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Original Article

Socket preservation using freeze-dried bone allograft with and without plasma rich in growth factors in dogs

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ABSTRACT

Background: Plasma rich in growth factors (PRGF) and freeze-dried bone allograft (FDBA) are shown to promote bone healing. This study was aimed to histologically and histomorphometrically investigate the effect of combined use of PRGF and FDBA on bone formation, and compare it to FDBA alone and control group.

Materials and Methods: The distal roots of the lower premolars were extracted bilaterally in four female dogs. Sockets were randomly divided into FDBA + PRGF, FDBA, and control groups. Two dogs were sacrificed after 2 weeks and two dogs were sacrificed after 4 weeks. Sockets were assessed histologically and histomorphometrically. Data were analyzed by Kruskal–Wallis test followed by Mann–Whitney U-tests utilizing the SPSS software version 20. $P < 0.05$ was considered statistically significant.

Results: While the difference in density of fibrous tissue in three groups was not statistically significant ($P = 0.343$), the bone density in grafted groups was significantly higher than the control group ($P = 0.021$). The least decrease in all socket dimensions was observed in the FDBA group. However, these differences were only significant in coronal portion at week 4. Regarding socket dimensions and bone density, the difference between FDBA and FDBA+PRGF groups was not significant in middle and apical portions.

Conclusion: The superiority of PRGF+FDBA overFDBA in socket preservation cannot be concluded from this experiment.

Key Words: Allografts, platelet-rich plasma, socket graft

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INTRODUCTION

Unfavorable dimensional and morphologic changes of the alveolar bone are inevitable subsequent to tooth extraction. The most destructive changes are reduction of socket walls, especially the buccal wall,[1,2] as well as replacement of the previous root space by the bone marrow.[1,3] It is reported that average horizontal volume reduction of 5–7 mm occurs within the 1st year after tooth removal which equals approximately to 50% of the original width.[4]

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Numerous materials including autograft, allograft, xenograft, and alloplastic bone graft have been utilized to maintain the alveolar ridge after tooth removal. Each of these materials has its own advantages and disadvantages.[5]

Guided bone regeneration (GBR) is shown to maintain ridge to some extent. However, in previous investigations performing GBR, ridge dimensions were not completely preserved.[6-8] In addition, neitherdecalcified freeze-dried bone allograft (DFDBA) with bioabsorbable membrane[9] nordeproteinized bovine bone material[5]achieved a complete ridge preservation. DFDBA is an osteoinductive and osteoconductive biomaterial. It is also claimed that DFDBA has osteopromotive properties.

Only a few studies have compared DFDBA and freeze-dried bone allograft (FDBA). Borg *et al*. observed a greater new bone formation with a combination of mineralized and demineralized allograft compared to mineralized FDBA in alveolar ridge preservation in humans.[10] Ogihara and Tarnow reported that enamel matrix derivate (EMD) + FDBA and EMD+DFDBA resulted in soft-tissue improvement compared to EMD alone, and both materials worked well in reconstruction of deep intrabony defects when combined with EMD.[11] Therefore, there are controversial data and no conclusive result on the better efficacy of each of these materials.

Platelet-rich plasma (PRP) is an autograft for regeneration of bone defects. It contains growth factors such as platelet-derived growth factor, transforming growth factor-β, fibroblast growth factor, insulin-like growth factor-I, epithelial growth factor, vascular endothelial growth factor, and other secretory proteins.[12] It is shown that PRP accelerates proliferation and differentiation of preosteoblasts, fibroblasts, and stromal stem cells. This leads to the promotion of soft- and hard-tissue regeneration, collagen production, calculus formation, and wound healing.[13] However, both positive and negative properties have been reported regarding the PRP. There are limited and controversial data for clinical usefulness of PRP.[14-16] While many studies reported increased bone maturation subsequent to use of PRP with various bone grafts,[12,17-20] results of other studies donot show similar findings.[21-25]

Plasma rich in growth factors (PRGF) is a similar recent product[26-28] which contains similar growth factors and platelets and has many advantages over PRP; in contrast with PRP, it is completely autologous and there is no need ofbovine thromboplastin in preparation of PRGF. Therefore, it poses no risk of disease transmission to the patient. In addition, preparation of PRGF is faster. While at least 30 min is needed to prepare PRP, it is only takes 15–20 min for PRGF. A marked disadvantage of PRP is that it may contain interleukins and a large number of leukocytes. In contrast, PRGF doesnot have this problem. Moreover, while 5 cc of blood sample is needed to purify PRGF, this amount is about 50–500 cc for PRP, which requires a hospital stay for taking blood sample. A considerable advantage of PRGF is that all platelets remain inactive before use. However, some platelets are activated in PRP because of the high speed of centrifugation.[28-30]

PRGF has been successfully used to enhance the regeneration of bone and epithelial tissues. It is shown that PRGF can decrease complications of surgeries as pain and inflammation. In addition, PRGF has been shown to enhance theosseointegration of the implants inserted in sockets.[29] While animal studies showed lack of improved bone healing after application of PRP alone,[20,30] combinationof PRGF and other biomaterials is shown to help socket preservation as well as healing of intrabony defects.[31]

As mentioned above, various biomaterials have been used to augment bone. Since the effects of PRGF and FDBA has been evaluated separately and it is shown that these biomaterials have a marked positive effect on bone healing and socket preservation, respectively,[26] the objective of the present study in dogs was to histologically and histomorphometrically investigate the effect of combined use of PRGF and FDBA on bone formation, as well as compare it to FDBA alone and control group.

MATERIALS AND METHODS

Ethical considerations

The ethical approval of the Ethics Committee of the Dental Research Center at Isfahan University of Medical Sciences was obtained. This *in vivo* study was performed following the Institutional Review Board guidelines for the use and care of laboratory animals.

Plasma rich in growth factor isolation, animal models, and surgery

A volume of 9 mL of peripheral blood of each dog was collected from saphenous vein. Tubes contained

an anticoagulant (3.8% sodium citrate). Using a Digital Apparatus (Model PRGF System IV, BTI, Biotechnology Institute, Spain), the plasma in 9ml tubeswas centrifuged at 2000 rpm for 8 min.

Fraction 1 (the supernatant, 2 mL) was plasma with a concentration of platelets comparable to peripheral blood (platelet-poor plasma) which was removed. Fraction 2 (intermediate layer, 1 mL) had a platelet concentration higher than physiologic level. Fraction 3 (PRGF, 1 mL) which was the richest in platelets (2–3 times more than the peripheral blood) and growth factors was collected.

A volume of 0.05 mL of $CaCl_2$ 10% (as a PRGF activator) was added to each mL of Fraction 3. Plasma was mixed with the graft material. A clot containing the graft and sticky in consistency (easy to handle and compact) formed within 2–5 min. PRGF and the activator were heated for 10 min by a heater to 37°C. The scaffold-like PRGF was ready to be mixed with FDBA.

Four disease-free 12-month-old female dogs, weighting 15–20 kg, were selected and kept in individual cages with similar conditions and standard diet during the experiment. Animals were first kept in quarantine for 2 weeks to perform antibacterial treatment and vaccination against common diseases.

The surgical procedures were under general anesthesia which was induced by intramuscular (IM) injection of 1% acepromazine (alfasan, 0.02 mL/kg) and 10% ketamine (10 mg/kg), followed by the administration of inhaled halothane. Local anesthesia was achieved using 2% lidocaine with epinephrine (1:100,000) (Darou Pakhsh, Tehran, Iran) in lower premolar regions.[31] Chlorhexidine 0.2% was utilized on the orofacial region.

Sulcular incisions were made in the premolar regions (second, third, and fourth [P2, P3, and P4]) in the right and left sides of the mandible after which a full-thickness flap was elevated to expose 1–2 mm of the alveolar crest.[32] Using a high-speed turbine bur (d and z Wiesbaden, Germany), P2, P3, and P4 of the left and right sides were hemi-sectioned while irrigated withnormal saline.

Distal roots were removed by a periotome and an elevator [Figure 1]. The teeth pulps were removed after which zinc oxide-eugenol was put in the pulp chamber as a dressing, and then dental amalgam was put on it.[32] Each dog had 6 sockets in lower right and left quadrants (24 sockets from 4 dogs). Sockets were divided into three groups:

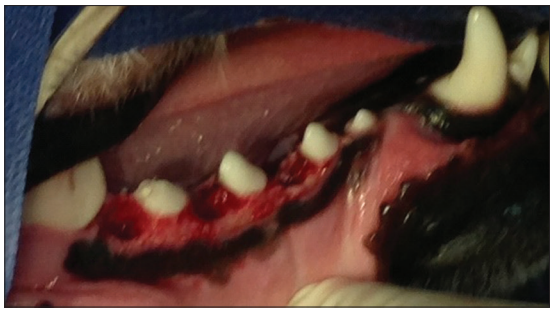

Figure 1: Clinical photograph illustrating distal sockets of mandibular premolars.

- Group 1 (experimental group): FDBA + PRGF
- Group 2: FDBA + saline
- Group 3 (control group):Filled with blood clot.

Eight sockets were randomly selected in each group. Following filling of all the extraction sockets, entrances of extraction sockets were covered by buccal and lingual flaps. Flaps were sutured in their original position with interrupted absorbable 3-0 Vicryl sutures (SUPA Medical Devices, Tehran, Iran).

Antibiotic therapy was administered postoperatively: ceftriaxone, 500 mg (Jaber Ebne Hayyan, Tehran, Iran) IM, four times a day for 5 days. 5 mg/kg of oral tramadol (Tehran Chemie, Tehran, Iran) was administered to relieve pain.

Dogs were given a soft diet, and a regular examination was performed daily to assess the systemic health or detect any problem, including suture opening and postoperative infection. Two dogs were sacrificed in 2 weeks after surgery with an intravenous overdose of thiopental sodium, leading to a painless and rapid death.[32]

The other two dogs were sacrificed in 4 weeks after surgery by the same method. Mandibles were removed, and the premolar sites (P2, P3, and P4), including the mesial root and distal socket area, were dissected by a diamond saw. Specimens were kept in 10% buffered formalin solution for 3 days after which they were placed in 10% nitric acid to be demineralized and prepared for histological and histomorphometric assessment.

Histology and histomorphometry, statistical analyses

Sections from premolar site (two sections from the mesial roots and two sections from the healed distal socket) were cut in the buccolingual plane perpendicular to the bone surface. Sections were

prepared from the central part of the socket or the root.

A series of sections of 5μm in thickness were obtained and then stained with hematoxylin and eosin (H and E).

Sections were observed and histologically evaluated under a light microscope (Nikon, YS100, Tokyo, Japan) at magnifications of ×10, ×100, and ×400. Type of newly formed bone (compact or cancellous) inside the socket, fibrous tissue, the epithelium, and the presence of inflammation were evaluated.

A stereomicroscope (Zoom Stereomicroscope, HP SMP 200, USA) software (Motocam 480 Digital camera SP 10/0224, Canada) was utilized to determine the histomorphometric criteria.

Following landmarks were identified [Figure 2]:

- BC: The crest of the buccal bone wall at the mesial root sites
- LC: The crest of the lingual bone wall at the mesial root sites
- A: Apical portion of the periodontal ligament of the mesial root
- BB: Base of the basal body of the mandible (vertical distance between A and the base of the mandible).

The image of the alveolar process (AP) at the root site was divided into three equal portions of apical, middle, and coronal area [Figure 2]. The cross-section area occupied by each portion was measured with a cursor and expressed in mm^2.

To determine the percentage of newly formed bone, histomorphometric study wascarried outby Nilu analyzer software and a microscope (Sand Optic BM 22, Isfahan, Iran) at ×10, ×100, and ×400 magnifications. The percentage of bone, fibrous tissue, and empty spaces of socket wascalculated.

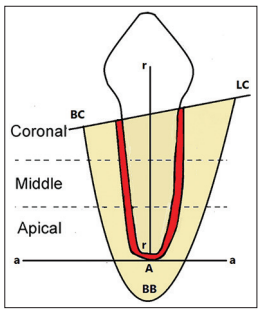

Figure 2: Diagram of landmarks used for histomorphometric measurements. LC:Lingual crest of tooth; BC:Buccal crest of tooth; r-r:Rooth length; BB:Thickness of the base of the mandible, a-a:Apical limit of alveolar process; A:Apical limit of periodontal ligament.

The outline of AP obtained from the sections representing the corresponding mesial root site (including the apical, middle, and coronal portions) was projected over the section using r-r as the reference line to estimate the size of the distal portion of edentulous area.[32]

Similarly, the area occupied by each of the apical, middle, and coronal portions was measured with a cursor and presented in mm^2. The changes of the size of the AP after tooth extraction in each dog was calculated by subtracting the value obtained at the extraction site from the corresponding value at the mesial root site.

The type of the new alveolar bone was determined by utilizing a point-counting procedure. Using the dog as the statistical unit, mean values and standard deviations (SDs) were calculated.

Statistical analysis was performed using Mann–Whitney U with the Kruskal–Wallis tests by SPSS software (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). Each value represents the mean ± SD. $P < 0.05$ was considered statistically significant.

RESULTS

After H and E staining, healing was observed in all the extraction sockets. Based on the blinded reading performed by a pathologist, no grafted particles were found in any of the specimens. In histologic sections, fibrous tissue and lamellar spongy mature bone were observed, and woven bone was not detected in any specimen. Bone marrow was observed in all the specimens [Figures 3 and 4]. While the difference in density of fibrous tissue in three groups was not statistically significant ($P = 0.343$), the bone density in both grafted groups was significantly higher thanthat of the control group[$P = 0.021$, Table 1].

In all three groups, the healed extraction sockets were covered with an oral mucosa with parakeratinized oral epithelium. The connective tissue of this mucosa contained a few inflammatory cells showing a mild inflammation.

The mean decrease in the coronal, middle, and apical area of sockets as well as the socket height in the FDBA+PRGF, FDBA, and control groups is shown in Table 2.

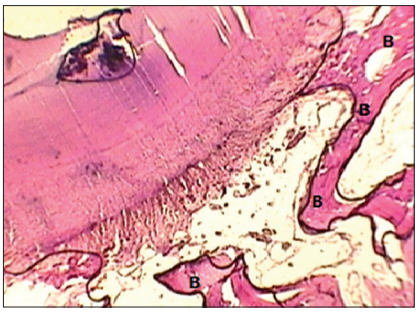

Figure 3: Microphotograph of healed sockets. B:Lamellar spongy mature bone(H and E, ×100).

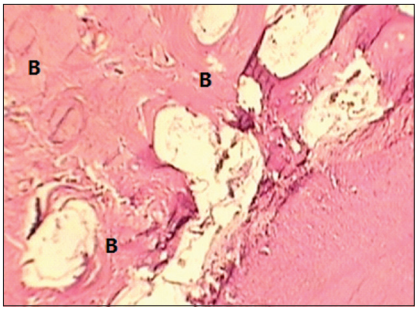

Figure 4: Microphotograph of healed sockets. B:Lamellar spongy mature bone(H and E, ×100).

Socket height of the FDBA group was the most preserved in comparison to the two other groups. However, according to the Kruskal–Wallis test, this difference between three groups was not statistically significant *(P* = 0.295).

As shown in Table 2, the least decrease in the coronal portion was observed in the FDBA group, followed by the FDBA+PRGF and control groups. According to the results of Mann–Whitney U-test, this difference between FDBA and FDBA+PRGF or control groups was statistically significant only after 4 weeks (*P* = 0.043, *P* = 0.021, respectively).

While the cross-section area of middle part of the socket was least decreased in the FDBA group (followed by the control and FDBA+PRGF groups), this difference was not significant; however, the difference in the middle surface of the sockets was statistically significant between the control and FDBA+PRGF groups (*P* = 0.038).

The least decrease in the apical portion was observed in the FDBA group, followed by the FDBA+PRGF and control groups. However, Kruskal–Wallis test showed that this difference in the apical portion of sockets was not statistically significant (*P* = 0.059).

Table 1: Mean percentage of fibrous tissue and bone in sockets

Group (*n*)	Week (*n*)	Bone (%)	Fibrous tissue (%)
Control (8)	Week 2 (4)		
	Mean	7.2500	19.6750
	SD	3.40343	2.45408
	Week 4 (4)		
	Mean	6.5000	16.6250
	SD	3.12943	4.06971
FDBA+PRGF (8)	Week 2 (4)		
	Mean	29.1250	13.0000
	SD	15.32087	1.47196
	Week 4 (4)		
	Mean	25.6750	14.0000
	SD	6.28828	3.48807
FDBA (8)	Week 2 (4)		
	Mean	23.5000	14.7500
	SD	2.38048	6.03462
	Week 4 (4)		
	Mean	20.5000	17.1250
	SD	3.48807	9.20484

FDBA: Freeze-dried bone allograft; PRGF: Plasma rich in growth factor; SD: Standard deviation

Table 2: Mean decrease of coronal, middle and apical portion of sockets (mm²) comparing to their mesial root as well as the socket height (mm) in three groups in 2 and 4 weeks

Group (*n*)	Week (*n*)	Height	Coronal	Middle	Apical
Control (8)	Week 2 (4)				
	Mean	1.5900	2.2125	1.9800	1.4800
	SD	0.56792	1.44935	0.67612	0.84111
	Week 4 (4)				
	Mean	1.0925	1.7625	0.5225	0.5250
	SD	0.84433	0.94461	0.57795	0.45735
FDBA+PRGF (8)	Week 2 (4)				
	Mean	0.8000	0.7100	1.0875	0.4025
	SD	0.89815	0.44774	0.39238	0.18081
	Week 4 (4)				
	Mean	1.1150	0.9700	0.6725	0.2650
	SD	1.33538	0.54906	0.26018	0.23101
FDBA (8)	Week 2 (4)				
	Mean	1.5100	1.2275	1.0250	0.3000
	SD	0.51904	0.27861	0.75993	0.44729
	Week 4 (4)				
	Mean	0.2075	0.1375	0.6425	0.3125
	SD	0.76173	0.41508	0.34529	0.20156

FDBA: Freeze-dried bone allograft; PRGF: Plasma rich in growth factor; SD: Standard deviation

To evaluate the effect of time on bone healing, socket dimensional changes were compared at 2 and 4 weeks. Mann–Whitney U-test revealed a significant difference in apical and middle change of control group at 2 and 4 weeks (*P* = 0.029). In addition, the coronal portion

of FDBA group changed significantly (P = 0.029). Other dimensional differences between 2 and 4 weeks were not statistically significant.

In general, grafted sockets showed less decrease in socket dimensions in comparison to the control group. Only significant changes were observed in FDBA group in coronal portion that was better preserved than two other groups, and FDBA+PRGF group which was better preserved in the middle portion than the control group. Summary of changes at 2 and 4 weeks is shown in Figure 5.

DISCUSSION

Several recent studies investigated the effect of PRP alone or in conjunction with other grafts. Although many studies suggested that PRP improves bone healing,[12,17-20] other studies found no enhancement in new bone formation either in quantity or in quality.[21-25] Since comparison of these results is difficult due to different study designs and methodologies (such as the animal species, platelet concentration, content of growth factors, and human racial properties), it is not possible to reach a definite conclusion yet which has made it a controversial issue.

The PRP material used in the present investigation is termed PRGF, and it differs from the other PRP systems which are commercially available in whichit does not include bovine thromboplastin and interleukins, and in whichconcentration of platelets and speed of centrifugation are different. As mentioned previously, PRGF is more advantageous than PRP. However, various and controversial results have been reported regarding its effect on bone regeneration.[29,33-34]

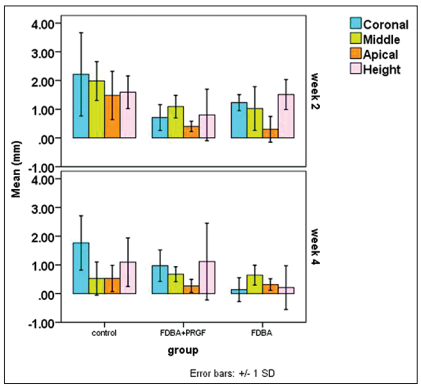

Figure 5: Mean dimensional changes of three groups at 2 and 4 weeks. Height in mm and cross-sections of coronal, middle, and apical portions in square mm^2.

In the present study, the effect of FDBA with and without PRGF on enhancing bone regeneration was evaluated both histologically and histomorphometrically. In general, whereas the reduction of the socket dimensions was observed in all three groups, it was better preserved in grafted groups than the control group[Table 2]. However, the only significant differences were between FDBA+PRGF and control groups in their middle portion, and between FDBA and two other groups in the coronal portion. These observations are in accordance with those of Thor*et al*.,[34] but in contrast with results of Anitua *et al*.[35]

In general, the most decrease was observed in coronal portion of control groups which is compatible with the results of other studies performed by Araújo and Lindhe,[36] Anitua *et al*.,[35] and Mogharehabed *et al*.[37]

In addition, we observed that the FDBA group was better preserved than FDBA + PRGF group in all dimensions; however, this difference was only significant in coronal portion at week 4.

The least decrease was in apical portion. This is also identical to the results of Schropp *et al*.[4] and Barone *et al*.[38] who found that the apical portion was the most preserved.

Regarding the middle portion, Mogharehabed *et al*., who compared DFDBA and DFDBA+PRGF with the control group in a similar study design, observed the most decrease in middle portion of control group followed by DFDBA+PRGF and then DFDBA group; however, these differences were statistically insignificant as well.[37] Similarly, no significant difference was observed in the middle portion of sockets in the study of Araújo and Lindhe.[36]

Regarding the changes in apical portions, a mean reduction of 0.31 mm was observed in grafted groups. This observation is in agreement with findings previously reported by Mogharehabed *et al*.[37] and Araújo and Lindhe.[36]

Although Anitua *et al*. reported that using PRGF with Bio-Oss leads to socket preservation,[35] the present study cannot claim that PRGF preserves the socket very definitely. It should be noted, however, that there aresome limitations in the present study which may lead to this uncertainty. A limitation of this study is that only mesiodistal cross-sections of the experimental sites were analyzed. In addition, the limited follow-up time and small sample size may have played a role.

However, since the present study was the first study investigating the effect of combination of FDBA and PRGF on socket preservation, it was neither ethical nor justifiable to sacrifice more dogs in this study. Moreover, since highly concentration of platelets (6–11-fold of physiologic range) can have an inhibitory effect on bone regeneration due to stimulating osteoclastogenesis,[39] a possible explanation would be that the concentration of 2–3-fold of normal range which was used in the present study has led to a similar effect, but with a lesser impact. Conclusively, it is strongly recommended to consider these factors in future studies.

The density of spongy bone in both groups of grafted sockets was significantly more than the control group. While this density was maximum in FDBA+PRGF group, it was not significantly more than FDBA group. As mentioned above, no grafted particles were found in any of the specimens, and this is in contrast with the report of Simon *et al*. who observed DFDBA in coronal portion of sockets.[39] The absence of FDBA particles in the present study may indicate the fast remodeling of grafted areas.

The dimensional differences of grafted groups were not significant between 2 and 4 weeks except in coronal portion of FDBA group. The similarity of bone dimensional changes at 2 and 4 weeks in FDBA+PRGF group may be because degranulation of platelets lasts 3–5 days and their primary growth factor activity stops in 7–10 days.[39] Therefore, it is expected that PRGF exerts its effect in early stages of bone regeneration.

CONCLUSION

Taken together, while the findings of the present study lend further support to the advantageous effects of both FDBA and PRGF in socket preservation generally, the superiority of PRGF or PRGF+FDBA to FDBA cannot be concluded from this experiment. However, this study does open up the need for further studies to investigate the effect of PRGF as an independent graft material or in combination with other materials.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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