

Original Article

Socket preservation using demineralized freeze-dried bone allograft with and without plasma rich in growth factor: A canine study

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ABSTRACT

Background: The accelerating effect of plasma rich in growth factors (PRGFs) in the healing of extraction sockets has been demonstrated by some studies. The aim of the present study was to histologically and histomorphometrically evaluate whether bone formation would increase by the combined use of PRGF and demineralized freeze-dried bone allograft (DFDBA).

Materials and Methods: In four female dogs, the distal root of the second, third and fourth lower premolars were extracted bilaterally and the mesial roots were preserved. The extraction sockets were randomly divided into DFDBA + PRGF, DFDBA + saline or control groups. Two dogs were sacrificed after 2 weeks and two dogs were sacrificed after 6 weeks. The extraction sockets were evaluated from both histological and histomorphometrical aspects. The data were analyzed by Mann-Whitney followed by Kruskal-Wallis tests using the Statistical Package for the Social Sciences version 20 (SPSS Inc., Chicago, IL, USA). Significant levels were set at 0.05.

Results: The least decrease in socket height was observed in the DFDBA + PRGF group (0.73 ± 0.42 mm). The least decrease in the coronal portion was observed in the DFDBA + PRGF group (1.38 ± 1.35 mm²). The least decrease in the middle surface was observed in the DFDBA group (0.61 ± 0.80 mm²). The least decrease in the apical portion was observed in the DFDBA group (0.34 ± 0.39 mm²).

Conclusion: The present study showed better socket preservation subsequent to the application of DFDBA and PRGF combination in comparison with the two other groups. However, the difference was not statistically significant.

Key Words: Allograft, growth factor, guided bone regeneration, plasma rich, socket graft

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INTRODUCTION

A significant dimensional change occurs during the healing phase of extracted sockets. The walls of the socket shrink and the changes are more apparent in buccal walls than in the lingual/palatal walls.^[1] Following tooth extraction, the ridge width

decreases to about 50% in 12 months.^[2] Two-thirds of this change occurs in the first 3 months after tooth extraction. The final position of the socket walls is determined by the bone surface of adjacent teeth.^[3]

Various graft materials, including autogenous and allogenic grafts, xenografts and alloplastic materials, have been employed for socket preservation procedures.^[4,5] Demineralized freeze-dried bone allografts (DFDBAs) are commonly used in periodontal regeneration procedures and in the preservation of extraction sockets.^[6] DFDBA is believed to act as an osteoconductive and osteoinductive material and also as a bone growth promoter.^[7]

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Growth factors are biological mediators that have an important role in tissue healing and bone formation. These molecules result in conversion of undifferentiated mesenchymal cells to mature bony cells.^[8] They also regulate a number of intracellular events that lead to bone regeneration.^[9]

Blood platelets contain alpha granules, which constitute a whole family of growth factors, including platelet derived growth factors (PDGF), transforming growth factor (TGF) beta 1, insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF), through which they have an important role in cell proliferation and wound healing.^[10] These factors stimulate extracellular matrix formation and regulate cell proliferation, adhesion and migration.^[11]

Plasma rich in growth factors (PRGFs) has a series of proteins which are derived from a certain volume of platelet-rich plasma (PRP) and have been claimed to accelerate wound repair and periodontal regeneration.^[12]

PRGF containing platelets and growth factors are involved in the repair process. It contains growth factors at a concentration of 2-3 times higher than peripheral blood capillaries.^[13]

Various methods have been introduced for preparation of concentrated platelet products.^[14] These products are often composed of a small volume of PRP, containing proteins and growth factors, which are released in the environment and accelerate soft-tissue healing and bone regeneration.^[15,16]

Clinical application of PRGF in different medical fields includes treatment of chronic ulcer, hard and soft tissue regeneration,^[17] gingival recession and sinus lifting procedures.^[18]

It also results in relief of pain, inflammation and others surgical complications.^[19,20]

Combined use of PRGF and other bone substitutes may improve the kinetic properties of autologous platelet-rich products, enabling a slower release of growth factors and facilitating the availability of growth factors for their target cells.^[21]

Furthermore, the combination of these materials is an accelerating tool for sealing the extraction sockets, which will promote complete epithelialization of soft-tissue.^[22] In addition, use of PRGF with dental implants in fresh sockets promotes osseointegration, bone-implant contact and soft tissue healing.^[23]

Considering the effects of DFDBA on preserving dimensional parameters of extraction sockets and the effects of growth factors that regulate events related to the healing process, it would be expected that PRGF treatment of an extraction socket might result in enhanced bone formation.^[24]

The primary aim of the present study was to evaluate the effect of DFDBA + PRGF on the preservation of socket dimensions and to evaluate the preserved sockets in both histological and histomorphometric aspects. The secondary objective was the comparison of effects of DFDBA + PRGF and DFDBA + saline on the socket preservation and the last objective was to compare the changes in socket dimensions separately after socket grafting.

The aim of the present study was to histologically and histomorphometrically evaluate whether bone formation would increase by the combined use of PRGF and DFDBA and also comparison of socket dimensional changes by using DFDBA with and without PRGF in a canine model.

MATERIALS AND METHODS

After obtaining the approval of the Local Ethics Committee, four 12-month-old female dogs, weighing 15-20 kg, were selected. All the animals were kept in individual cages during the whole experimental period under similar conditions and standard diet.

Prior to the procedures, the animals were kept in quarantine for 2 weeks and antibacterial treatment was performed along with vaccination of the dogs against common diseases. The surgical procedures were performed under general anesthesia induced by intramuscular injection of 1% acepromazine (Alfasan, 0.02 ml/kg) and 10% ketamine (10 mg/kg), followed by the administration of inhaled halothane. Local infiltration of 2% lidocaine with epinephrine (1:100,000) (Darou Pakhsh, Tehran, Iran) was performed in mandibular premolar regions.^[25] In addition to prophylaxis against infection, 0.2% chlorhexidine was used on the face and the oral cavity.

Sulcular incisions were made in the premolar regions (P2, P3 and P4) in both quadrants of the mandible. A full-thickness flap was elevated to expose 1-2 mm of the alveolar crest.^[26]

Second, third and fourth (P2, P3 and P4) premolars on one side were hemi-sectioned by a high-speed turbine

bur (D and Z, Wiesbaden, Germany) with irrigation of normal saline.

The distal roots were removed by using a periosteal elevator and an elevator. The root canal of mesial roots were filed, reamed and then filled with Gutta-percha (Aryadent, Tehran, Iran) and was sealed with dental amalgam^[26] [Figure 1].

To prepare the PRGF, 9 ml of the peripheral blood was drawn from the saphenous vein into two tubes. The sterile tubes contained 3.8% sodium citrate as an anticoagulant. The plasma was centrifuged using a digital apparatus (Model PRGF System Iv-BTI, Biotechnology Institute, Spain); normal centrifugation time was 8 min at 2000 rpm for the 9-ml tubes. The plasma was then separated into fractions by careful pipetting.

The first layer (2 ml) was plasma with a number of platelets similar to peripheral blood, which was called fraction 1. It was used for autologous membrane preparation. The intermediate fraction (fraction 2) was approximately 1 ml in volume and had a platelet concentration higher than that in the peripheral blood.

Finally, fraction 3 or the plasma rich in platelets and growth factors was approximately 1 ml in volume, located immediately above the white line, with a platelet concentration of 2-3 times higher than the physiologic level.

Fraction 3 was the richest in platelets and growth factors. For PRGF preparation, 0.05 ml of PRGF activator (10% CaCl₂) was added to each ml of fraction 3. The clot formed within 5 min [Figure 2].

During the mixing of plasma with any other graft material, first PRGF activator was added and then mixed with the graft. Within 2-5 min a clot containing

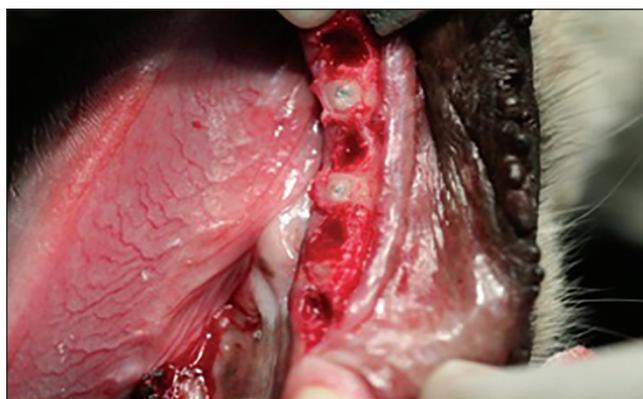


Figure 1: A clinical photograph illustrating the distal sockets and mesial roots in the mandible of dog

the graft formed and yielded a gluey consistency, making it easy to handle and compact. Then the platelet-rich layer and the activator were heated for about 10 min on a heater to about 37°C and the scaffold-like PRGF was achieved. This scaffold-like PRGF was mixed with DFDBA and covered by an autologous membrane. There were 24 sockets from four dogs; each dog had six sockets in mandibular right and left quadrants. The sockets were divided into 3 groups: Group 1, DFDBA + PRGF + membrane; group 2, DFDBA + saline; group 3, control, filled with a blood clot.

In each group, eight sockets were randomly selected. After filling all the extraction sockets, the buccal and lingual flaps were replaced to cover the opening of the extraction socket. The flaps were retained in their original position with interrupted absorbable sutures (3-0 vicryl, SUPA Medical Devices, Tehran, Iran).

Post-operatively, the dogs were given antibiotics: Intramuscular ceftriaxone, 500 mg (Jaber Ebne Hayyan, Tehran, Iran), 4 times for 5 days. In order to relieve pain, 5 mg/kg of oral tramadol (Tehran Chemie, Tehran, Iran) was administered.

The animals were given a soft diet and underwent regular examination on a daily basis in order to evaluate the systemic health or any other problems, like suture opening, post-operative infection etc. Two dogs were euthanized after 2 weeks of healing with an intravenous overdose of thiopental sodium, causing a painless and quick death.^[26]

Two dogs were euthanized after 6 weeks of healing with the same method. The mandibles were removed. The premolar sites (P2, P3 and P4), including the mesial root and distal socket region, were dissected using a diamond saw. The specimens were placed in 10% buffered formalin solution for 3 days. Then they

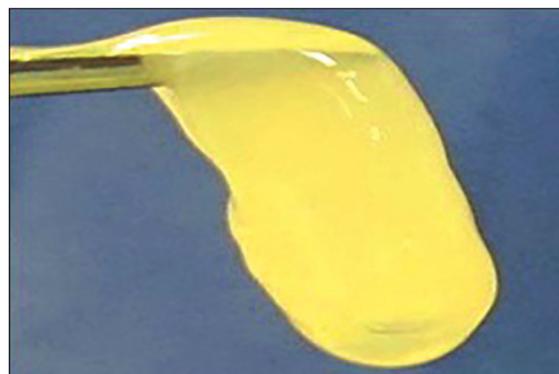


Figure 2: Scaffold-like plasma rich in growth factors

were demineralized in 10% nitric acid and prepared for histological and histomorphometric evaluations.

Sections were prepared from each premolar site (two sections from the mesial roots and two sections from the healed socket). These sections were cut in the buccolingual plane and were sampled from the central area of either the root or the socket.

The specimens were serially sectioned in 5- μ m thickness and stained with H and E.

Histological evaluation

Histological evaluations were performed under a light microscope (Nikon, YS100, Tokyo, Japan) at 100 \times 400 magnification and type of newly formed bone in the socket, the overlying epithelium, fibrous tissue and inflammation were evaluated.

Histomorphometric evaluation

In order to determine the histomorphometric criteria, the stereomicroscope (Zoom Stereomicroscope, HP SMP 200, USA) software (Motocam 480 Digital camera SP 10/0224, Canada) was used and the following landmarks were identified:

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 [Figure 3].

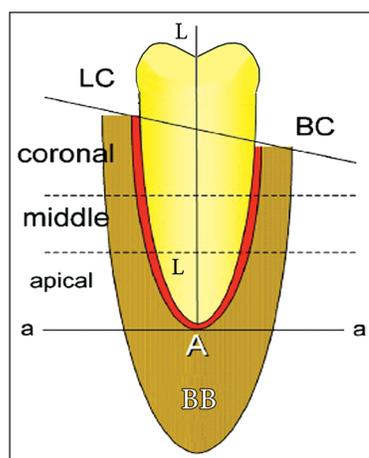


Figure 3: Schematic drawing illustrating the various landmarks used for the histomorphometric measurements. BC: Buccal crest of the root site; LC: Lingual crest of the root site; A: Apical end of the periodontal ligament of the root site; BB: base of the basal body of the mandible; L-L: Long axis of the root; a-a: Apical limit of the alveolar process

Then, the vertical distance between A and the BB was determined. The image of the alveolar process at the root site was subsequently divided into three equal portions: Apical, middle and coronal. The cross-section area occupied by each portion was measured with a cursor and presented in mm².

In order to determine the percentage of newly formed bone, a microscope (Sand Optic BM 22, Isfahan, Iran) at 10 \times 100 \times 400 magnification was used and the percentage of bone, fibrous tissue and empty spaces of socket were calculated.

In order to estimate the size of the edentulous distal portion, the outline of AP obtained from the sections representing the corresponding mesial root site, including its apical, middle and coronal portions, was projected over the section using L-L as the reference line.^[26]

The area occupied by each of the apical, middle and coronal portions was measured with a cursor and expressed in mm². The relative alteration of the size of the alveolar process that had occurred in each dog after tooth extraction was estimated by subtracting the value obtained at the extraction site from the corresponding value at the mesial root site.

The composition of the alveolar bone was determined by using a point-counting procedure. The mean values and standard deviations were calculated using the dog as the statistical unit.

Statistical analysis

Statistical analysis was performed by Mann-Whitney with the Kruskal-Wallis tests using Statistical Package for the Social Sciences version 20 (SPSS Inc., Chicago, IL, USA). Each value represents the mean \pm standard error of the mean. Differences with $P < 0.05$ were considered to be significant.

RESULTS

Histologic analysis

After H and E staining and section preparation it was observed that all the extraction sockets had healed uneventfully. In all groups, the healed sockets were covered with an oral mucosa composed of parakeratinized oral epithelium. In the connective tissue of this mucosa, mild inflammation was detected. Based on analysis by a blind pathologist, no grafted particles were found in any specimens. In histologic sections, lamellar spongy mature bone was observed and woven bone was not detected

in any specimen. Bone marrow was observed in all specimens [Figures 4 and 5].

Histomorphometric analysis

The present study showed the least decrease in socket height in the DFDBA + PRGF group followed by the DFDBA + saline and control groups.

The results showed that the socket height in the DFDBA + PRGF group was better preserved in comparison to the two other groups; however, the differences between the three groups were not statistically significant.

In the present study, the least decrease in the coronal portion was observed in the DFDBA + PRGF group, followed by the DFDBA and control groups; however, the coronal portion of the socket under the experiment in the DFDBA + PRGF group was better preserved in comparison to the two other groups, but the difference was not statistically significant ($P = 0.168$).

The least decrease in the middle surface was observed in the DFDBA group, followed by the control and DFDBA + PRGF groups; however, the decrease in the middle surface of the sockets was not statistically significant between three groups ($P = 0.317$) or in each groups before intervention comparison with after intervention.

The least decrease in the apical portion was observed in the DFDBA group, followed by the DFDBA + PRGF and then control groups. However, the decreases in the apical portion of sockets before and after intervention in control and DFDBA groups were statistically significant ($P = 0.018$), ($P = 0.04$), but

the decrease in the apical portion in the DFDBA + PRGF group before and after the intervention was not significant ($P = 0.167$).

Generally speaking, it could be concluded that socket height and coronal portion of the socket were better preserved with DFDBA + PRGF + membrane in comparison to the other groups and apical portion changes were similar between the two graft sites and better than the control group. (Tables 1-3)

Table 1: Mean socket dimensional change in control group in 2 and 6 weeks (in mm²)

Week	Height	Coronal	Middle	Apical
2	1.41±0.35	2.48±1.58	1.10±0.90	1.09±1.01
N	4	4	4	4
6	0.89±0.84	1.92±0.95	1.34±5.22	0.92±0.58
N	4	4	4	4
Total	1.15±0.66	2.20±1.24	1.22±3.70	1.00±0.77
N	8	8	8	8

Table 2: Mean socket dimensional changes in DFDBA group in 2 and 6 weeks (in mm²)

Week	Height	Coronal	Middle	Apical
2	0.31±0.19	1.27±0.48	0.31±0.55	0.38±0.53
N	4	4	4	4
6	1.56±0.88	1.62±1.95	0.91±0.98	0.31±0.26
N	4	4	4	4
Total	0.94±0.89	1.44±1.32	0.61±0.80	0.34±0.39
N	8	8	8	8

DFDBA: Demineralized freeze dried bone allograft

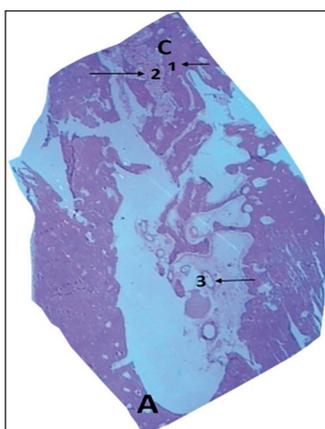


Figure 4: Histological view of socket grafted by using of demineralized freeze-dried bone allograft + plasma rich in growth factors. C: Coronal; A: Apical; 1: Spongy bone; 2: Capillary; 3: Alveolar socket bone; 4: Bone marrow formation

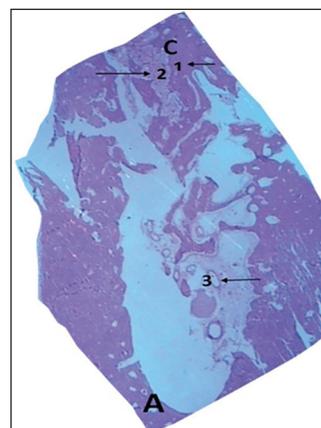


Figure 5: Histological view of socket grafted by using of demineralized freeze-dried bone allograft. C: Coronal; A: Apical; 1: Spongy bone; 2: Fibrous tissue; 3: Capillary

Table 3: Mean socket dimensional changes in DFDBA+PRGF group in 2 and 6 weeks (in mm²)

Week	Height	Coronal	Middle	Apical
2				
	0.91±0.50	1.82±1.82	0.70±0.94	0.51±0.99
N	4	4	4	4
6				
	0.56±0.27	0.94±0.65	2.80±4.92	0.21±0.58
N	4	4	4	4
Total				
	0.73±0.42	1.38±1.35	1.75±3.47	0.36±0.66
N	8	8	8	8

DFDBA: Demineralized freeze dried bone allograft; PRGF: Plasma rich in growth factor

In the DFDBA group, the middle portion was best preserved in comparison to the other groups; it also experienced the least dimensional changes. However, the differences between the three groups were not statistically significant.

All the changes in dimensions during 2 and 6 weeks were not statistically significant either between groups or in the three portions of the socket. Apart from the apical portion of the sockets, the differences between the three groups were not statistically significant ($P > 0.05$); in other variables the grafted sockets showed less decrease in socket dimensions in comparison to the control group.

DISCUSSION

Some studies have demonstrated that early bone loss following tooth extraction can be reduced by socket grafting.^[27] Recent studies have shown that PRGF can accelerate the hard and soft-tissue healing of dental sockets after extraction that result in preserved socket dimensions.^[28] In addition, the use of PRGFs combined with an immediate implant placement procedure can be considered a safe, effective and predictable treatment option for the rehabilitation of fresh post extraction infected sockets.^[29]

PRGF has more advantages in comparison with PRP. Therefore, it has been used recently. However, there are controversies about its effects on the healing process.^[30]

In the present study, histological and histomorphometric analyses were used to evaluate tooth socket dimensions grafted by PRGF. Based on the results, grafted sockets had significantly less decrease in their height and coronal portions compared with the control group after 2, 6 weeks, [Figure 6] and the highest reduction in the socket

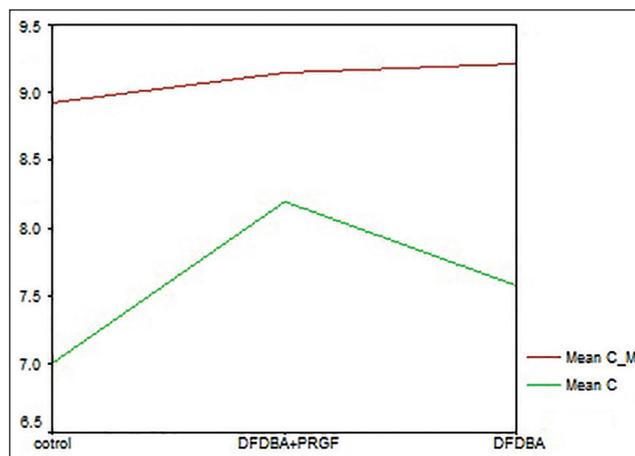


Figure 6: Mean coronal changes in three groups after 6 weeks (mean coronal portion of root)

height was 21.5% in the control group. Although socket height reduction occurred in all the groups, the socket height was significantly better preserved in the two grafted groups compared with non-grafted groups ($P = 0.029$). While the reduction in the socket height of the DFDBA + PRGF group was less than that in the DFDBA group after 2 and 6 weeks, the difference was not statistically significant ($P = 0.566$). These observations confirm the results of a study by Thor *et al.*^[31]

However, the considerable clinical differences observed may be attributable to limited sample size. Furthermore, the bony graft material, too, might have exerted a confounding effect on the results. A decrease in the middle part of sockets was observed in all three groups ($P = 0.317$) after 2 and 6 weeks, in descending order, in the control, DFDBA + PRGF and DFDBA groups. Araújo and Lindhe observed no differences between these groups in relation to a decrease in the middle part of sockets.^[26]

There was a reduction in the apical part of sockets before and after intervention in all three groups. While this decrease was significant in control and DFDBA groups ($P < 0.05$), it was not statistically significant in the DFDBA + PRGF group ($P = 0.167$), suggesting that using DFDBA and PRGF combination can help preserve the socket in the apical part. The maximum decrease in the apical part was seen in the control group after 2 and 6 weeks while this was equal in two other groups (4% or 0.3 mm²). It could be concluded that the apical portion of the socket in the DFDBA + PRGF group was preserved similar to the DFDBA group after 2 and 6 weeks. These findings support the results of a study by Araújo and Lindhe,

who reported changes of $4 \pm 4\%$ in the apical part of sockets after extracting teeth.^[26]

In the DFDBA + PRGF group, the decrease in the middle and apical parts of sockets was not statistically significant, ($P = 0.196$), ($P = 0.167$) and the socket dimensions were preserved desirably. The socket height and its coronal part underwent significant changes after using PRGF. The slightest changes in all the dimensions were related to the DFDBA + PRGF group, except for the middle part. However, the changes subsequent to the use of PRGF were not significant, which might be attributed to the limited sample size as well as to the limited duration of the study.

The least changes of socket dimensions were related to the apical part of the socket. This decrease was 4% (mean 0.3 mm^2) in grafted groups and 11% ($1.00 \pm 0.77 \text{ mm}^2$) in the control group after 2 and 6 weeks. Based on the histomorphometrical analysis, there were slight changes in the apical and middle parts of sockets during the healing period of grafted groups while changes in the coronal part of the non-grafted group were considerable [Tables 2 and 3]. The observations of Schropp *et al.* and Barone *et al.* are compatible with these findings.^[3,32] However, the differences between the groups were not statistically significant due to the probable reasons mentioned above ($P = 0.168$). For the height of sockets between three groups, only apical portion changes after 2 and 6 weeks were statistical significant ($P = 0.024$).

The decrease in all the dimensions was less notable in the grafted groups in comparison with the control group. However, the grafted groups were not significantly different in any parameter. The DFDBA particles were not found in 24 sockets, which cannot confirm the observations of Araujo and Lindhe, who could observe Bio-Oss particles in the grafted site.^[26] The absence of DFDBA particles can be considered a sign of rapid remodeling of grafted sockets in dogs in comparison to the control group. The differences between the dimensions in 2 and 6 weeks were not statistically significant, which can be attributed to rapid releasing of growth factors. PRGF is provided based on the manufacturer's instructions using the patient's own proteins. PRGF contains growth factors, including IGF1, EGF, TGF, PDGF, fibroblast growth factor and VEGF, which affect the biologic processes and accelerate migration, growth and morphogenesis of the cells, leading to tissue regeneration.^[33]

Based on the literature, after extraction, the coronal part of sockets undergoes resorption more than other parts. Schropp *et al.* observed the most decrease in height in the coronal part.^[3] Similarly, Araujo and Lindhe reported a maximum change in the coronal part of non-grafted sockets ($35 \pm 15\%$).^[26]

In the present study, the most decrease was observed in the coronal part of sockets particularly in the control group after the surgery (21.5%) and it is compatible with the literatures.

The socket height decreased in all three study groups and it is in accordance with findings of Barone *et al.* which found that the socket height decreased in both control and the ridge preserved groups.^[32] However, in the present study the least decrease before and after the intervention was related to the apical part of sockets.

In this study, it was hypothesized that all socket dimensions will have fewer changes in the grafted group than the non-grafted group. Results revealed that there was less decrease in the grafted group except in the middle part of sockets. However, this difference was not statistically significant.

There are limitations in animal studies, which can lead to different results. These limitations include the sample size, duration of the study and different potential of healing in animals. Moreover, other factors such as the selected material and PRGF formulation and manipulation can make a difference.

Obviously, there should be sufficient osteoblasts and precursors to respond to these factors during the 1st week. Therefore, the application technique and preparation method of PRGF is a critical factor influencing the final results.^[34]

CONCLUSION

The present study showed better socket preservation subsequent to application of DFDBA and PRGF combination in comparison with the two other groups. However, this difference was not statistically significant. PRGF can accelerate the formation of bony trabeculae as well as bone remodeling. Therefore, it can be used in immediate implantation cases.

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