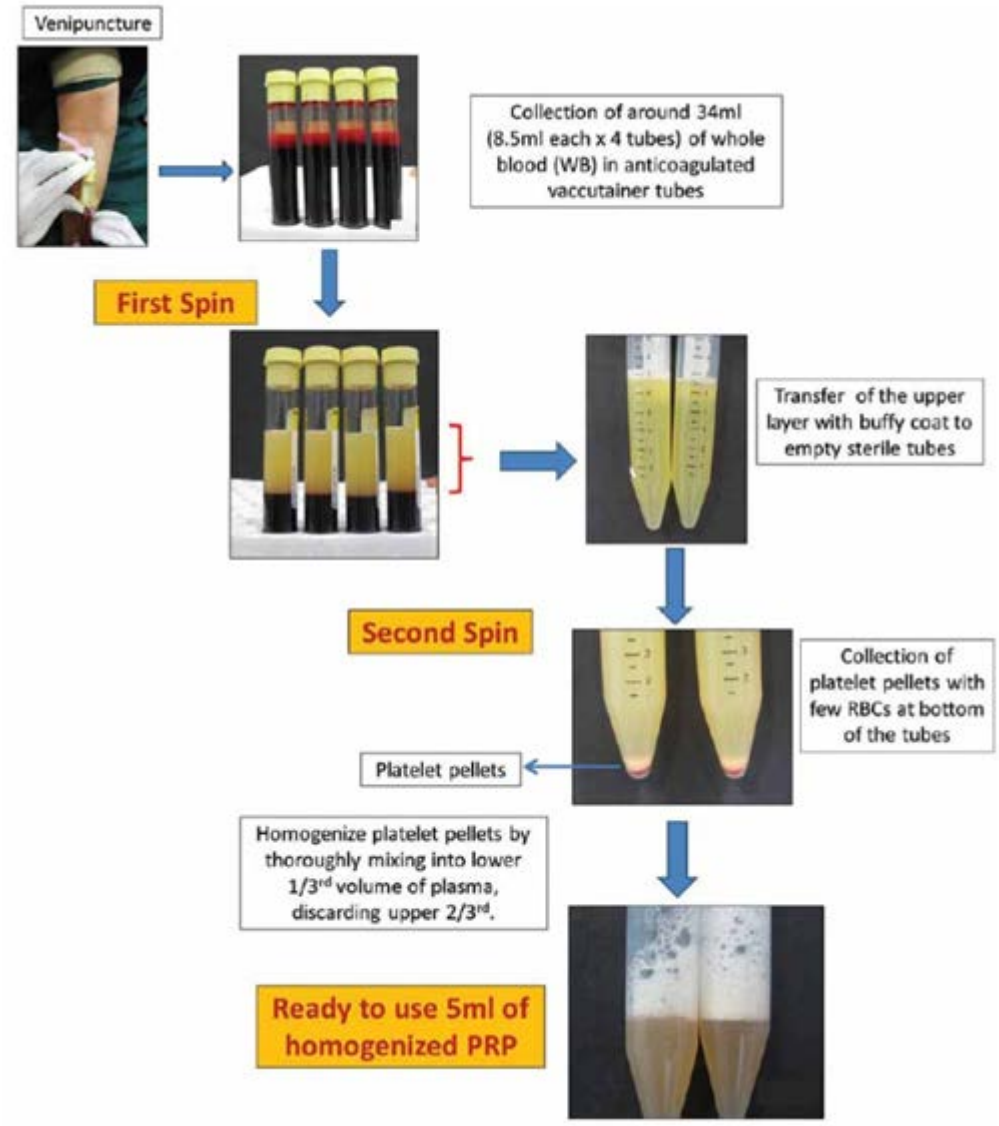

FIGURE 1. Flow diagram describing preparation of platelet rich plasma (PRP). Figure reproduced from Dhurat R, Sukesh M. Principles and methods of preparation of platelet-rich plasma: A review and author's perspective. J Cutan Aesthet Surg 2014;7(4):189-197. [3]

After an immediate centrifugation at 3000 rpm (approximately 400g) for 10 minutes, a dense fibrin clot is formed in the middle of the tube, between acellular plasma at the top and the red blood corpuscles at the bottom. This fibrin clot is then picked up from the tube and transferred to a flat sterile surface for a compression to obtain the final fibrin membrane.[8,14] (Figure 2)

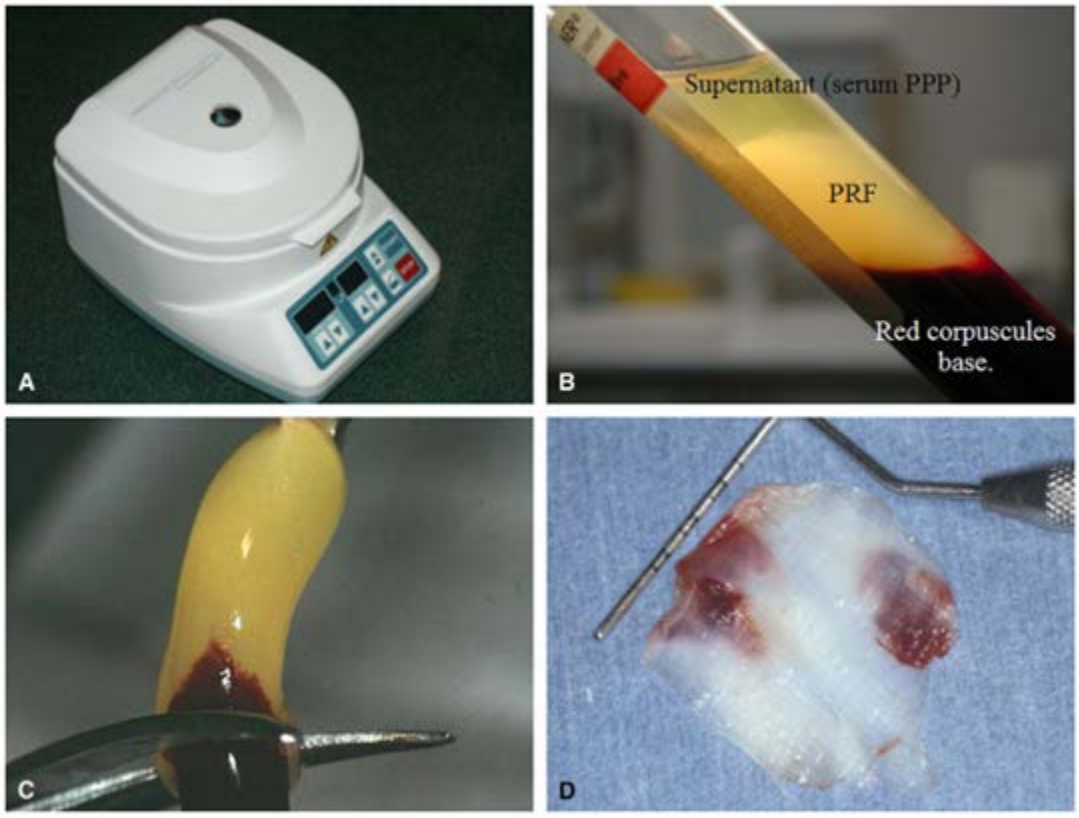

FIGURE 2. (A) Blood processing with a centrifuge for PRF allows the composition of a structured fibrin clot in the middle of the tube (B), which easily makes resistant autologous fibrin membranes by driving out the serum from the clot (D). Figure and caption reproduced from Dohan DM, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I. Technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101(3):e37-44.[8]

Higher values of platelets, growth factors and leukocytes. A comparison study in 2015[2] found a greater quantity of platelets and the growth factors PDGF, VEGF and TGF in PRF compared to PRP. Moreover, about 65% of white blood cells from the total blood sample become entrapped in the fibrin matrix after centrifugation in PRF, whereas less than 50% of white blood cells are concentrated in PRP. The authors of the study concluded that PRF membrane has a higher potential to more efficiently and effectively regulate the local immune response and with better feedback control of the inflammation. Clinically this leads to the better control of postoperative infections when the PRF is utilized in the surgical procedure. They also mentioned that three-dimensional PRF fibrin matrix conformation is optimized to start the neo-angiogenesis from rapid invasion by leukocytes, which contain the VEGF that acts as a potent vascular growth factor.[2]

More gradual release of growth factors. An in vitro study,[13] quantified the levels of platelet-derived growth factor AB (PDGF-AB) and transforming growth factor 1 (TGF-1) in PRP and PRF produced from human blood sample. These products were used to culture 96-well plates of rat calvaria osteoblasts, with the biologic characteristics of osteoblasts being analyzed for 14 days. Results showed that both growth factors were released at the highest amounts on the first day in PRP followed by significantly decreased release later on. On the other hand, PRF released the highest amount of PDGF-AB at day 7 and of TGF-1 at day 14. Further investigation on the cell mineralization revealed PRF treated osteoblasts reached peak mineralization at day 14, which was significantly higher than control and PRP treated cells. The authors of the study concluded that PRF is superior to PRP in terms of gradual release of growth factors and expression of more significant cell proliferation and differentiation of rat osteoblasts in vitro.[13] The gradual growth factor release of PRF is

deemed due to the nature of fibrin matrix that biodegrade gradually, unlike the liquid form of PRP. According to Kawase et al.,[16] it takes approximately 1 to 2 weeks for PRF membrane to completely resorb in vivo.

1.1.3 Clinical Applications

The efficacy of platelet concentrates on soft and hard tissue regeneration has been studied in diverse disciplines of dentistry including periodontics, endodontics, and oral and maxillofacial surgery. Applications include endodontic pulp regeneration, free gingival graft, connective tissue graft, surface treatment of implant for the stimulation of osseointegration, the treatment of peri-implantitis and intrabony defects, socket preservation, maxillary sinus augmentation, treatment of orofacial clefts, medication-related osteonecrosis of the jaw (MRONJ), and guided bone regeneration (GBR).[17-41]

1. Endodontics

Endodontic treatment of necrotic immature teeth is not always easy due to thin dentinal walls that frequently fracture. Calcium hydroxide formally had been widely used, but its long-term use reduced micro-hardness of the dentin by disruption of the links between collagen fibers and hydroxyapatite crystals, which increased risk of fracture. A newer material, mineral trioxide aggregate (MTA), was introduced but fracture is still a challenging issue due to relatively unchanged dimension of thickness of dentinal walls and length of the roots after the treatment. As several studies have evaluated feasibility of regenerative procedures to overcome these problems, platelet concentrate products have been recommended as a potential scaffold.[17,18]

Keswani and colleagues evaluated seventy children who needed pulpotomy with incomplete root development for 24 months after treatment with MTA (control) and PRF (test).[19] They reported that clinically and radiographically evaluated success was not significantly different between the groups. But at the 24-month follow up, radiographic evaluation revealed that complete apical closure was observed in 88.8% of the PRF group and 80.07% of roots in the MTA group. The authors concluded PRF is a good alternative to MTA in pulpotomy procedures of permanent teeth with incomplete root development.

2. Palatal wound after autogenous soft tissue graft harvest

When 40 wounds were compared between PRF (N=20) and gelatin (N=20) sponge, the PRF group showed a significantly faster complete re-epithelialization of the palatal wound ($P < 0.001$).[20] At the end of week 2, 35% of the test group patients showed complete re-epithelialization whereas only 10% of the gelatin sponge group showed complete healing. At the end of week 3, all palatal wounds in the test group epithelialized completely, whereas only 25% of the control group showed complete re-epithelialization. PRF patients also reported significantly less discomfort and took a significantly lower dose of analgesics ($P = 0.02$). The authors concluded that PRF significantly accelerates palatal wound healing and reduces patients' morbidity.

3. Coronally advanced flap

A randomized, split-mouth, controlled study compared PRF membrane (test) with subepithelial connective tissue graft (SCTG; control) for a modified coronally advanced flap (MCAF) in treatment of a total of 60 defects in 20 patients with Miller Class I and II bilateral multiple gingival recessions.[21] The percentage of root coverage was significantly greater in

the control group (84%) relative to the test group (77%). By comparison, complete root coverage differed only marginally between the two groups (60% and 50% in control and test groups, respectively). Keratinized tissue width (KTW) and gingival thickness (GT) significantly increased in both groups at 6 months after the procedures; KTW was significantly wider in the control group whereas GT was significantly thicker in the test group. Additionally, PRF group showed significant decrease in patients' self-reported postoperative discomfort (visual analog scale) compared to SCTG group. The author concluded that the PRF is a valid alternative to SCTG to treat localized gingival recessions with an additional benefit of giving less discomfort during the healing period.[21]

Another study compared the addition of PRF membrane for modified coronally advanced flap (MCAF) (test) with MCAF only (control).[22] The study recruited twenty subjects who had multiple Miller class I and II multiple gingival recessions on both sides of the mouth. At 6 months, 74.6% of the treated sites showed complete root coverage in control group, whereas only 52.2% of the treated sites showed completed root coverage in the test group. But increase of gingival thickness in the test group (1.1 ± 0.3 mm → 1.4 ± 0.5 mm) was statistically significant when compared to the control group (1.1 ± 0.3 mm → 1.1 ± 0.3 mm). The authors concluded that addition of PRF membrane under the MCAF is less predictable for root coverage but more predictable for gain in gingival thickness at 6 months.

A recent meta-analysis study based on six previous studies followed up for ≥6 months, however, reported that the use of PRF membranes did not give any additional benefits compared to the other treatment modalities in terms of the root coverage,

keratinized tissue width, or clinical attachment level in Miller Class I and II gingival recessions.[23]

4. Intrabony defects

A study of chronic periodontitis patients evaluated the efficacy of PRF on treatment of a total of fifty-six 3-wall periodontal intrabony defects.[24] The test group received PRF and open flap debridement and the control group received open flap debridement only. Probing depth (PD) and periodontal attachment level (PAL) were recorded at baseline and 9 months postoperatively. Results showed that the PRF group demonstrated greater PD reduction (4.55 ± 1.87 mm) and CAL gain (3.31 ± 1.76) than the control group (3.21 ± 1.64 mm and 2.77 ± 1.44 mm, respectively). They also compared the bone fill using the baseline and 9 month postoperative radiographs, and reported a significantly greater percentage of mean bone fill in PRF group (48.26% ± 5.72%) compared to the control group (1.80% ± 1.56%). The authors concluded that addition of PRF was beneficial for treatment of intrabony defects.

Another study investigated the effectiveness of PRF in the treatment of intrabony defects with open flap debridement (OFD) only (control) or with PRF (test), and reported similar findings.[25] The results revealed that the test group was superior to the control group on all the clinical and radiographic parameters that they evaluated and the difference was statistically significant.

The above studies proved the effectiveness of PRF on intrabony defects when it is used with open flap debridement. However, PRF membrane resorption time is approximately 1-2 weeks,[16] which therefore would not serve as a good scaffold if one is

expecting to gain some bone regeneration. A split-mouth study was then performed to compare PRF only with PRF with bone graft material[26] and investigate the effect of the actual bone particulate when it is used with PRF. Seventeen paired intrabony defects were treated with PRF only on one site of the mouth (PRF) and PRF with bovine porous bone mineral (PRF-BPBM) on the other site of the mouth. Re-entry surgeries were performed at 6 months. Results showed that PRF treatment improved the defect conditions on defect fill, PD reduction and CAL gain, but PRF-BPBM treatment improved more significantly as compared to the PRF only group. The authors of the study concluded that PRF can be used to treat periodontal intrabony defects, and one can expect even more significant effect by adding BPBM to PRF.

5. Socket preservation

Many socket preservation studies evaluate the effectiveness of PRF treatment on soft tissue healing, bone fill and discomfort and pain level following third molar extractions.[27-33] Currently there is mixed evidence on the effect of PRF in socket preservation, especially post-extraction pain. One of the impacted third molar extraction studies failed to show any beneficial effect of PRF on pain relief,[27] but some other studies suggested that surgical removal of impacted third molar with PRF significantly reduced not only the pain but also the other postoperative complications such as swelling and trismus, and it promoted soft tissue wound healing after tooth extraction.[27-33]

Another possible complication exists when the impacted mandibular third molar is extracted; a delayed compromised healing causing distal bone loss and prolonged sensitivity due to root exposure or increased probing depth.[31] Kumar and colleagues

evaluated the efficacy of PRF treatment on probing depth after impacted third molar extractions.[31] Results showed that the application of PRF led to a larger average decrease in postoperative pocket depth at 3 months relative to treatment without PRF (PRF, 5.94 mm to 3.40 mm; control 6.09 mm to 4.78 mm). Results also indicated that pain, swelling, and trismus on day 1 postoperative were significantly less in the PRF group compared with the control group. The authors additionally reported that bone density scores at 3 months follow-up were higher in the PRF group than in the control group, even though the difference was not statistically significant.

A different split-mouth study published in 2012[30] reported that PRF was significantly effective for both soft and hard tissue regeneration. Twenty patients who need bilateral mandibular third molar extractions were evaluated after PRF clot was placed in the socket. Soft tissue healing and bone regeneration were evaluated for 3 months. Soft tissue healing was significantly superior in PRF group based on the soft tissue healing index by Landry, Turnbull and Howley.[34] At the 4 week postoperative radiographic evaluation, it was noted that trabecular bone formation started earlier in the PRF site as compared to the counterpart on the other side of mouth. At 3 months, postoperative radiographic evaluation of bone density showed that the grey level value in the PRF site was significantly higher than the control sites. The authors concluded that PRF significantly improved soft tissue healing, bone regeneration and bone density after the extraction sockets.

6. Sinus lift

Seventy-two maxillary sinus elevation grafting procedures using either Bio-Oss® and PRF (test group) or Bio-Oss® only (control) were followed up with bone core

harvesting at the time of implant placement surgery for histologic evaluation at day 106, 120 and 150.[35] The PRF clot was obtained, with part of it being used as a filling material in a form of "amorphous PRF" to be placed inside the sinus, and the rest flattened to make fibrin membrane out of it to be transferred onto *Schneiderian* membrane.

The results revealed that bone maturation time was decreased with the use of PRF from the average healing time of 120-150 days as noted in previous literature for optimal bone regeneration,[36,37] to 106 days—a reduction of 30%. At 106 days, it was already possible to place the implants with good primary stability, though without functional loading. The authors suggested that the gelatinous consistency of PRF made the clot stability better and the fibrin membrane created a natural "barrier effect" on the bone breaches opened in the surgical site.

7. Orofacial clefts

Twenty-four patients with unilateral alveolar cleft were evaluated after bone reconstruction with PRF and autogenous anterior iliac crest bone graft (test) and autogenous bone graft alone (control).[38] Bone quality and quantity were assessed with computed tomography at 6 months postoperatively. The newly formed bone quantity was significantly superior in the PRF group (mean 82.6% ± 3.9%) compared to the control group (mean 68.4% ± 6.7%). The newly formed bone density (quality) was inferior in the PRF group than the control group, but the difference was not statistically significant. The authors concluded that addition of PRF in alveolar cleft reconstruction improved the volume of newly formed bone but was not significantly beneficial for bone density.

8. Medication-Related Osteonecrosis of the Jaw (MRONJ) or Osteoradionecrosis (ORN)

According to a 2014 AAOMS position paper, a MRONJ diagnosis is established under these conditions: 1) Current or previous treatment with antiresorptive or antiangiogenic agents; 2) exposed bone or bone that can be probed through an intraoral or extraoral fistula(e) in the maxillofacial region that has persisted for more than eight weeks; and 3) no history of radiation therapy to, or obvious metastatic disease in the jaw bones.[39]

ORN is diagnosed when the previously irradiated field shows the clinical findings of exposed necrotic bone without evidence of tumor recurrence. ORN can happen spontaneously but a lot of times is triggered by traumatic events such as tooth extraction or ill-fitting denture.

Once MRONJ or ORN happens, it is very challenging to manage and usually only conservative treatments are rendered such as patient education, antibacterial mouth rinse, systemic antibiotics, and debridements depending on the severity of the condition.

A recent case study evaluated PRF use to manage 3 patients with MRONJ and 4 patients with ONJ. The treatment protocol was 1) debridement to bone with piezoelectric handpiece; 2) placement of PRF; and 3) primary closure without surgical flaps. Full soft tissue coverage was noted in 5 out of 7 patients at 2, 8, 10, 13 and 15 weeks respectively. The authors of the study concluded that PRF can be a good adjunct therapy for MRONJ or ORN. [40]

9. Guided Bone Regeneration

PRF is also used for guided bone regeneration procedure as an adjunct material to be mixed with the bone particulate as a liquid form or chopped membrane form, or as a sole or additional membrane as a fibrin membrane form. A case report evaluated the potential of platelet-rich fibrin (PRF) membranes on the bone and soft tissue regeneration.[41] PRF membrane was placed on top of the combination of autogenous bone, bovine hydroxyapatite and PRF membrane that was cut into small pieces. They evaluated the surgical site radiographically for bone gain based on CBCT and clinically for soft tissue maturation 4 months after the surgery. The authors concluded that PRF was effective in terms of period of time for healing, gingival maturation and bone regeneration.

In the present study, efficacy of PRF on guided bone regeneration (GBR) will be evaluated for soft tissue healing and pain. A more detailed discussion of the use of PRF in GBR procedures is presented below.

1.2 GUIDED BONE REGENERATION

As the demand for dental implant procedures increases, pre-implant bone augmentation procedures to increase the height and width of alveolar bone are also on the rise. In 1957, Murray and his team[42] was one of the first research groups to come up with the idea of protecting bone defects from the overlying soft tissue by using a plastic cage barrier for new bone regeneration on decorticated dog femur. And since Dahlin and colleagues first introduced the successful bone regeneration by utilizing Teflon membrane

in mandible of rats in 1988,[43] guided bone regeneration (GBR) has been developed into one of the most common bone augmentation procedures for the edentulous ridge. Many clinicians and researchers have tried with different materials and methods for GBR to achieve better results, but the main principles of GBR has not changed.

According to "PASS" principles by Wang et al.,[44] four major biologic principles need to be met:

1. *Primary closure*: Primary closure should be accomplished for an optimal environment away from bacterial infiltration and along with tension free closure for better chance to keep the site unexposed.

2. *Angiogenesis*: Adequate blood supply and angiogenesis are essential for bone regeneration, with decortication if one thinks it is beneficial for better blood supply, though it is controversial.

3. *Space creating/maintenance*: Space needs to be created and maintained for bone to form while unwanted epithelial and connective tissue cells are excluded. Prevention of membrane collapse is also important, especially for higher amounts of bone regeneration.

4. *Stability of wound*: Stabilizing the blood clot underneath the membrane is an important key because this initial blood clot has ample amount of cytokines, growth factors, and signaling molecules for initial healing and this clot is the precursor of granulation tissue which eventually turns into bone.

1.2.1 Membrane Types

There are two main types of membranes, resorbable and non-resorbable, and their subgroups depending on the source and processing method.

For a list of typical commercially available membranes, please refer to the publication by Rakhmatia et al.[45]

Collagen membranes are made of natural source from bovine, porcine or human, while polyglycolic acid/polylactic acid (PGA/PLA) membranes are made of synthetic aliphatic polyesters. Resorbable membrane does not require a second surgery for removal, but space maintenance has been a main issue due to rapid degradation and flexibility. Cross-linking techniques are used to overcome the rapid degradation, but some fixatives used to treat the membrane for this feature such as glutaraldehyde are known to be not completely cytotoxic free.[46] A new technique was recently introduced by Urban et al.[47] to overcome this drawback of the resorbable membrane by tacking the stretched native collagen resorbable membrane to stabilize and protect the particulate bone graft inside until it completes initial graft maturation.[47]

Non-resorbable membranes including titanium mesh, ePTFE (expanded polytetrafluoroethylene) and dPTFE (high-density polytetrafluoroethylene) are mainly used in current dentistry. ePTFE was first developed in 1969 and became the standard in bone regeneration in early 1990s but discontinued for dental use once dPTFE came out in 1993 to enhance the strength of ePTFE and make up for the weakness of it.[45]

Premature membrane exposure during initial healing with non-resorbable membranes is one of the most common complications of regenerative procedures[48] and immediate removal of membrane was necessary to prevent bacterial infiltration to the underlying bone graft through the pores of the ePTFE since its pore sizes are bigger (0.5-30 microns) than most of the oral bacteria (approximately 0.2-10 microns).[45] On the other hand, dPTFE has smaller pore sizes (0.2-0.3 microns) to prevent bacterial passing through, so the manufacturers claim that the membranes are not required to be removed even with exposure, as long as the exposure is not extending to the edge of the membrane.[45] Some studies reported that food and bacteria were completely blocked even when the soft tissue dehiscence exist to cause membrane exposure, so one can still leave the membrane as exposed.

1.2.2 Membrane Exposure

In a study involving 102 guided tissue regeneration surgeries,[49] the average time for Gore-tex membrane exposure was 16.2 days. Membrane exposure usually happens during the initial healing phase prior to soft tissue maturation; thus if membrane exposure can be prevented during the initial healing phase, more desirable outcomes are expected.

In a meta-analysis conducted by Machtei et al.,[50] the overall incidence of membrane exposure in GBR procedures was 60% with either resorbable or non-resorbable membrane and this exposure resulted in a significant decrease in the amount of new bone regeneration.

Further evidence by Simion et al.[51] quantified the effect of e-PTFE membrane exposure around implants and found that the desired level of bone was regenerated in 96.6% of cases when membranes were completely covered by tissue flaps at the time of implant placement one-month post-extraction, while only 41.6% of cases gained the desired level of bone when membranes were left exposed after immediate implant placement.

More recent studies show that bone regeneration is not affected by premature membrane exposure if d-PTFE barrier membrane is used.[52,53] But according to multiple case reports, once membrane is exposed prematurely, even if bone still regenerates, clinicians may encounter multiple problems, such as compromised patient comfort level, additional office visits for closer follow-ups, additional surgical procedures to remove exposed membrane before implant placement when indicated.[54-56] Therefore, membrane exposure prevention should be attempted whenever it is possible.

1.2.3 Can platelet concentrate products help prevent membrane exposure?

In GBR procedures utilizing titanium mesh, the test group with titanium mesh in combination with PRP had no membrane exposure, whereas the control group had 28.5% exposure.[57] Moreover, height and width of bone augmentation was significantly superior in the PRP group (3.5 ± 0.7 mm height and 4.1 ± 0.6 mm width) compared to control group (3.1 ± 0.8 mm height 3.7 ± 0.6 mm width).[57] The author of this study concluded that addition of PRP to the GBR procedure when Ti-mesh is used as a membrane, it is beneficial to prevent complications such as mesh exposure and consequent graft failure.

1.3 PURPOSE OF STUDY

The present study evaluates whether the addition of PRF to conventional GBR procedures has a beneficial effect on the following clinical outcomes: incidence of membrane exposure, incidence of suppuration at the graft site, soft tissue consistency, severity of soft tissue inflammation, and severity of postoperative pain. The evaluation on the quantity and quality of bone based on radiographic and histologic analysis will be discussed on the next part of the study. The null hypothesis for this whole research project is that there is no difference in clinical outcomes between GBR procedures with and without PRF treatment.

2.0 MATERIALS AND METHODS

2.1 STUDY POPULATION

Twenty patients requiring GBR prior to implant placement will be eventually enrolled in the study based on the inclusion and exclusion criteria outlined in Table1. Half of the group will be treated with GBR+PRF (test group) and half of the group will be treated with GBR alone (control group); randomization of patients will be conducted by the RAND function in Excel. In this particular study, A total of seven patients were recruited and five of the seven were assigned to the test group and two patients were assigned to the control group. When the projected sample size of 20 patients is obtained, half will have been assigned to the test group and half to the control group. A succeeding provider will continue to see more patients until all twenty patients are treated.

TABLE 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• Age ≥18 years old • Bone deficiency from single or multiple missing teeth • Bone regeneration with xenograft (Bio-Oss®) • Treatment with non-resorbable membrane	• Systemic diseases that compromise wound healing including but not limited to, history of IV bisphosphonate treatment, autoimmune disorders and uncontrolled diabetes mellitus • Pathology present near the site of the procedure • Allergy to Penicillin antibiotics • Contraindication to Ibuprofen use • Smoker status

2.2 PROCEDURES

In both groups, Cone Beam Computed Tomography (CBCT) was taken prior to the surgery to obtain baseline bone information. All GBR procedures were performed by making a crestal incision with two buccal/facial vertical releasing incisions as needed and raising a full-thickness mucoperiosteal flap. An additional periosteal releasing incision was made for coronal flap advancement. For the test group only, the PRF products was prepared to obtain liquid-form PRF to mix with the particulate bone graft material and PRF membranes to cover the dPTFE membrane (see Table 2 and Figure 1A-D).

TABLE 2. Brief outline of PRF protocol

1. Blood is drawn and collected in 10mL collection tubes. Amount of blood drawn varies depending on the defect size from 2 tubes to 5 tubes in total.
2. Tubes are immediately centrifuged for 3 minutes for liquid-form PRF and 10 minutes for PRF clot.
3. The liquid-form PRF layer is extracted from the tube using a syringe and mixed with bone graft materials, and the PRF clot is taken out of the tubes and placed on the grid in the PRF BOX and covered with compressor for 5 minutes to form a thin flat PRF membrane.
4. The remaining bottom blood cell layer is disposed of according to OSHA guidelines.

For this study, one tube of blood was spun for 3 minutes to obtain liquid-form PRF. This liquid-form PRF was then mixed with the particulate bone (Bio-Oss®), and placed at the defect site. The mixture of bone graft and liquid-form PRF was covered with the non-resorbable dPTFE membrane and the PRF membrane was placed above the dPTFE membrane, just beneath soft tissue periosteum. The mucoperiosteal flaps was then approximated using PTFE sutures to obtain tension-free primary closure. Postoperative appointments and oral hygiene instructions were scheduled in the dental office at weeks 1, 2, 4, 6, 8, 12, 16 post-GBR surgery. All patients received the same antibiotic and analgesic medications, as well as instructions for their use while postoperative instructions are given at the end of the surgery. Sutures were removed at 4-week postoperative appointment.

Patients were also provided with a visual analogue scale (VAS) on which to record their daily pain level for the first week post-GBR. Briefly, patients were instructed to indicate their current experience of pain by placing a vertical line along a 100-mm line wherein 0 indicates no pain and 100 indicates the worst pain the patient has ever experienced. This self-evaluation of postoperative pain was instructed to record in the evening, immediately before they go to the bed. Patients were also asked to provide the time of day and time interval since their last dose of analgesic medication. Clinical measurements and photographs were taken at baseline and each follow-up visit.

2.3 MEASURES

2.3.1 Clinical parameters:

- Membrane exposure measured in the longest dimension: 0mm, <3mm, 3-5mm, 5-10mm, >10mm
- Soft tissue inflammation according to a modified version of the Gingival Index (GI)[58]
- Firm or boggy soft tissue consistency
- Absence or presence of suppuration

At 5 months post-GBR, a CBCT is planned to be taken on all patient groups and will be analyzed blindly for bone quantity and quality comparing to the baseline CBCT. At the time of implant placement at 6 months, a core of bone is planned to be trephined and analyzed histologically. Among a total of 7 patients who received GBR procedure, only 4 patients have reached 5-month and 6-month point by the time the provider finishes the periodontics residency program. In present study, only soft tissue healing and pain will be evaluated, and analysis on CBCT and bone core sample histology will be investigated by a succeeding resident provider with more patient data.

3.0 RESULT

Because of the small sample size, no formal statistical analyses were performed on the data collected from this study. Accordingly, only descriptive information (mean ± standard deviation) is presented here.

3.1 SELF-REPORTED PAIN

One patient was not compliant in returning the pain questionnaire report. Thus, data from only six patients were used to analyze the pain level. Figure 3 displays patients' responses to the 7-day pain self-report, and a table that shows actual type of medications and time that medications were taken is attached in Appendix A. With a single exception (Subject 1004), patients in both groups reported moderate to severe pain on the first postoperative day (test, mean SD = 4.16 ± 3.10; control mean SD = 5.98 ± 1.87). However, patients in the test group showed a more rapid decline in self-reported pain, with ratings for the test group being substantially lower than those for the control group (test, mean SD = 0.65 ± 0.68; control, mean SD = 3.00 ± 1.41). While four patients showed consistently reducing pain level, two patients (Subject 1001 and 1007) reported increased level of pain in the middle of the week on day 4, and day 3 and 5, respectively.

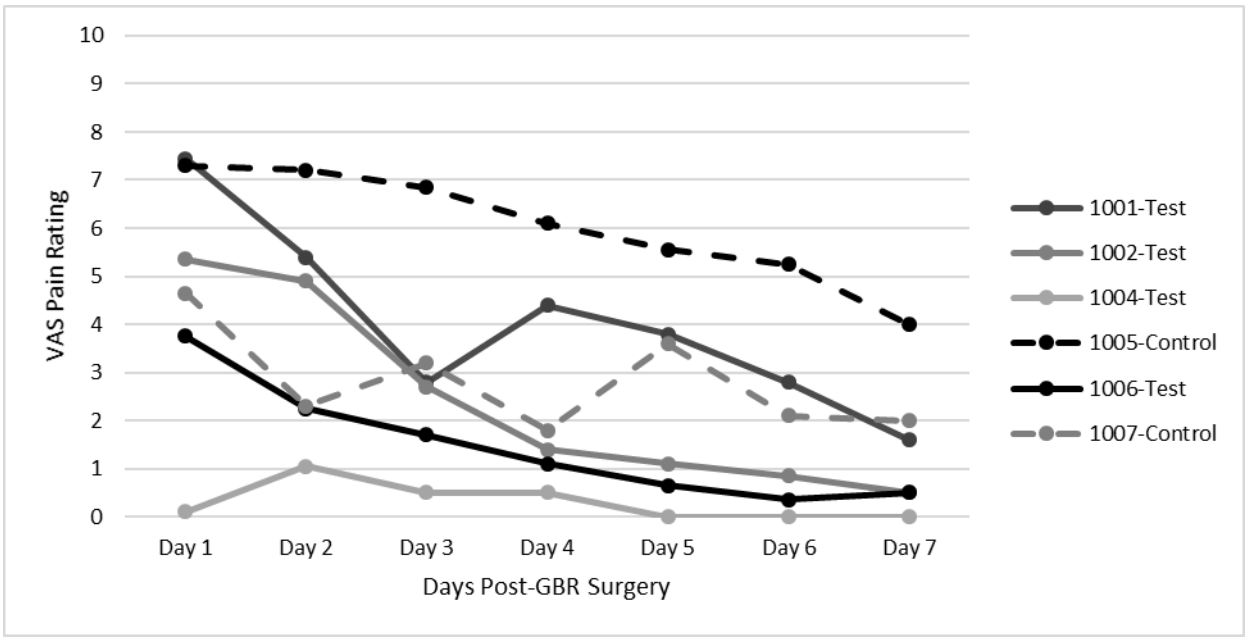

FIGURE 3. Patient self-reported pain during the first week post-GBR surgery (n=6). Individual lines represent responses from a single patient

3.2 SOFT-TISSUE HEALING

Soft-tissue healing was examined across the first four postoperative study visits (+1 week, +2 weeks, +4 weeks and +6 weeks) because all patients had been returned for at least four postoperative visits at the time of this writing. To summarize these data, scores on each of the four soft tissue measures were dichotomized to indicate whether patient examination revealed any membrane exposure, any abnormal color, boggy consistency indicative of poor healing or infection, or presence of suppuration, respectively, during any of the four postoperative study visits. Summary data for three of these measures are

displayed in Figures 2A, B, and C. Suppuration was not noted for any of the patients during any of the four visits.

Unexpectedly, two patients in the test group displayed the evidence of membrane exposure during the six-weeks of follow up (Figure 2A). Whereas no exposure was noted for either control patient, moderate (3-5 mm) to severe (5-10 mm) exposure was noted for two of the five test patients (ID 1001 and ID 1002) during two or more follow-up visits.

In contrast to membrane exposure, evidence of gingival inflammation was noted in all patients (Figure 2B). However, whereas control patients only experienced mild inflammation, two of the five test patients (ID 1002 and ID 1003) experienced moderate and/or severe inflammation.

Similar to the findings for membrane exposure, abnormality in soft tissue consistency was noted for participants in the test group only (Figure 2C). In three of the four test patients with abnormal findings (ID 1001, ID 1003, and ID 1004), boggy consistency was noted during the one-week postoperative visit only. For the fourth patient (ID 1002), by comparison, boggy soft tissue consistency was noted during the one-, two-, six-week postoperative visits.

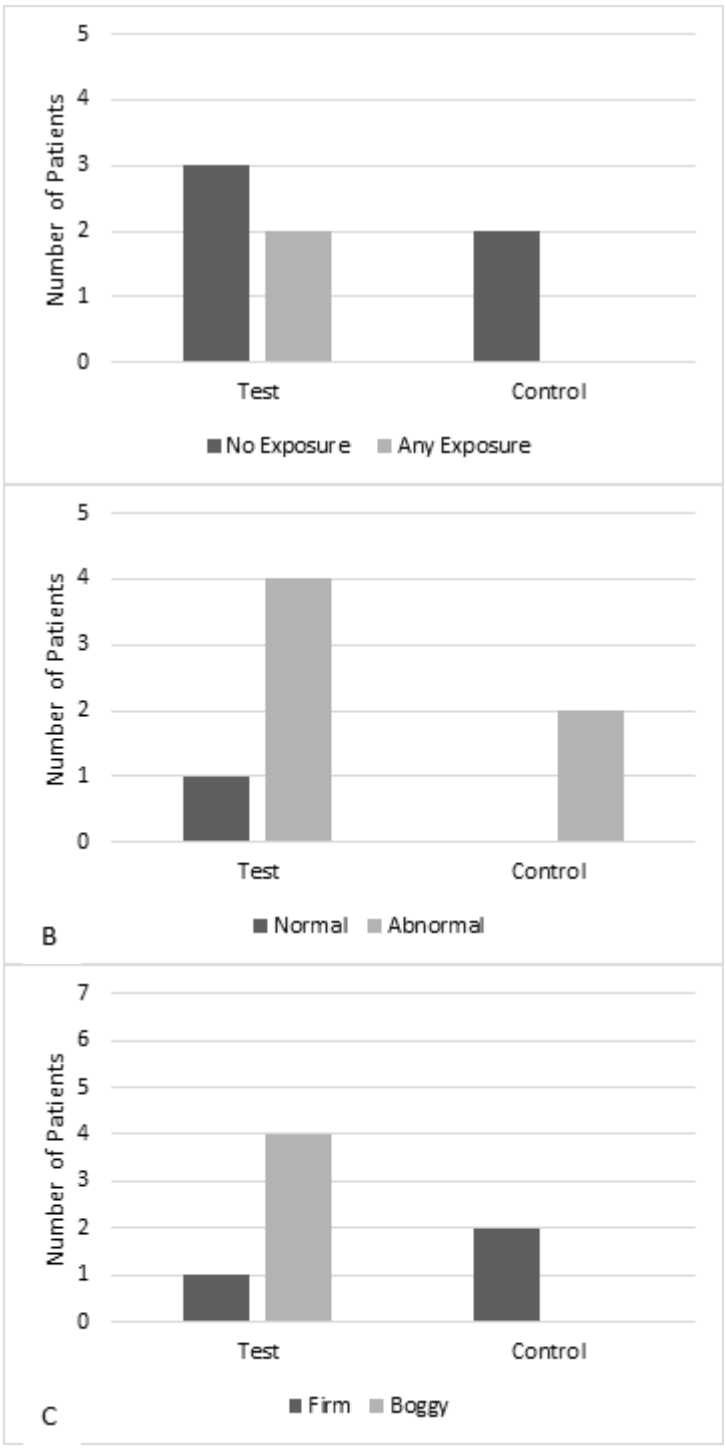

FIGURE 4. Abnormal soft-tissue findings. A. Number of patients exhibiting membrane exposure during any of the four follow-up visits. B. Number of patients exhibiting abnormal (mild, moderate, or severe) gingival inflammation during any of the four follow-up visits. C. Number of patients exhibiting boggy tissue consistency during any of the four follow-up visits.

4.0 DISCUSSION

The purpose of the present study is to evaluate whether the addition of PRF is beneficial for conventional GBR procedures on the clinical outcomes including incidence of membrane exposure, incidence of suppuration at the graft site, gingival consistency, severity of gingival inflammation, and severity of postoperative pain during the healing phase.

By comparison, the present study's soft tissue findings are not consistent with previous research. Multiple soft tissue evaluations showed the benefit of PRF and other platelet concentrate products on wound healing for palatal wound management after autogenous soft tissue graft,**Error! Bookmark not defined.** tooth extraction,[30,31] and GBR with titanium membrane.[57] In the present study, two patients among five test group patients developed membrane exposures while no exposures were developed in two control group patients during the six-weeks of follow up. More gingival inflammation and boggy tissue consistency was noted in the test group as well.

A couple of factors might explain why the present study did not find improvements in wound healing with PRF. Two of the five PRF group patients (ID 1001 and ID 1002) had either recent extraction (1-month prior) or simultaneous extractions when the GBR was performed, and only these two subjects showed the membrane exposure during the initial

healing among all seven patients. The tension-free primary closure required relatively more involved soft tissue handling to advance the flap more coronally, and the surgical sites had significantly less keratinized tissue to handle during the procedure. Kfir et al.[59] evaluated the effect of immediate extraction and implant placement on the soft tissue and bone regeneration after the simultaneous GBR. They mentioned that complete soft tissue coverage of the extraction socket is difficult to obtain, and even if primary closure was accomplished after tooth extraction, it can result in early membrane exposure by epithelial dehiscence. They reported that out of the 15 patients, 7 patients had early membrane exposure, which was approximately 47% of the cases.

Thus, if the study is redesigned, an additional exclusion might be added to the current exclusion criteria: simultaneous extractions. If both extraction and GBR were required for implant therapy, extraction should be performed prior to the GBR, and GBR procedure should not be initiated until the soft tissue remodeling is completed, which is up to 6 months.[60] One of the test group patients (ID 1006) also received recent extractions, but it was 7 weeks prior to the GBR and did not show any membrane exposure. Our suggestion is to wait about 2 months after the extractions before the GBR procedure.

Another assumption that may explain the membrane exposures among PRF group was the provider's surgical skill. The first four out of seven patients were randomized to become test group and that is when the provider just started performing GBR procedures in the periodontics residency program. It is possible that the higher rate of compromised soft tissue healing and membrane exposure within test group is accounted for by the limited experience and lack of established techniques of the treatment provider, especially the first two subjects (ID 1001 and ID 1002) who showed the membrane exposure.

The soft tissue healing pattern looking at the soft tissue inflammation and consistency was slightly inferior in the test group patients. However, more inflammation and boggy tissue consistency have to be linked with the existence of membrane exposures because these findings were noted mainly in the membrane exposure patients. After the first follow-up, boggy consistency only appeared in the two membrane exposure patients. Tissue inflammation was a little more mixed, but moderate inflammation only turned up for one of the exposure patients and all the rest were mild inflammation.

The present postoperative pain findings are consistent with the findings of multiple previous pain analyses that showed a positive effect of PRF on pain after the surgery.[20,21,28-33] Patients in the test group reported substantially less pain during the first week of healing after the GBR procedure compared to the control group. There were spikes in the middle of the week shown in Subject 1001 and 1007, and these patients are the only patients who took a combination of ibuprofen and hydrocodone/acetaminophen (see Appendix A). All the patients were instructed to take ibuprofen as regularly as possible at least for the first 3 days postoperatively and also take hydrocodone/acetaminophen additionally only if ibuprofen was not enough to control the pain. These two patients freely took ibuprofen only, hydrocodone/acetaminophen only, or combination of two medications based on their subjective pain level. It could be explained that the inconsistent decrease of the pain level is due to the inconsistent administration of the drugs and different potency and effectiveness of two different drug modalities.

One thing to note about this study is that by necessity it could not be blinded. Assuming it were possible to blind the subject, the pain result may have been different. Before the surgeries are started, all of the research patients hear and read the information

about PRF treatment with previous study showing potentially better and faster healing capacity and lesser postoperative pain. The better postoperative pain control in the test group that was shown in this study could have been the placebo effect because patients who received or did not receive the PRF already knew what they may expect.

A limitation of this study was the sample size. Any patients who needed sinus lift at the time of GBR were excluded from the research recruitment because resorbable membrane was preferred for sinus lift. And many patients who need implant therapy already lost their teeth from previous periodontal disease with multiple contributing factors, which include smoking. Any smokers no matter how many cigarettes they smoke per day were excluded as well. Some patients were excluded because they were allergic to the medications that we needed to use, especially Penicillin. Due to the small sample size, it was difficult to generalize the treatment results.

Another limitation will be the multiple treatment providers that will participate in this research project. When a succeeding resident continues the research until we recruit a total of 20 patients, it would be important to calibrate the new resident so all the procedures are performed in a similar fashion, in terms of the speed of the venipuncture, blood collection, and surgery itself, and the way to handle the materials and soft/hard tissue.

5.0 CONCLUSION

One of the most important keys to prevent the membrane exposure after the GBR procedure is to follow the fundamental principles of the GBR which include 1) primary closure, 2) blood supply, 3) space maintenance and 4) stability of the wound. The addition of PRF treatment to properly performed GBR procedure may be beneficial for the wound healing and the pain control due to the healing potentials that PRF has. In the present study, it showed a trend that addition of PRF to GBR procedure lowers the postoperative pain level. But due to the small sample size, it was difficult to draw a conclusion on the effect of PRF on the membrane exposure and other soft tissue parameters. Once the study continues with a next investigator, with the additional data we may be able to draw more definitive conclusions. The next report will also look at the bone quality and quantity in the histologic and radiographic analysis.

APPENDIX A

PATIENT REPORTS OF ANALGESIC MEDICATION USE DURING THE SEVEN DAYS FOLLOWING GBR SURGERY – RAW DATA

ID	time1	pmed1	pmed1t	time2	pmed2	pmed2t	time3	pmed3	pmed3t	time4	pmed4	pmed4t	time5	pmed5	pmed5t	time6	pmed6	pmed6t	time7	pmed7	pmed7t
1001	22:15	HC/IB	22:15	23:15	HC/IB	23:30	23:20	HC	20:14	21:30	HC	21:30	0:15	IB	0:15	23:10	IB	19:30	22:00	IB	22:00
1002	22:45	IB	17:30	22:00	IB	21:45	22:15	IB	22:00	22:00	IB	11:30	22:00	IB	7:30	22:00	IB	7:30	22:00	N	
1004	20:46	IB	13:00	8:00	IB	23:00	11:30	IB	11:00	10:00	IB	0:00	10:00	IB		11:00	IB			IB	
1005	**22:00**	**IB**	**18:00**	**22:00**	**IB**	**18:00**	**22:00**	**IB**	**18:00**	**22:00**	**IB**	**18:00**	**22:00**	**IB**	**18:00**	**22:00**	**IB**	**18:00**	**22:00**	**IB**	**18:00**
1006	22:40	IB	22:40	23:00	IB	22:50	23:05	IB	21:50	23:00	IB	13:30	22:10	IB	20:20	22:36	IB	12:40	23:50	IB	19:40
1007	**21:30**	**HC/IB**	**21:30**		**HC/IB**	**21:55**		**HC**	**19:00**		**N**			**IB**	**20:30**		**IB**	**21:15**		**IB**	**23:30**

time() = time patient filled out form

pmed() = type of pain medication patient took (HC=hydrocodone; IB=ibuprofen; HC/IB=combination; N=no medication)

pmed()t = time patient took their last dose of medication

filled cells = missing data

bold type = control patient

BIBLIOGRAPHY

1 *FERNÁNDEZ-DELGADO N, HERNÁNDEZ-RAMÍREZ P, FORRELLAT-BARRIOS M.* Platelet functional spectrum: from hemostasis to regenerative medicine. Rev Cubana Hematol Inmunol Hemoter 2012; 28: 200-216.

2 *GIANNINI, S. CIELO, A. BONANOME, L. RASTELLI, C. DERLA, C. CORPACI, F. FALISI, G*. Comparison between PRP, PRGF, and PRF: lights and shadows in three similar but different protocols. Eur Rev Med Pharmacol Sci 2015; 19(6):927

3 *DHURAT R, SUKESH M.* Principles and Methods of Preparation of Platelet-Rich Plasma: A Review and Author's Perspective. Journal of Cutaneous and Aesthetic Surgery. 2014;7(4):189-197. doi:10.4103/0974-2077.150734.

4 *BIELECKI T AND DOHAN EHRENFEST DM*. Leukocyte- and Platelet-rich plasma/fibrin in medicine- past, present, future. Curr Pharm Biotechnol 2012; 13:i-ii

5 *HOTWANI K, SHARMA K.* Platelet rich fibrin - a novel acumen into regenerative endodontic therapy. Restorative Dentistry & Endodontics. 2014;39(1):1-6. doi:10.5395/rde.2014.39.1.1

6 *WHITMAN DH, BERRY RL, GREEN DM.* Platelet gel: An autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. Journal of Oral and Maxillofacial Surgery, Volume 55 , Issue 11 , 1294 – 1299

7 *CHOUKROUN J, ADDA F, SCHOEFFLER C*, VERVELLE A. Une opportunite´ en paroimplantologie: le PRF. Implantodontie. 2001;42:55-62.

8 *DOHAN DM, CHOUKROUN J, DISS A, DOHAN SL, DOHAN AJ, MOUHYI J, GOGLY B.* Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I. Technological conecepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006 Mar;101(3):e37-44.

9 *DOHAN DM, CHOUKROUN J, DISS A, DOHAN SL, DOHAN AJ, MOUHYI J, GOGLY B.* Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006 Mar;101(3):e45-50.

10 *DOHAN DM, CHOUKROUN J, DISS A, DOHAN SL, DOHAN AJ, MOUHYI J, GOGLY B.* Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part III: leucocyte activation: a new feature for platelet concentrates? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006 Mar;101(3):e51-55.

11 *CHOUKROUN J, DISS A, SIMONPIERI A, GIRARD MO, SCHOEFFLER C, DOHAN SL, DOHAN AJ, MOUHYI J, DOHAN DM.* Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006 Mar;101(3):e56-60.

12 *DOHAN DM, CHOUKROUN J, DISS A, DOHAN SL, DOHAN AJ, MOUHYI J, GOGLY B.* Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006 Mar;101(3):299-303

13 *HE L, LIN Y, HU X, ZHANG Y, WU H.* A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009 Nov; 108(5):707-13

14 *BORIE E, OLIVÍ DG, ORSI IA, GARLET K, WEBER B, BELTRÁN V, FUENTES R.* Platelet-rich fibrin application in dentistry: a literature review. International Journal of Clinical and Experimental Medicine. 2015;8(5):7922-7929.

15 *DOHAN DM, DEL CORSO M, CHARRIER JB.* Cytotoxicity analyses of Choukroun's platelet-rich fibrin (PRF) on a wide range of human cells: The answer to a commercial controversy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;103:587–583.

16 *KAWASE T, KAMIYA M, KOBAYASHI M, TANAKA T, OKUDA K, WOLFF LF, YOSHIE H.* The heat-compression technique for the conversion of platelet-rich fibrin preparation to a barrier membrane with a reduced rate of biodegradation. J Biomed Mater Res B Appl Biomater. 2015 May;103(4):825-31. doi: 10.1002/jbm.b.33262. Epub 2014 Aug 14.

17 *BAKHTIAR H, ESMAEILI S, FAKHR TABATABAYI S, ELLINI MR, NEKOOFARAND MH, DUMMER PM.* Second-generation Platelet Concentrate (Platelet-rich Fibrin) as a Scaffold in Regenerative Endodontics: A Case Series. J Endod. March 2017Volume 43, Issue 3, Pages 401–408

18 *YEOM KH, ARIYOSHI W, OKINAGA T, WASHIO A, MOROTOMI T, KITAMURA C, NISHIHARA T.* Platelet-rich plasma enhances the differentiation of dental pulp progenitor cells into odontoblasts. Int Endod J. 2016;49:271–278.

19 *KESWANI D, PANDEY RK, ANSARI A, GUPTA S.* Comparative evaluation of platelet-rich fibrin and mineral trioxide aggregate as pulpotomy agents in permanent teeth with incomplete root development: a randomized controlled trial. J Endod. 2014;40:599–605.

20 *FEMMINELLA B, IACONI MC, DI TULLIO M, ROMANO L, SINJARI B, D'ARCANGELO C, DE NINIS P, PAOLANTONIO M.* Clinical comparison of platelet-rich fibrin and a gelatin sponge in the management of palatal wounds following epithelialized free gingival graft harvest: A randomized clinical trial. J Periodontol. 2015;87:1–17.

21 *ÖNCÜ E.* The Use of Platelet-Rich Fibrin Versus Subepithelial Connective Tissue Graft in Treatment of Multiple Gingival Recessions: A Randomized Clinical Trial. Int J Periodontics Restorative Dent. 2017 Mar/Apr;37(2):265-271. doi: 10.11607/prd.2741.

22 *AROCA S, KEGLEVICH T, BARBIERI B, GERA I, ETIENNE D.* Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: a 6-month study. J Periodontol. 2009 Feb;80(2):244-52. doi: 10.1902/jop.2009.080253.

23 *MORASCHINI V. BARBOZA EDOS S.* Use of Platelet-Rich Fibrin Membrane in the Treatment of Gingival Recession: A Systematic Review and Meta-Analysis. J. Periodontol. 2016, 87, 281–290.

24 *SHARMA A, PRADEEP AR.* Treatment of 3-wall intrabony defects in patients with chronic periodontitis with autologous plateletrich fibrin: a randomized controlled clinical trial. J Periodontol 2011;82(12):1705–12.

25 *THORAT M, PRADEEP AR, PALLAVI B.* Clinical effect of autologous platelet-rich fibrin in the treatment of intra-bony defects: a controlled clinical trial. J Clin Periodontol 2011;38(10):925–32.

26 *LEKOVIC V, MILINKOVIC I, ALEKSIC Z, JANKOVIC S, STANKOVIC P, KENNEY E, CAMARGO P.* Platelet-rich fibrin and bovine porous bone mineral vs. platelet-rich fibrin in the treatment of intrabony periodontal defects. J. Periodontal Res. 2012, 47, 409–417.

27 *OZGUL O, SENSES F, ER N, TEKIN U, TUZ HH, ALKAN A, KOCYIGIT D, AND ATIL F.* Efficacy of platelet rich fibrin in the reduction of the pain and swelling after impacted third molar surgery: Randomized multicenter split-mouth clinical trial. Head & Face Medicine. 2015 Nov 11:37

28 *ALISSA R, ESPOSITO M, HORNER K, OLIVER R.* The influence of platelet-rich plasma on the healing of extraction sockets: an explorative randomised clinical trial. Eur J Oral Implantol. 2010;3(2):121–34.

29 *MOZZATTI M, MARTINASSO G, POL R, POLASTRI C, CRISTIANO A, MUZIO G, ET AL.* The impact of plasma rich in growth factors on clinical and biological factors involved in healing processes after third molar extraction. J Biomed Mater Res. 2010;95(3):741–6. doi: 10.1002/jbm.a.32882.

30 *SINGH A, KOHLI M, GUPTA N*. Platelet rich fibrin: a novel approach for osseous regeneration. J Maxillofac Oral Surg. 2012;11(4):430–4. doi: 10.1007/s12663-012-0351-0.

31 *KUMAR N, PRASAD K, RAMANUJAM L, K R, DEXITH J, CHAUHAN A.* Evaluation of Treatment Outcome After Impacted Mandibular Third Molar Surgery With the Use of Autologous Platelet-Rich Fibrin: A Randomized Controlled Clinical Study. J Oral Maxillofac Surg. 2014 Dec 13. doi: 10.1016/j.joms.2014.11.013.

32 *UYANıK LO, BILGINAYLAR K, ETIKAN I,* Effects of platelet-rich fibrin and piezosurgery on impacted mandibular third molar surgery outcomes. Head & Face Medicine (2015) 11:25

33 *AL-HAMED FS, TAWFIK MAM, ABDELFADIL E.* Clinical effects of platelet-rich fibrin (PRF) following surgical extraction of lower third molar. The Saudi Journal for Dental Research.May 2016.

34 *LANDRY RG, TURNBULL RS, HOWLEY T.* Effectiveness of benzydamyne HC1 in the treatment of periodontal post-surgical patients. Res Clin Forum. 1988;10:105–118.

35 *TATULLO M, MARRELLI M, CASSETTA M, PACIFICI A, STEFANELLI LV, SCACCO S, DIPALMA G, PACIFICI L, INCHINGOLO F.*. Platelet Rich Fibrin (P.R.F.) in Reconstructive Surgery of Atrophied Maxillary Bones: Clinical and Histological Evaluations. Int J Med Sci 2012; 9(10):872-880.

36 *CHIAPASCO M, ROMEO E.* "La Riabilitazione Implantoprotesica nei casi complessi". UTET; 2003. Chirurgia preimplantare nelle atrofie dei mascellari; pp. 142–159.

37 *ADELL R, LEKHOLM U, GRÖNDAHL K, BRÅNEMARK PI, LINDSTRÖM J, JACOBSSON M.* Reconstruction of severely resorbed edentulous maxillae using osseointegrated fixtures in immediate autogenous bone grafts. Int J Oral Maxillofac Implants. 1990 Fall; 5(3):233-46.

38 *SHAWKY H, SEIFELDIN SA.* Does Platelet-Rich Fibrin Enhance Bone Quality and Quantity of Alveolar Cleft Reconstruction? Cleft Palate Craniofac J. 2015 Oct 9.

39 *RUGGIERO SL, DODSON TB, FANTASIA J, GOODDAY R, AGHALOO T, MEHROTRA B, O'RYAN F.* American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw--2014 update. J Oral Maxillofac Surg. 2014;72:1938–1956.

40 *YOUNG H, PATEL D, KWOK J.* Primary Wound Closure Using L-PRF in Patients With Mronj and Osteoradionecrosis - a Case Series. Journal of Oral and Maxillofacial Surgery , Volume 74 , Issue 9 , e35 - e36

41 *MONTANARI M, CALLEA M, YAVUZ I, MAGLIONE M.* A new biological approach to guided bone and tissue regeneration. BMJ Case Reports. 2013;2013:bcr2012008240. doi:10.1136/bcr-2012-008240.

42 *MURRAY C, HOLDEN D, ROACHLAU W.* Experimental and clinical study of new growth of bone in a cavity. Am j surg. 1957; 95: 385-7

43 *DAHLIN C, LINDE A, GOTTLOW J, NYMAN S.* Healing of bone defects by guided tissue regeneration.Plast Reconstr Surg. 1988 May;81(5):672-6.

44 *WANG HL, BOYAPATI L.* "PASS" principles for predictable bone regeneration. Implant dent. 2006;15:8–17.

45 *RAKHMATIA YD, AYUKAWA Y, FURUHASHI A, KOYANO K.* Current barrier membranes: titanium mesh and other membranes for guided bone regeneration in dental applications. J. Prosthodont. Res., 57 (2013), pp. 3–14 https://doi.org/10.1016/j.jpor.2012.12.001

46 *LEE SW, KIM SG.* Membranes for the Guided Bone Regeneration. Maxillofacial Plastic and Reconstructive Surgery. 2014;36(6):239-246. doi:10.14402/jkamprs.2014.36.6.239.

47 *URBAN IA, NAGURSKY H, LOZADA JL, NAGY K.* Horizontal ridge augmentation with a collagen membrane and a combination of particulated autogenous bone and anorganic bovine bone-derived mineral: a prospective case series in 25 patients. Int j periodontics restorative dent. 2013 may-jun;33(3):299-307. DOI: 10.11607/PRD.1407.

48 *NEWMAN MG, TAKEI H, KLOKKEVOLD PR, CARRANZA FA.* Carranza's Clinical Periodontology. Elsevier Health Sciences 2014. Twelfth Edition

49 *MURPHY KG.* postoperative healing complications associated with Gore-Tex periodontal material. Part I. Incidence and characterization. Int J Periodontics Restorative Dent 1995; 15:363-75

50 *MACHTEI EE.* The effect of membrane exposure on the outcome of regenerative procedures in humans: a meta-analysis. J periodontal 2001; 62:512-6

51 *SIMION M, BALDONI M, ZAFFE D.* Guided tissue regeneration in osseointegrated implants II: extraction sockets. Ital J Osseointegration 1991; 1:40-45.

52 *BARBER HD, LIGNELLI J, SMITH BM.* Using a dense PTFE membrane without primary closure to achieve bone and tissue regeneration.Journal of oral and maxillofacial surgery : J Am Assoc Oral Maxillofac Sur. 2007;65:748–52.

53 *HOFFMANN O, BARTEE BK, BEAUMONT C.* Alveolar bone preservation in extraction sockets using non-resorbable dPTFE membranes: a retrospective non-randomized study. J Periodontol. 2008;79:1355–69.

54 *BELLEGGIA F*. Exposure of a dense PTFE Membrane in a GBR Procedure: Proposal of a Treatment Proto 2014.

55 *MARIDATI P, CREMONESI S, FONTANA F, MAIORANA C*. Treatment of a dense PTFE membrane exposure: a case report 2014.

56 *MARIDATI PC, CREMONESI S, FONTANA F, CICCIU M, MAIORANA C* Management of d-PTFE Membrane Exposure for Having Final Clinical Success. 2016

57 *TORRES J, TAMIMI F, ALKHRAISAT MH, MANCHÓN A, LINARES R, PRADOS-FRUTOS JC, HERNÁNDEZ G, LÓPEZ CABARCOS E.* Platelet-rich plasma may prevent titanium-mesh exposure in alveolar ridge augmentation with anorganic bovine bone. J Clin Periodontol. 2010 Oct; 37(10):943-51

58 *HERALD LOE.* The Gingival Index, the Plaque Index and the Retention Index Systems. J Of Periodontol 1967;38(6:610-616)

59 *KFIR E, KFIR V, KALUSKI E.* Immediate bone augmentation after infected tooth extraction using titanium membranes. J Oral Implantol. 2007;33(3):133-8.

60 *RAMZI VA, MAJZOUB ZAK, HOLMES CM, GEISINGER ML, GEURS NC.* Healing Time for Final Restorative Therapy After Surgical Crown Lengthening Procedures: A Review of Related Evidence. Clinical Advances in Periodontics 2015 5:2, 131-139