



RESEARCH ARTICLE

EVALUATION OF BONE HEALING AND RIDGE PRESERVATION IN MANDIBULAR AND MAXILLARY EXTRACTION SOCKET FOLLOWING BONE GRAFTING WITH PLATELET RICH PLASMA AND PLATELET RICH FIBRIN - A CLINICAL AND RADIOLOGICAL STUDY

*Dr. Himanshu C Soni, Dr. Raghuvveer H.P., Dr. Dilip Kumar R., Dr. Shobha E.S.,
Dr. Prashanth N.T., Dr. Vinod Rangan and Dr. Nikhila G.

Department of Oral and Maxillofacial Surgery, Dayananda Sagar College of Dental Sciences, Shavige Malleshwara Hills, Kumaraswamy layout, Bangalore, Karnataka

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ABSTRACT

Introduction: Background and objectives: The purpose of this study was to compare the efficacy of demineralized freeze dried bone graft with platelet rich fibrin (PRF), and demineralized freeze dried bone graft with platelet rich plasma (PRP) and collagen membrane in healing of extraction socket of maxillary and mandibular anterior and posterior teeth.

Materials and method: 60 extraction sites were selected and divided into group I and group II. Group I consisted of DFDBG and PRF and group II consist of DFDBG and PRP along with collagen membrane. Each group comprising of thirty extraction sites were subjected to one of the two modalities (DFDBF with PRF and DFDBG with PRP) of treatment for, maxillary and mandibular extraction socket. Post-operatively the patients were evaluated for bone healing, pain and swelling following extraction and bone grafting, and to evaluate the bone height, width and density radiographs were taken at interval of 1st month, 3rd month and 6th month.

Results: Results did show statistical differences ($p > 0.05$) for parameters like bone height, width and density with group I, when compared with group II. Radiographically Bone height and Bone density was significantly higher with DFDBG and PRF group when compared with DFDBG and PRP group. Clinically here was no difference in terms of soft tissue healing with both the groups.

Interpretation and Conclusion: Our study suggested that even though both modalities used in this study were effective for grafting extraction sockets, DFDBG and PRF group proved to be better in terms of bone height, width and density postoperatively. We conclude that the overall summation of the results of the study showed that DFDBG and PRF seems to offer better significant results both clinically and radiographically than DFDBG and PRP in ridge preservation of maxillary and mandibular extraction sockets.

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INTRODUCTION

Socket preservation is a procedure to preserve hard and soft tissue of the alveolar ridge after extraction. The alveolar process is a tooth-dependent structure, so after the extraction bone loss occurs very rapidly in the initial 6 months. This results in reduction of 40% of alveolar height, and 60% of alveolar width. Evidences have proved that bone loss occurs after extraction which is more on the labial side of the alveolar process when compared to the lingual or the palatal side (Weng, 2011). Alveolar ridge resorption refers to the bone remodeling that occurs following tooth extraction. Araujo *et al.* (Araujo, 2005) found that the coronal aspect of buccal bone

was often comprised only of bundle bone and hypothesized that bone resorption would occur after tooth extraction. Other authors proposed that surgical trauma during extraction results in the separation of the periosteum from the underlying bone, causing vascular damage and an acute inflammatory response, which mediates bone resorption (Staffileno, 1966 and Wood, 1972). Alveolar ridge resorption following tooth extraction may lead to esthetic and functional defects. The defects can be so severe that good Prosthodontic rehabilitation will become a challenge for removable or fixed prosthetic rehabilitation and implant placement can be difficult or impossible without using augmentation procedures (Bartee, 2001 and Ashman, 1995). Those defects also can interfere with the use of removable dentures. Leblebicioglu *et al.* (2013) have shown that ridge height loss is greater in mandibular than maxillary sites, and ridge width loss is greater on the buccal plate in both the

*Corresponding author: Dr. Himanshu C Soni,

Department of Oral and Maxillofacial Surgery, Dayananda Sagar College of Dental Sciences, Shavige Malleshwara Hills, Kumaraswamy layout, Bangalore, Karnataka, India.

mandibular and maxillary sites. Thinner buccal plates also appear to be associated with more post-extraction resorption. Other studies have shown that elevating a full mucoperiosteal flap may be associated with bone loss following tooth extraction (Van der Weijden, 2009), resulting in approximately 0.6 mm of crestal bone loss. Multiple procedures are employed for prevention of post-extraction bone loss and predictable implant placements after extraction, including socket preservation with grafts (biomaterials), and immediate or early implant placements. While there are number of graft materials to choose from, some bone graft materials need longer healing time to achieve even a small amount of new bone incorporation into the graft site (Norton and Wilson, 2002). Irrespective of the reason for socket preservation, sufficient alveolar ridge width and height are essential to meet the functional and esthetic demands. The purpose of this study was design to compare efficacy of Demineralized Freeze Dried Bone Graft with Platelet Rich Fibrin and Platelet Rich Plasma in socket preservation and to evaluate bone healing and graft uptake, clinically and radio-graphically after removal of maxillary and mandibular teeth.

MATERIALS AND METHODS

The present study was undertaken on patients requiring extraction reporting to the Department of Oral and Maxillofacial Surgery, Dayananda Sagar College of Dental Sciences and Hospital Bangalore, in maxillary and mandibular anterior as well as posterior teeth. A clinical radiographic study was planned after due ethical approval from Ethics Committee. The study involved both male and female patients.

Inclusion criteria

- Patients indicated for extraction of maxillary and mandibular anterior and posterior teeth
- Patients in the age group 19-60 years.

Exclusion criteria

- Medically compromised patients.
- Patients unwilling to be part of the study.

A custom made case sheet was designed for the study to record the case history. After obtaining the complete history, patients were examined clinically and were explained about the procedure, its complications and the follow up period involved in the study. A Written Informed consent was obtained from all the patients. A total of 60 extraction sites both in maxillary and mandibular anterior and posterior teeth indicated for extraction was included in study. They were divided into Group I and Group II. 30 extraction sites requiring demineralized freeze dried bone graft with platelet rich fibrin as GROUP I and 30 extraction sites requiring demineralized freeze dried bone graft with platelet rich plasma as GROUP II. Patient of both the groups was assessed clinically on 7th day and 1 month for soft tissue healing. Radiographic assessment for bone healing was done at 7th day, 1st month and 3rd month and 6th month post-operatively.

Preparation of Platelet Rich Fibrin (PRF)

- Routine haematological investigation and informed consent were taken before withdrawal of blood for platelet rich fibrin preparation.

- Tourniquet was placed on the hand from which blood was to be drawn.
- In all patients, cephalic vein in the ante-cubital fossa was used for blood withdrawal.
- 18gauge needle was used for drawing blood
- 5ml of blood was drawn from the patient and placed in test tube with no anticoagulant.

The tubes are then placed in a centrifugal machine at 3,000 revolutions per minute (RPM) for 10 min, after which it settles into the following three layers:

- Upper fraction - straw-colored Acellular plasma,
- Middle fraction - containing the fibrin clot.
- Lower colored fraction - containing red blood cells (RBCs),

The upper straw-colored layer is then removed and middle fraction is collected, 2 mm below to the lower dividing line, which is the PRF. PRF can be obtained in the form of a membrane by squeezing out the fluids in the fibrin clot.

Preparation of Platelet Rich Plasma (PRP) Gel

1st STEP: Collection of blood

Under all aseptic techniques 5 ml of venous blood will be collected from the anticubital region. 3.6 ml of the collected blood will be placed in a vial containing 0.4 ml CPDA (Citrate Phosphate Dextrose Adenine) anticoagulant solution. The blood will be gently mixed with the anticoagulant. The vials will be thoroughly shaken to ensure mixture of anticoagulant with the drawn blood.

2nd STEP: Preparation of PRP

- The tube will be placed in a lab centrifuge machine and counter balanced. The first centrifuge cycle will be done at 2,000 rpm for 15 min. The result will be separation of the whole blood into a lower red blood cell region and upper straw colored plasma. This plasma contains relatively low concentration of platelets (platelet poor plasma) in the uppermost region and higher concentration of platelets and WBC in the boundary layer often called as 'Buffy coat'.
- With a micropipette the PPP and the buffy coat layer including 1 mm below the boundary layer will be collected in a sterile test tube and centrifuged at 3,000 rpm for 10 min. After the second centrifuge the upper half will be discarded and the lower half will be used as PRP.

3rd STEP: PRP GEL Preparation

The PRP will be activated with CaCl_2 to form a PRP gel.

Surgical procedure

Prior to the surgical procedure, alveolar ridge height and width were calculated on pre-operative RVG obtained. After anesthetizing the surgical area it was ensured that minimum trauma was caused while extracting the teeth. Forceps and elevators were used with great care taken to maintain the buccal bone and surrounding soft and hard tissues. Following

extraction, granulation tissues were removed with the help of curettes, and the socket was irrigated with sterile normal saline.

- **Group I patients** – Demineralized Freeze Dried Bone Graft (DFDBG) was condensed into extraction sockets until crestal level, and a platelet rich fibrin (PRF) was used to cover the graft material as a membrane.
- **Group II patients** –Demineralized Freeze Dried Bone Graft (DFDBG) mixed with PRP was condensed into extraction socket until crestal level and similarly, collagen membrane was used to cover the extraction socket.

In both the groups the flaps were sutured with hidden X suture technique, to cover as much as possible of the biomaterials. The postoperative instruction was given, and the patients were recalled at the interval of 7th day, 1st month, 3rd month and 6th month and clinical and radiographic measurements were recorded.

Clinical Evaluation

- Patient of both the groups was assessed on 7th day and 1 month for soft tissue healing.
- Soft tissue healing was assessed based on the criteria given by Landry et al.

Radiographic Evaluation

Extraction sockets were evaluated radiographically using Radiographs for parameters –Height, Width and Bone Density. The height, width and surface area (density) of the extraction socket were measured using computer graphic software program- ImageJ. The size of the extraction socket were calculated by the technique described by Chaipasco and Rossi (2000). The radiographic images were transferred to ImageJ software and converted to grayscale tonalities 256. Linear measurement tool option available in a software was used to measure height and width of extraction socket. Then tracing of the size of the residual cavity using a tool was done for each defect. The area marked was converted into a histogram which gave the number of pixels in the residual cavity. The number of pixels in the residual cavity was calculated on all RVGs. This indicated the size of defect. The number of pixels in the residual defect in the immediate post-operative radiograph was fixed at 100%. The decreasing number of pixels in the surgical defect overtime gave us the relative bone filling in the area of the socket. The percentage of bone filling was then calculated. Height width and bone regeneration results of the participants on Group A and Group B at 1st month, 3rd month and 6th months were compared and statistically analyzed.

Post-operative procedure

- Antibiotics (cap Amox 500mg t.i.d for five days) and Anti-Inflammatory drugs (Tab Divon plus t.i.d for three days) were prescribed along with oral hygiene maintenance instruction.
- Patients were checked for any pain/swelling/infection/wound break down in the grafted region on the seventh day and one month postoperatively.
- Suture removal was done on the seventh day.

RESULTS

A total of 60 extraction sites requiring extraction of teeth both in the maxillary and mandibular anterior and posterior region reporting to Department of Oral and Maxillofacial Surgery were included in the study. Patients were divided into two groups 30 extraction sites requiring demineralized freeze dried bone graft with platelet rich fibrin as GROUP I and 30 extraction site requiring demineralized freeze dried bone graft with platelet rich plasma as GROUP II. Both male and female patients between the age group of 18years-60 years with mean age of 29.2years were included in the study. Non restorable teeth with chronic irreversible pulpitis was the leading cause for extraction followed by Dento-alveolar abscess and trauma in the anterior teeth. Patient of both the groups were assessed on 7th day and 1 month Post-extraction for soft-tissue healing. Radiographic assessment for bone healing was done at 7th day, 1st month 3rd month and 6th month after extraction.

Group I- 30 extraction site were divided as follows

Maxillary anteriors- 10
 Maxillary posteriors- 5
 Mandibular anteriors- 5
 Mandibular posteriors-10

Group II- 30 extraction site were further divided as follows-

Maxillary anteriors- 10
 Maxillary posteriors- 5
 Mandibular anteriors- 5
 Mandibular posteriors-10

Analysis of result

The result of the study were statistically scored for three parameters namely bone height, bone width and bone density. The patients were evaluated at specific postoperative intervals at 1st 3rd and 6th month using the custom made evaluation form

DISCUSSION

To achieve a predictable esthetic and functional restoration, it is important to preserve the dimensions of alveolar ridge width and height after tooth extraction. Following extraction of tooth, various patterns of bone resorption occurs especially on the buccal side, therefore socket preservation plays a very crucial role in maintaining adequate bone height, width and density.

Although one study has shown that ridge preservation does not completely prevent bone loss, it aids in reducing the extent of that loss (Ten Heggeler, 2011). In a systematic review, Vittorini *et al* concluded that ridge preservation has a slight advantage over no treatment due to less horizontal and vertical bone loss. In their meta-analysis (Vittorini Orgeas, 2013), they noted that following tooth extraction, it is preferable to perform ridge preservation at esthetic areas where the buccal bone thickness is less than 1.5 to 2 mm when several teeth are extracted or when anatomical structures such as the maxillary sinus and mandibular canal are located in the immediate proximity. Barone *et al.*, 2013, found that an alveolar ridge preservation technique with collagenated porcine bone and a resorbable membrane was able to limit the vertical changes after tooth extraction. Aimetti *et al* (Aimetti, 2009).



Armamentarium



Demineralized Freeze Dried Bone Graft



Bone graft mixed with PRP



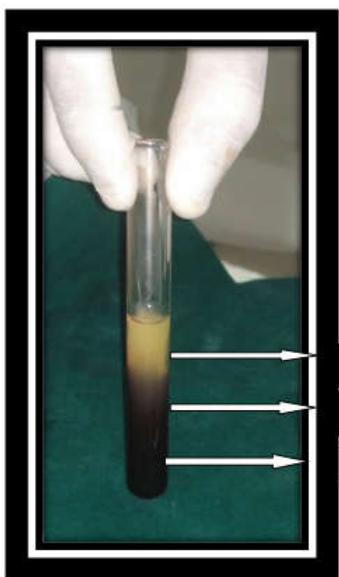
Collagen Membrane



Collection of blood for PRP and PRF preparation



Centrifuge Machine

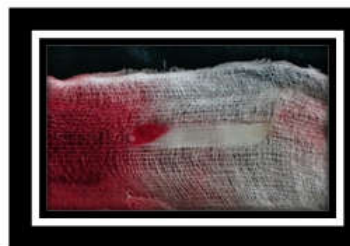


Layers of Centrifuged Blood

- PRF clot
- Platelet poor plasma
- RBC's base



PRF clot



PRF Membrane

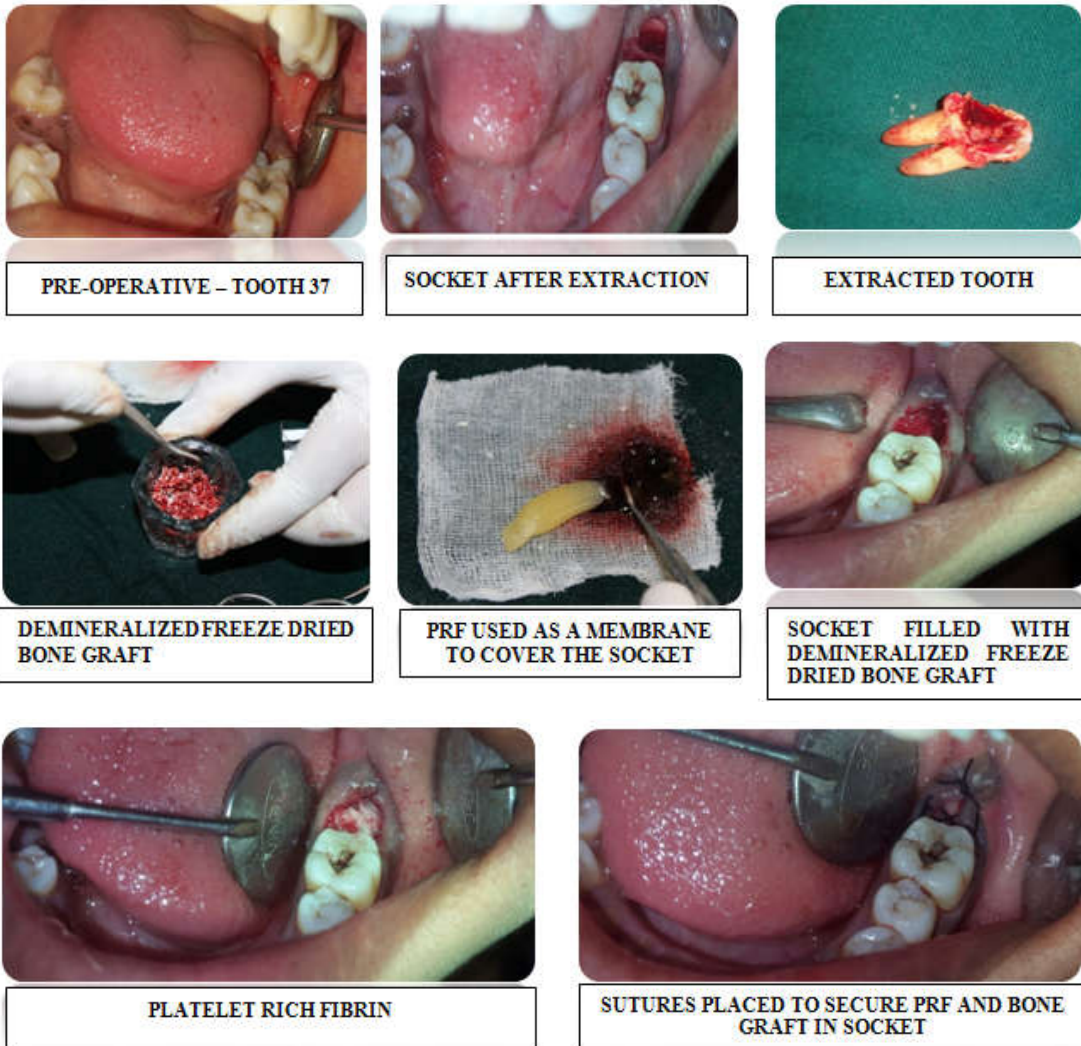
also found less vertical and horizontal changes when ridge preservation was performed using calcium sulfate hemihydrate than extraction with no preservation. Ultimately, the indications for ridge preservation included maintenance of the existing hard and soft tissues of the alveolar ridge, and to simplify subsequent treatment (such as implant or denture placement). Allografts can be divided as fresh-frozen, freeze-dried, or demineralized freeze-dried. The use of freeze-dried bone allografts (FDBA) and demineralized freeze-dried bone allografts (DFDBA) has reduced the problem of immunogenicity that was associated with fresh-frozen bone. They are the most common allografts used currently for ridge preservation. Al-Ghamdi *et al.* (AlGhamdi, 2002), suggested that FDBA is only osteoconductive, while DFDBA can be both osteoconductive and osteoinductive. DFDBA also showed more vital bone and less residual grafting material compared to

FDBA when placed in extraction sockets 19 weeks after extraction. DFDBA also acts as a scaffold for osteoconduction. As DFDBA fulfills the criteria of an ideal graft material so in this study, it was used in both the groups. During the last decade, there have been several *in vivo* animal studies, which have used biological mediators such as polypeptide growth factors to expedite soft tissue and bony healing. It is therefore a reasonable hypothesis that increasing the concentration of platelets in bone defects may lead to improved, faster healing and stimulate new bone formation. Surgical sites enhanced with PRP have been shown to heal at two to three times that of normal surgical sites. PRP can be a great adjunct to many surgical procedures, and PRP accelerates wound maturity and epithelialization, hence decreased scar formation. PDGF and epidermal growth factor (EGF) are the main growth factors involved in fibroblast migration, proliferation, and collagen

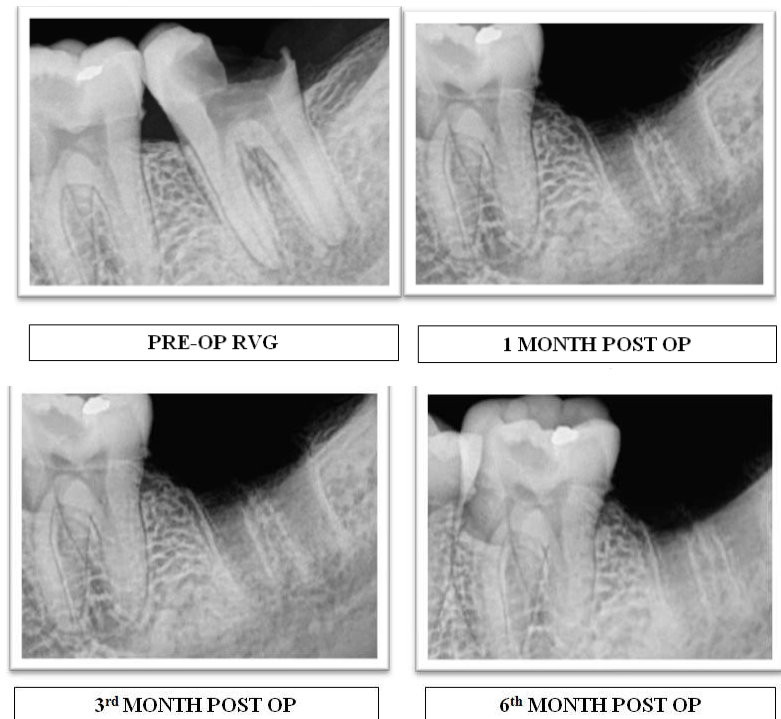
synthesis. Increased concentrations of these growth factors is a likely reason for the accelerated soft tissue wound healing (Wang, 2007). PRF first described by Choukroun *et al.* is a new second generation of platelet concentrate.

Simplified processing technique without any complex handling makes it superior to PRP. PRF can be used to promote wound healing, bone regeneration, graft stabilization, wound sealing, and hemostasis.

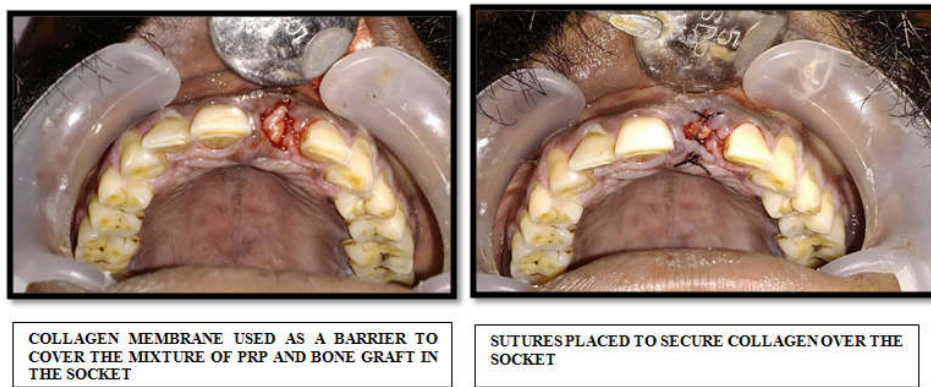
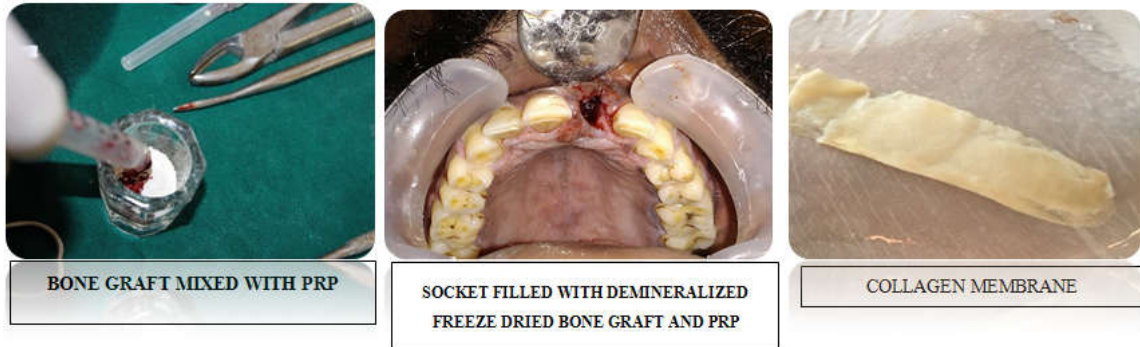
Group I- Demineralized Freeze Dried Bone Graft with Platelet Rich Fibrin



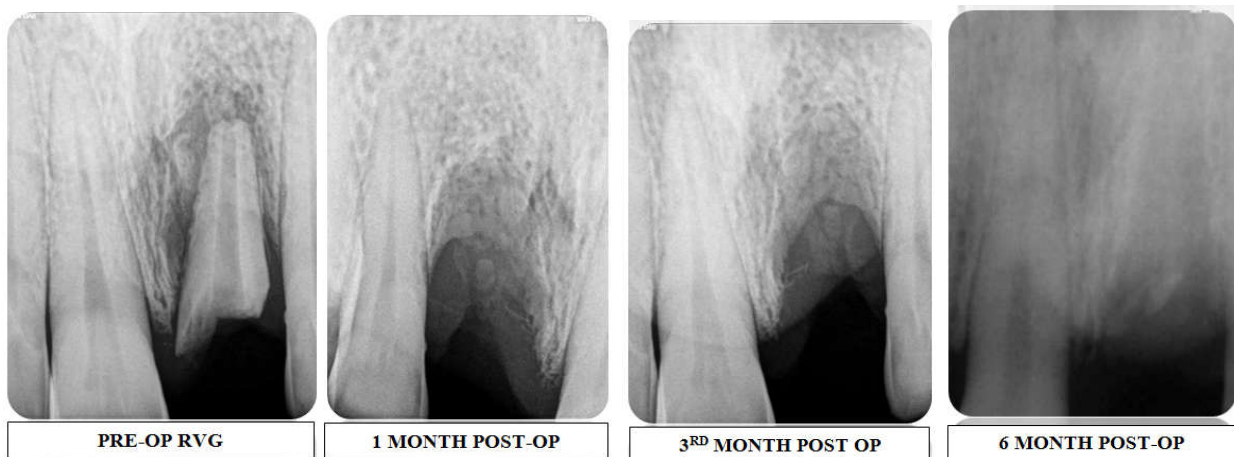
RADIOGRAPHS



Group II- Demineralized freeze dried bone graft with platelet rich plasma and collagen membrane as a barrier



RADIOGRAPHS



As the fibrin matrix is better organized, it is able to more efficiently direct stem cell migration and the healing program. Release of growth factors from PRF through *in vitro* studies and good results from *in vivo* studies led to optimize the clinical application of PRF. It was shown that there are better results of PRF over PRP. The nature of extraction socket is such that it can cause the loss of the majority of bone graft. Therefore, to avoid the loss of graft material, the use of collagen membrane was used in a group 2 (demineralized

freeze dried bone graft and Platelet rich plasma) not only to avoid the loss of graft material but also induce stabilize wound and promote blood clot formation. Among all the available membrane collagen membrane was preferred due to its high biocompatibility and hemostatic activity that can facilitate clot formation and wound stabilization. Collagen also has a high chemotactic function for fibroblasts. This promotes cell migration, and primary wound coverage maximum efforts were made to achieve complete coverage of membrane, but

complete coverage was not obtained in all cases. In a study done by Nam and Park in 2009 showed that if membrane exposure occurs during the healing phase, it does not affect the outcome of ridge preservation. In the present study there was exposure of the membrane in one patient with mandibular posterior region. Patient came with complain of pain in the area on 5th day following extraction. Patient gave history of smoking from the 2nd day of extraction even after verbal and documented post-op instruction. After careful evaluation presence of dry socket was excluded. The exposed membrane was removed, socket irrigated with saline and left to healing by secondary intention. Although we could not find any possible correlation of smoking and membrane exposure. Smoking causes epithelial margin break down which causes loosening and breakage of sutures holding the membrane leading to membrane exposure. Uneventful healing was noted in all other the cases. While comparing the results of group 1 and group 2 it was seen that there was increase in bone height in maxillary and mandibular anterior & posterior region with group 1 (demineralized freeze dried bone graft and PRF) when compared to group 2 (demineralized freeze dried bone graft, PRF and collagen). However, statistically significant improvement was noted in respect to height from baseline to 180 days in both the groups. This is consistent with the earlier studies done using DFDBA alone for the purpose of socket preservation. The use of PRF along with DFDBA has significant advantages over the use of DFDBA alone. Use of PRF aids in retaining of the bone graft material within the walls of the socket, as it is a fibrin clot, it aids in the arrest of bleeding as well. Also there was increase in bone width with Maxillary & Mandibular anteriors and posteriors in group 1.

Similar findings were reported in the study by Simon et al (Simon and Gupta 2015) showing mean width socket resorption of 0.57mm with PRF after 4 months and confirmed a significant advantage in the preservation of post extraction alveolar ridge dimension with the use of PRF. The present study also showed increased in bone density in maxillary and mandibular anterior and posterior region in group 1 when compared with group 2. Although group 2 shows increased in bone density but not significant when compared with group 1. This is similar to a study carried out by , (Yelamali and Saikrishna, 2015) which shows mean values of bone density for PRF groups were significantly higher as compared to PRP groups at four months follow up. The results of our study in terms of bone density contradicts with the study carried by Anitua et al¹⁶ which reported improved epithelization and bone density when PRP was placed in extraction sockets. In the present study when evaluating efficacy of PRF and PRP along with bone graft in maxillary and mandibular extraction socket the results shows that there was increase in all parameters of bone healing with PRF. This was similar to the study which was done by Tejesh Yelamali & D. Saikrishna (2014) to evaluate and compare the utility and effectiveness of platelet rich fibrin (PRF) with that of platelet rich plasma (PRP) on soft tissue healing and bone tissue healing of extracted third molar sockets which shows PRF is significantly better in promoting soft tissue healing and also faster regeneration of bone after third molar extraction, in comparison with PRP. This could be attributed to simpler preparation protocols of PRF over. Another study by Dohan et al. 2006 proved a slower release of growth factors from PRF than PRP and observed better healing properties with PRF. It was observed and shown that the cells are able to migrate from fibrin scaffold; while some authors demonstrated the PRF as a supportive matrix for bone

morphogenetic protein as well. However, despite all attempts being made to carry out a study which considers all the required parameters, following are some limitations that do exist in this study as well. In this study, intraoral radiographic technique was used to measure the bone width and height changes. However, the use of cone beam computed tomography could have been done to achieve more accurate results. Several studies have carried out histomorphometric analysis which was not done in this study. Placement of implants was not done at the follow-up and hence, histological analysis could not be done.

Conclusion

DFDBG and PRF have exhibited significantly better radiographic parameters when compared with DFDBG and PRP along with collagen membrane in the management of ridge preservation in maxillary and mandibular extraction sockets, while evaluating patients on radiographic parameters like bone height, width and density during the follow up period. Although not statistically significant, but DFDBG and PRP showed less bone height, width and density when compared to DFDBG and PRF in maxillary and mandibular anterior and posterior region. The results of this study showed significant upsurge in ridge width and height for both groups at 180 days. Our observations showed that the extent of bone density was found to be greater in DFDBG and PRF when compared to DFDBG and PRP. When both groups were compared PRF combined with DFDBA preserved ridge width better than DFDBA alone. This procedure would benefit the patient by providing ridge form to meet functional and esthetic needs and spare from future ridge augmentation procedure. It can be concluded that the overall summation of the results of the study showed that DFDBG and PRF seem to offer better clinically and radiographically significant results than DFDBG and PRP in the management of ridge preservation in mandibular and maxillary extraction sockets. Moreover, DFDBG & PRF definitely promote better osseous regeneration over DFDBG & PRP in terms of uniformity and density of regenerated bone which is statistically significant.

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