BONE AND SOFT TISSUE CHANGES AFTER ALVEOLAR RIDGE PRESERVATION USING PRF SOCKET PLUG TECHNIQUE VERSUS SOCKET PLUG TECHNIQUE: A SPLIT-MOUTH RANDOMISED CONTROLLED TRIAL

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ABSTRACT

Aim: The aim of this study was to compare platelet rich fibrin “PRF socket plug technique” versus the traditionally used “socket plug technique” to improve bone quality without affecting its volumetric stability. Research question: In mandibular premolars socket preservation, will “PRF socket plug” technique improve bone quality without affecting bone quantity, when compared to ‘socket plug” technique? Materials and Methods: This was a split-mouth randomized controlled trial conducted on 9 patients. For each patient, bilateral socket preservation was performed. PRF socket plug technique (intervention) was performed for one side, while the other was performed using the traditional socket plug technique (control). After 6 months, bone and soft tissue changes were measured. Horizontal and vertical alveolar ridge loss and loss percentage were measured using cone-beam CT. Bone quality was measured by histomorphometric analysis of area percentages of mineralized trabecular bone of core biopsies. Keratinized mucosa changes were measured using Williams graduated periodontal probe. Results: Intervention group showed slightly higher horizontal bone loss, loss percentage, vertical bone loss, loss percentage (1.36 mm, 16.98 %, 1.07 mm, 7.99 %) with no statistically significant difference when compared to the control group (1.14 mm, 13.89 %, 0.97 mm, 7.21 %). Histomorphometric analysis showed higher new bone formation (34.11 %) in intervention group compared to the control group (30.78 %) with no statistically significant difference. Both groups showed keratinized mucosa gain (1.28 mm intervention, 1 mm control) with no statistically significant difference. Conclusions: Socket plug technique is an effective technique for alveolar ridge preservation; PRF clot represents an easy, successful, and economical method to cover the graft in socket plug technique; PRF socket plug technique represents a promising alternative to the routinely used socket plug technique.

KEYWORDS: Socket preservation, Socket plug technique, Xenograft, Platelet concentrates, Platelet rich fibrin.

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INTRODUCTION

Dental implants are currently considered a routine treatment modality for replacing missing teeth. This enthusiastic intention has often clashed with the progressive tissue loss occurring after teeth extraction. Bone loss can reach up to 50% of the alveolar ridge width. The majority of these changes occur in the first 6 months. Moreover, this process is concentrated on the buccal bone results in the re-location of the ridge to a more lingual position. Therefore, alveolar ridge preservation after tooth extraction has been introduced to maintain alveolar ridge, eliminating the need for different augmentation procedures before implant placement.

Alveolar ridge preservation involves any procedure performed to eliminate or limit post-extraction resorption, maintain ridge contour, promote bone formation and facilitate future implant placement. The first attempts to preserve alveolar ridge was performed using submerged root concept. Thereafter, different techniques have been introduced for alveolar ridge preservation. These techniques mainly differ in flap management, grafting material, and graft coverage.

Traditionally alveolar ridge preservation was performed by socket filling (socket grafting) technique with or without GBR. In socket filling technique, extraction socket is grafted by different materials. The grafted socket is covered with barrier membrane in socket filling/GBR technique. Finally, the graft or/and membrane coverage is achieved by primary soft tissue closure. While in GBR, barrier membranes are used with ungrafted sockets. The principle of GBR involves the placement of barrier membrane to block in growth of soft tissue to the bone defect.

The main drawback of previous techniques is the need of flap advancement to achieve primary soft tissue closure, leading to migration of mucogingival junction, reduction of available keratinized mucosa, increasing the risk of flap dehiscence and graft exposure, and increasing postoperative discomfort. In a trial to overcome this drawback, socket seal technique was introduced. This technique utilizes a palatal soft tissue graft to cover the grafted socket. Although, socket seal technique eliminates the need of primary tissue closure, it is still associated with postoperative discomfort due to soft tissue donor site morbidity. Moreover, soft tissue graft harvesting needs skillful operator, and showed unpredictable results.

Lately, “socket plug” technique has been introduced. “Socket plug” technique was primarily proposed by Sclar as “Bio-Col” technique. In the original technique Sclar suggested: (1) atraumatic extraction, (2) socket grafting with bovine bone, (3) collagen plug placement for graft coverage, (4) horizontal mattress suture to secure the plug, (5) cyanoacrylate over the collagen to harden and decrease collagen permeability, (6) oval pontic placement over surgical site. Thereafter, various modifications have been introduced to improve and simplify the “Bio-Col” technique. The proposed modifications were concerned with handling and stabilization of collagen plug, suturing technique, and grafting materials. In 2014, Kotsakis et al introduced the term “socket plug” to describe different techniques using collagen dressing to cover grafted sockets. They recommended 4 distinct steps for this technique: (1) atraumatic flapless extraction, (2) socket grafting, (3) socket coverage with collagen dressing, (4) suturing.

Although autogenous bone represented for years the gold standard in bone grafting procedures (for its osteogenic, osteoinductive and osteoconductive properties), its availability is countered by the morbidity associated with graft harvesting. Bone substitutes have been used as alternatives or supplements to autogenous bone. Those materials represent an attractive alternative for autogenous bone especially in implant grafting procedures. In such procedures, it should always be a priority...
to reduce patient morbidity to a minimum. It may be considered an aggressive method to harvest autogenous bone for small defects as extraction socket. Furthermore, bone substitutes showed higher volumetric stability compared to autogenous bone.

Hard and soft tissue healing has been dramatically improved by the modulation of growth factors. The application of growth factors in bone tissue regeneration is mainly focused on the osteoinductive factors (as PDGF and BMPs) aiming to accelerate bone formation. Platelet concentrates are considered as an alternative source of growth factors for its availability and cost. They are autologous fibrin adhesives with a high platelet concentration and high quantities of key growth factors (such as PDGF, TGFβ1, TGFβ2, VEGF, IGF, EGF, FGF).

PRP and PRGF represents the first generation of platelets concentrates. All available PRP and PRGF techniques have some points in common: (1) blood collection from the patient with anticoagulant to avoid platelet activation and degranulation, (2) differential centrifugation, (3) platelet concentrate application, together with activator. In 2001, PRF was developed by Choukroun et al. PRF is neither fibrin glue nor classical platelet concentrate. PRF protocol needs no anticoagulant or activator. The blood is collected into glass tubes without anticoagulant, and immediately centrifuged. Unlike other platelets concentrates, platelets activation during the centrifugation process leads to substantial embedding of growth factors within the formed fibrin matrix. Being simply prepared and of low cost compared to other growth factors, PRF remains on the top of the list when choosing a reliable and a convenient regenerative material.

Ever though socket plug technique showed promising results, almost all previously published clinical trials are case reports and series. In this randomised controlled trial, we introduce the use of “PRF socket plug technique” and compared it to the traditionally used “socket plug technique” in a trial to improve bone quality without affecting its volumetric stability.

**PICO question**

In mandibular premolars socket preservation, will “PRF socket plug” technique improve bone quality without affecting bone quantity, when compared to ‘socket plug” technique?

**MATERIALS AND METHODS**

**A) Trial design**

This was a split-mouth randomised controlled clinical trial. For each patient bilateral socket preservation was performed. Socket plug technique was performed for one side (control side), while the other (intervention side) was performed using the PRF socket plug technique (Fig. 1).

**B) Participants**

This study was conducted on 9 patients (6 female, 3 male aged between 29 and 47 years) selected from the out-patient clinic, Department of Oral and Maxillofacial Surgery, Faculty of Oral and Dental Medicine, Cairo University from December 2016 to January 2018.

Patients were selected according to the following criteria: Adult patients with bilateral non restorable mandibular premolars indicated for extraction; normal blood counts; free from any systemic disease or local condition that may affect normal bone and soft tissue healing, and predictable outcome, or contraindicate implant placement; non-smokers; sockets maintaining a five wall defect after extraction. Any patients with acute infection or periapical lesion related to the area to be grafted were excluded.

**C) Interventions**

Patients were assessed clinically to assure their correspondence with eligibility criteria. Complete
blood count was requested to assure normal blood counts. Preoperative panoramic radiograph was performed for each patient to assess root morphology, and exclude the presence of any periapical lesions. Every patient has undergone scaling and root planning to assure a more hygienic environment before the extraction procedure, and minimizing the risk of infection. Patient specific radiographic stent was fabricated for each patient using 2 mm thick clear vacuum sheets. Stents were fabricated to cover the lower dental arch (including non-restorable teeth). Gutta percha radiographic markers were fixed at the coronal, buccal, and lingual aspects of the stent at the treatment sites to act as reference points for measurements standardization. Surgical procedures were performed under local anesthesia using Articane with Epinephrine 1:100,000 (Artinibsa, Inibsa, Barcelona, Spain). Patients were instructed to rinse their mouth with Chlorohexidine Gluconate 0.1%* mouth wash (Antiseptol, Kahira Pharma Co, Cairo, Egypt). Flapless atraumatic extraction was performed using periotomes. Periotomes were used to cut periodontal fibers at the cervical area. The tip of the periotome was inserted at the root mesial and distal line angles parallel to the root, with the blade located at the tip of the crestal bone. The periotome was then inserted into the periodontal ligament space and moved around the whole root surface area. The periotome was then directed apically towards the root apex and the process continued up to two thirds of the root. The final step of the extraction was performed using conventional extraction forceps. Forceps was used to extract the tooth (remaining root) with minimal force applied to forceps handles to avoid fracture or crushing of the tooth. Finally, extraction socket was examined to ensure the presence of five walls defect, and bone curette was used to debride the socket and remove any soft tissue remnants (Fig. 2).

Socket preservation was performed with traditional “socket plug” technique for the control side, and with “PRF socket plug” technique for the other. For the control side, xenograft particles (0.25-1 particles, Tutobone, RTI Biologics™ Tutogen medical GmbH, Germany) were hydrated with saline and used to fill the socket. Collagen plug (Collaplug, Zimmer Dental Inc, Carlsbad CA, USA) was trimmed and adapted over the socket to cover the graft. Finally, the plug was stabilized with figure of eight suture using 3/0 black silk. Minimal tension was applied to the suture to preserve soft tissue architecture (Fig. 3).

For intervention side, 10 ml venous blood was drawn from the patient using plastic syringe to prepare PRF. Blood was immediately collected in 10 ml dry glass test tube. The tube was immediately centrifuged at 3000 rpm (about 400 x g) for 12 minute. After centrifugation, PRF (middle layer) was formed with acellular platelet poor plasma (top layer), and red blood cells (base layer). PRF clot was removed from the tube and the attached red blood cells layer was scraped off and discarded. Scissors was used to cut PRF clot to 2 pieces. One clot was pressed using PRF piston and cylinder to form PRF plug. While the other was cut into small pieces and mixed with grafting material. The socket was filled with the hydrated graft particles mixed with PRF. Finally, PRF plug was adapted over the socket to cover the graft, and stabilized with figure of eight suture 3/0 black silk (Fig. 4).

Postoperative instructions and medications were: extraoral ice packs for first postoperative six hours, non-steroidal anti-inflammatory analgesic (Diclofenac potassium 50mg, Catafast tablets, Novartis Pharma AG, Cairo, Egypt) for three days, antibiotic (Clindamycin 300 mg, Clindam capsules, Sigma pharmaceutical industries, Egypt) for five days, Regular oral hygiene measures were resumed after 24 hours. Patients were recalled 1 week postoperatively for suture removal and clinical evaluation. The clinical evaluation included assessment of postoperative complications and soft
tissue healing. Next visits were scheduled at 1, 3 and 6 months postoperatively.

Postoperative CBCT radiographs were performed for every patient 1 week (T₀) and 6 months (T₆) postoperatively. The radiographs were made with the same machine and same exposure parameters, with the radiographic stent seated in place to standardize radiographic measurements. Image reconstruction was performed using special software (Ondemand 3D version 1.0.9, Cybermed, Korea).

Second stage surgery was performed 6 months after socket grafting. It involved biopsy harvesting and implant placement. Bone core biopsy was collected from each socket before implant placement using a 3 mm diameter trephine bur. Biopsy samples were fixed immediately in 10% buffered formalin. Decalcification of the specimens were achieved by suspension in EDTA 10% solution for one week with regular renewal of the solution daily. After decalcification the specimens were dehydration using ascending alcohol, followed by clearing in xylol, then they embedded in paraffin wax to form a block. The paraffin block was sectioned longitudinally using a microtome into thin paraffin sections, each of approximately 5 microns thick. Sections were stained using Masson Trichrome stain for histomorphometric analysis.

**D) Outcomes**

The primary end point of radiographic analysis was the change in alveolar bone width. Additional analysis was done for the alveolar bone height change. Radiographic measures were performed on the cross-sectional cuts of CBCT. To assess horizontal alveolar bone loss (width loss), the alveolar bone width was measured at below the alveolar crest at the base line (W₀) and after 6 month (W₆). Then, horizontal bone loss and loss percentage were calculated as follow: Horizontal bone loss (L₆), the difference between bone width at base line and after 6 months (W₀ - W₆); Horizontal bone loss percentage (PL₆), percentage of the bone loss (L₆) to the base line width (W₀).

To assess vertical alveolar bone loss (height loss), the alveolar bone height was measured from the deepest point of the socket base to the bone crest at the base line (H₀) and after 6 month (H₆). Then, vertical bone loss and loss percentage were calculated as follow: Vertical bone loss (L₆), the difference between bone width at base line and after 6 months (L₀ - L₆); Vertical bone loss percentage (PL₆), percentage of the bone loss (L₆) to the base line width (W₀). The radiographic stent was used to replicate the same measuring points at different time points for each patient (Fig. 5).

The end point of histomorphometric analysis was percentage of newly formed bone. Histomorphometric analysis was performed using image analyzer computer system (Leica QWin 500, Leica Microsystems Inc. Buffalo Grove, IL 60089 United States). Additional analysis was performed for remnants of bone substitute. Areas of newly formed bone, remnants of bone substitute were measured as a percentage of the total area for each core biopsy harvested from grafted sockets. In Masson Trichrome stained samples, new bone was identified in blue, and bone substitute remnants in red (Fig. 6).

Keratinized mucosa was measured at the mid buccal aspect of the tooth using a Williams graduated periodontal probe. Base line keratinized mucosa was performed between the mucogingival line and the gingival margin, while after 6 months the measurement was done between mucogingival junction and the most crestal part of the edentulous ridge. To determine the keratinized mucosa gain for each socket, 6 months measure was subtracted from the base line measure.

**E. Sample size**

Based on previous study by Festa et al 46, the difference in horizontal alveolar bone loss between
the 2 groups was 1.9 mm ± 1.2. Using power of 80% and 5% significance level, we needed to study 7 sockets in each group. The number was increased to a total sample size of 9 in each group to compensate for 20% losses during follow up. Sample size calculation was achieved using PS: Power and Sample Size Calculation software Version 3.1.2 (Vanderbilt University, Nashville, Tennessee, USA).

F) Randomisation

For each patient, one side was randomly allocated to control group, and the other side to intervention group. Randomisation was performed using 9 sheets of standard size paper. “Control/right side” was written on 5 sheets, “Control/left side” was written on the other 4 sheets. Paper sheets were then placed in 9 opaque sealed envelopes till start of surgical procedures. For each patient, an envelope was opened after patient eligibility assessment, preoperative preparation, and before starting the surgical procedure.

G) Blinding

This was a double blinded study. Outcome assessor and statistical data analyst were kept blinded. Whereas, participants and interventionists involved in the surgical procedures were aware with group allocation due to the nature of the procedure.

H) Statistical analysis

Statistical analysis was performed using SPSS (Statistical package for the social sciences-IBM® SPSS® Statistics Version 20 for Windows, IBM Corp., Armonk, NY, USA). The data were represented as mean ± standard deviation. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. For parametric data; Paired t-test was used to compare variables between the two groups. For non-parametric data; Wilcoxon signed-rank test was used. The results were considered statistically significant if the p value was less than 0.05.

Fig. (1) a. Socket plug technique: the socket is filled with bone substitute particles and covered by socket plug. b. PRF socket plug technique: the socket is filled with bone substitute/PRF mixture and covered by PRF clot.

Fig. (2) a. Periotome inserted at the root line angle parallel to the root. b. Extraction socket after flapless atraumatic extraction.
Fig. (3) Socket plug technique. a. Socket filled by xenograft particles. b. Collagen plug covering bone graft. c. Plug stabilized with figure of eight suture.

Fig. (4) PRF socket plug technique. a. PRF (middle layer) between acellular plate poor plasma (top layer), and red blood cells (base layer). b. PRF box kit with the piston and cylinders. c. PRF clot covering grafted socket. c. PRF clot stabilized with figure of eight suture.
RESULTS

Intervention group showed slightly higher horizontal bone loss, horizontal bone loss percentage, vertical bone loss, and vertical bone loss percentage (1.36 ± 0.24 mm, 16.98 ± 2.39 %, 1.07 ± 0.31 mm, 7.99 ± 2.1 %) when compared to the control group (1.14 ± 0.35 mm, 13.89 ± 3.48 %, 0.97 ± 0.18 mm, 7.21 ± 1.25 %). There was no statistically significance difference between the 2 groups for all measures (P value > 0.05) (Fig. 7, Table 1).

Histomorphometric Analysis

Intervention group showed higher new bone formation (34.11 ± 6.07 %) compared to the control group (30.78 ± 5.93 %), while control group showed higher bone substitute remnants (31.89 ± 6.39 %) compared to intervention group (29.33 ± 5.1 %). There was no statistically significance difference between the 2 groups for both measures (P value < 0.05) (Fig. 8).

Clinical assessment

Normal healing process was observed in both groups. Intervention group showed higher gain in the keratinized mucosa (1.28 ± 0.26 mm) when compare to the control group (1 ± 0.5 mm). There was no statistically significance difference between the 2 groups (P value > 0.05) (Fig. 9, Table 2)
TABLE (1) Radiographic measures (Mean ± standard deviation)

<table>
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<tr>
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<th>$W_0$</th>
<th>$W_6$</th>
<th>$L_w$</th>
<th>$PL_w$</th>
<th>$H_0$</th>
<th>$H_6$</th>
<th>$L_H$</th>
<th>$PL_H$</th>
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<tr>
<td><strong>Intervention</strong></td>
<td>7.97 ± 0.67</td>
<td>6.61 ± 0.55</td>
<td>1.36 ± 0.24</td>
<td>16.98 ± 2.39</td>
<td>13.29 ± 1.09</td>
<td>12.22 ± 0.98</td>
<td>1.07 ± 0.31</td>
<td>7.99 ± 2.1</td>
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<tr>
<td><strong>Control</strong></td>
<td>8.14 ± 0.5</td>
<td>7 ± 0.22</td>
<td>1.14 ± 0.35</td>
<td>13.89 ± 3.49</td>
<td>13.42 ± 1.04</td>
<td>12.46 ± 0.99</td>
<td>0.97 ± 0.18</td>
<td>7.21 ± 1.25</td>
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$W_0$: Bone width at base line; $W_6$: Bone width after 6 months; $L_w$: Horizontal bone loss; $PL_w$: Percentage of horizontal bone loss; $H_0$: Bone height at base line; $H_6$: Bone height after 6 months; $L_H$: Vertical bone loss; $PL_H$: Percentage of vertical bone loss

TABLE (2) Keratinized mucosa measures (Mean ± standard deviation)

<table>
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<th>Baseline</th>
<th>6 months</th>
<th>Gain</th>
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<tr>
<td><strong>Intervention</strong></td>
<td>2.44 ± 0.58</td>
<td>3.72 ± 0.67</td>
<td>1.28 ± 0.26</td>
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<tr>
<td><strong>Control</strong></td>
<td>2.28 ± 0.44</td>
<td>3.28 ± 0.57</td>
<td>1 ± 0.5</td>
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Fig. (7) Bar charts showing radiographic measures for both groups. a. Alveolar ridge width change. b. Alveolar ridge height change.

Fig. (8) Pie charts showing histomorphometric results of both groups.
Alveolar ridge preservation has been introduced to maintain the existing soft and hard tissues, simplifying upcoming procedures, enhancing functional and esthetic results. Various techniques and protocols have been investigated, however the selection of the ideal technique still questionable. This split-mouth randomised study was designed to introduce the use of “PRF socket plug” technique and test its efficiency for alveolar ridge preservation compared to the “socket plug” technique. The study report was prepared following the CONCORT 2010 guidelines for reporting randomised trials.

The split-mouth design has been used in different clinical studies. The major advantage of this design is the isolation of treatment comparisons from the inter-subject variation; subsequently it obtains the same power with lower sample size when compared to parallel arm design. In split-mouth studies, each patient should have more than one site indicated for treatment. To achieve this in our study, all patients were selected with bilateral non restorable mandibular premolars indicated for extraction and socket preservation.

In this study, the grafted sockets were covered by collagen plugs (control group) and PRF clots (Intervention group). The collagen dressings were utilized to cover grafted sockets, prevent graft wash out, and induce clot formation. Furthermore, it enhances soft tissue coverage and encourages cell migration as collagen shows chemotactic properties for fibroblasts. In “PRF socket plug” technique, we induced the use of PRF clot instead the collage plug. PRF is not only an optimized blood clot to cover the graft, but also it is a matrix containing various elements enhancing migration of endothelial cells and fibroblasts, and tissue healing. A recent systematic review by Miron et al highlighted this positive effect of PRF soft tissue healing. Although, PRF group showed slightly higher keratinized mucosa gain, both groups showed higher results (1-1.28 mm gain) when compare to unassessed healing (0.9 mm loss to 0.4 mm gain).

Recent histomorphometric meta-analysis by De Risi et al showed that xenograft ARP results in lower bone percentage after 6 months compared to other grafting materials. To the contrary, xenograft slow biodegradation seems to maintain graft volume and prevent bone loss. The optimal time for implant placement after socket grafting represents a true dilemma. With time, the alveolar ridge volume gradually decreases, while the bone quality gradually increases. Consequently, the implants should be placed as early as possible, but late enough to allow proper bone formation. In this study, PRF fragments were mixed with xenograft particles in a trial to accelerate bone maturation without affecting its volumetric stability. Although, Histomorphometric analysis after 6 months showed higher new bone and lower xenograft remnants in the PRF group compared to control group, there was no statistically difference between both groups.

Both groups showed a high percentage of residual bone substitute. This result was correlated with various studies conducted on bovine bone showed retention of graft up to 3 years.
A SPLIT-MOUTH RANDOMISED CONTROLLED TRIAL

grafting material. Consequently, the final result of the grafted socket is formed of newly formed bone and the residual bone substitute. It has been proven that such remaining material wouldn’t hinder implants osteointegration. Berglundh and Lindhe investigated in an experimental study bone healing around implants placed in bone defects grafted with bovine bone. Histological results showed the presence of mineralized bone separating graft particles from implants surfaces.

Radiographic outcomes of our study showed comparable results in both groups. It showed that the addition of PRF to xenograft particles doesn’t adversely affect its volumetric stability. Both groups showed vertical and horizontal bone loss. This result corresponds with various systematic reviews that demonstrated that different ARP techniques minimize, but don’t totally eliminate post extraction alveolar bone resorption. However, both groups showed great results compared to unassisted socket healing. Tan et al. conducted a meta-analysis to evaluate dimensional changes after 6 months of unassisted socket healing. Non grafted sockets showed horizontal dimensional reduction of 3.79 mm (ranging from 29-63 % of the original site width), vertical dimensional reduction of 1.24 mm (ranging from 11-22 % of the original site height).

Although, PRF doesn’t prove to have beneficial effect on xenograft maturation, “PRF socket plug” technique showed promising results compared to “socket plug” technique. It reduces the quantity of bone substitute used, eliminates the need of collagen plug; reducing the overall cost of the procedure without affecting volumetric stability. Despite the great advantages of both techniques, they have a major limitation. These techniques can be used only in five bony wall sockets. Any bony wall damages, dehiscence or fenestration indicates the need of barrier membrane to cover the graft at the defect site and prevent soft tissue in growth.

CONCLUSIONS

Within the limitation of this study, we concluded that: Socket plug technique is an effective technique for alveolar ridge preservation; PRF clot represent an easy, successful, and economical method to cover the graft in socket plug technique; PRF socket plug technique represents a promising alternative to the routinely used socket plug technique.

LIST OF ABBREVIATIONS

PRF Platelet rich fibrin
GBR Guided bone regeneration
PDGF Platelet-derived growth factor
BMPs Bone morphogenetic proteins
TGFβ Transforming growth factor beta
VEGF Vascular endothelial growth factor
IGF Insulin-like growth factor
EGF Epidermal growth factor
FGF Fibroblast growth factor
PRP Platelet-rich plasma
PRGF Plasma rich in growth factors
CBCT Cone beam computed tomography

REFERENCES


