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Socket Preservation Using Deproteinized Bovine Bone Matrix with and without Plasma Rich in Growth Factors: A Canine Study

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Abstract

Background and aims. The accelerating effect of plasma rich in growth factors in the healing of extraction sockets has been shown by some studies. The aim of the present investigation was to evaluate the effect of deproteinized bovine bone matrix (DBBM) with and without plasma rich in growth factors (PRGF) in canine extraction socket.

Materials and methods. Distal roots of second and third upper premolars were extracted bilaterally in six mongrel dogs. Buccolingual (BL) and apico-coronal (AC) dimensions of extraction sockets were measured. The extraction sockets were randomly grafted with DBBM+PRGF or DBBM. The ungrafted extraction sockets were allowed to be filled with clot which served as control. The dogs were sacrificed after 3 months and the extraction sockets were evaluated from clinical and histological viewpoints.

Results. There were significant differences in post extraction vertical dimensions of the sockets among the study groups. The mean AC changes were 6.25±2.13 mm, 6.83±1.83 mm, and 3.83±1.63 mm in DBBM, BDDM+PRGF, and control groups, respectively (P=0.034). The mean BL dimension reduction in both DBBM+PRGF and DBBM groups was 2.00±1.09 mm, whereas this amount was 2.66±1.47 mm in the control group (P=0.627). The greatest amount of bone resorption occurred in the control group whereas the PRGF group exhibited the least amount of bone resorption. The control group exhibited a complete bone fill and bone formation rate which was more than that in the experimental groups.

Conclusion. The findings of this study indicate that using of DBBM with and without PRGF might successfully maintain socket dimensions.

Key words: **Plasma rich in growth factors, deproteinized bovine bone matrix, socket preservation.**

Introduction

A significant dimensional change occurs through the healing phase of extraction sockets. These changes are more prominent in buccal walls than the lingual walls.[1-5] Subsequent to tooth extraction, the ridge width is reduced about 50% or 6.1– 12 mm in 12 months. Two-thirds of this ridge width reduces in the first 3 months after tooth extraction. The final position of the socket walls is determined by the bone level of adjacent teeth.[3]

Various graft materials, including autogenous and allogenic grafts, xenografts, and alloplastic materials, have been employed for socket preservation procedures.[6-17] DBBM as a xenogenic bone substitute has been used in ridge augmentation and socket preservation with 1.17–1.73 mm of horizontal resorption.[11,18,19]

Growth factors are biological mediators that regulate the events related to tissue repair, bone formation, and remineralization. These molecules have an induction role in differentiation of undifferentiated mesenchymal cells to mature bone cells. They also regulate a series of intracellular reactions that release cell-enhancing factors for bone regeneration. Growth factors have local and systemic effects. These factors regulate cell migration, adhesion, proliferation, and differentiation and stimulate extracellular matrix through binding to specific receptors on the cells surface.[20] PRGF includes an array of proteins which are obtained from a certain volume of platelet-rich plasma (PRP). They contain platelets and growth factors involved in the repair process with fibrin and fibrinogen. In this method PRP and leukocyte-free plasma (LFP) are provided.[21] Growth factors are also used in combination with different graft materials. Various methods have been used to prepare concentrated platelet products. These products are generally composed of a limited volume of PRP containing growth factors and proteins released into the environment, leading to accelerated bone regeneration. PRGF, as a contributing factor, has been considered to increase bone regeneration and epithelial tissues in oral surgery. It effectively stimulates regeneration of bone and soft tissue and also decreases pain, inflammation and other side effects of surgeries.[22,23]

Combined use of PRGF and other bone augmenting materials might accelerate the availability of growth factors for their target cells. Furthermore, the combinations of these materials are facilitating tools for sealing the extraction socket and will promote complete epithelialization of soft tissues.[24]

Considering the effect of DBBM in preserving dimensional parameters of extraction socket and the effect of growth factors that regulate events related to healing process, it would be expected that PRGF treatment of an extraction socket might result in enhanced bone formation. The aim of the present study was to clinically and histologically evaluate whether bone formation would increase by the combination of PRGF and DBBM or not and to compare socket dimension changes by using DBBM with and without PRGF in canine model.

Materials and Methods

After obtaining the approval of the local ethics committee the study was conducted using six mixed-breed female dogs weighing 20–25 kg, randomly selected for this experiment. All the animals were kept in individual cages during the whole experimental period, under similar conditions and maintained with standard diet and water ad libitum.

Prior to surgery the animals were kept in quarantine for two weeks and anti-parasite treatment was rendered and the dogs were vaccinated against common diseases. The surgical procedures were performed under general anesthesia induced by intramuscular injection of Xylazin® (2% Xylazin, Alfasan, Worden-Holland, 0.5 mg/kg) and ketamine (10% Ketamine, Alfasan, Woerden-Holland, 5 mg/kg) followed by the administration of inhaled halothane. In addition, local infiltration of lidocaine (2%) with epinephrine (1:80,000) (Daroupakhsh, Tehran, Iran) was administered in the second and third maxillary premolars ($_3P_3$, $_2P_2$).[25]

Subsequent to sulcular incisions, a full-thickness flap was elevated to expose 1– 2 mm of the alveolar crest.[26] Randomly two premolars on one side and one premolar on the other side of maxilla were hemi-sected by a high-speed turbine bur (D&Z, Wiesbaden, Germany) with profuse irrigation of normal saline. The distal roots were removed using elevators and the canal of the mesial roots was reamed and filled with gutta-percha (Aryadent, Tehran, Iran). BL width and AC depth of extraction socket were measured with the use of a periodontal probe. The examination tool was the same (a Williams probe) and all the measurements were carried out from a notch that was created by a bur on the mesial root and served as a reference point for measurements (Figure 1).[25]

To prepare the PRGF, 10 mL of peripheral blood was drawn into two tubes from the cephalic vein. The tubes contained 3.8% sodium citrate as an

anticoagulant. The tubes were centrifuged at 460 g for 8 minutes at room temperature. Then the blood was separated into distinct layers, with the cellular portion located at the bottom of the tube and the plasma portion at the top of the tube.

Plasma volume consisted of 1 mL and was located just above the red blood cell line. This portion appears to be very rich in growth factors. Then 50 µL of 10% calcium chloride ($CaCl_2$) was added to PRGF. $CaCl_2$ activates PRGF and stimulates the formation of a semi-solid mass which functions as a matrix for progenitor cells and contributes to tissue regeneration (Figure 2).[27]

The mixture of platelet-rich layer and activator was heated for about 10 minutes on a heater at about 37°C and the scaffold-like PRGF was achieved. This scaffold-like PRGF mixed with Bio-Oss® (Geistlich Biomaterials, Wolhusen, Switzerland). DBBM was placed in the extraction sockets. The flaps were then reflected to their original position and sutured with absorbable suture material (4-0 Vicryl® Ethicon, Johnson & Johnson, Piscataway, NJ) using a combination of horizontal mattress and simple loop sutures.

Postoperatively, the dogs were given 500 mg of Cephalexin (Daroupakhsh, Tehran, Iran) orally four times daily for one week. Furthermore, to reduce the possible pain, 1 mg/kg of Ketoprofen (Shahr Daru, Tehran, Iran) was given orally.

The animals were given a soft diet and underwent veterinarian examination on a daily basis in order to evaluate the systemic health or any other problems, like suture opening, surgical site infection etc. One dog died after 1 month and the intervention was performed on another substitute animal.

The remaining experimental sites were randomly assigned for inserting either DBBM or left alone to heal by natural formation of blood clot without any other intervention. After 3 months of healing the dogs were euthanized with an intravenous injection of an overdose of thiopental sodium, which caused a quick and painless death after perfusion through the carotid arteries, with a fixative (5% glutaraldehyde and 4% formaldehyde).[26]

Following resection of the animals' maxilla the premolar sites, including the mesial roots and the distal sockets were dissected by a diamond saw. Buccolingual and apico-coronal dimensions of the healed sockets were measured. The specimens were placed in 10% buffered formalin solution for 3 days. They were then demineralized in 10% nitric acid and prepared for histological evaluations.

Gross-sections were prepared from each premolar site (two sections from the mesial root and two sections from the healed socket). These sections were cut in the buccolingual plane and were prepared from the central part of healing root or socket. The specimens were serially sectioned with a thickness of 5 µm and were stained with hematoxylin-eosin. Then the extraction socket dimensions were examined and histological examinations were performed under a light microscope (Nikon E400, Nikon Corporation, Tokyo, Japan) at ×40, ×100, and ×200 magnifications.

To perform the histomorphic analysis, light micrographs (×40 magnification) of biopsy sections were obtained with a digital camera (Nikon E8400, Nikon Corporation, Tokyo, Japan) and analyzed using IHMMAVer.1 histomorphic software (SBMU, Tehran, Iran) to evaluate new bone formation, inflammatory cells, remaining of DBBM granules and bone type (lamellar or woven).

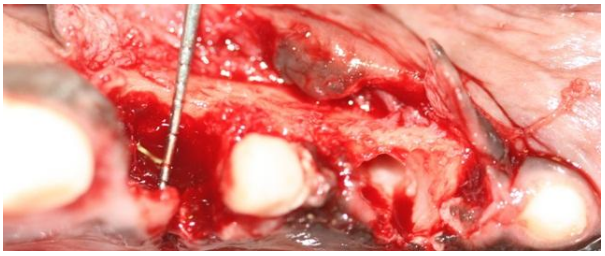

Figure 1. Distal root extraction and measurement in mid buccal point of extraction site using a periodontal probe.

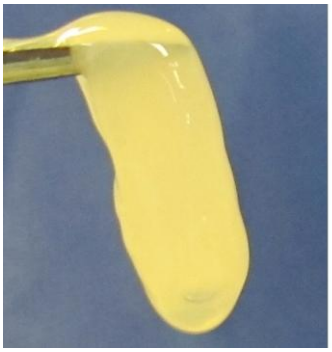

Figure 2. The scaffold-like PRGF is prepared.

Statistical analysis

The variables were presented as means ± standard deviations. The between-group postoperative differences of the variables before and after the treatment were compared based on the Wilcoxon test. In addition, Mann-Whitney U test was used for the comparison of the within-group differences at baseline and 3 months postoperatively. In the present study statistical significance was defined at $P<0.05$.

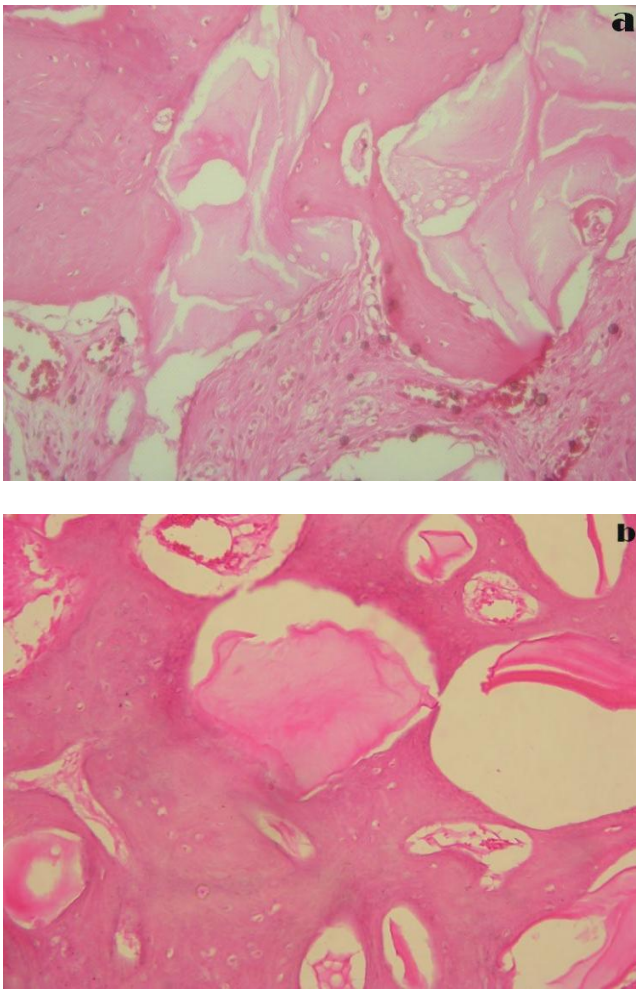

Figure 3. Histologic evaluation for socket healing using DBBM (a), and DBBM+ PRGF (b) (3 months after treatment).

Results

In this study, all the 18 extraction sockets healed uneventfully. The mean BL dimension reduction in both DBBM+PRGF and DBBM groups was 2.00±1.09 mm whereas this amount was 2.66±1.47 mm in the control group. Therefore use of DBBM or DBBM+PRGF was more effective in preserving socket dimensions. However, these differences were not statistically significant (P=0.627).

Significant changes were observed in vertical healing of extraction sockets in the study groups. The mean AC dimensional reduction were 6.25±2.13 mm, 6.83±1.83 mm and 3.83±1.63 mm in DBBM, BDDM+PRGF and control groups, respectively (P=0.034). The greatest bone resorption was recorded in the control group and the PRGF group exhibited the least amount of bone resorption. The details of BL and AC dimensions are presented in Tables 1 and 2.

In all the three groups, the healed extraction sockets were covered with a keratinized oral epithelium. In the DBBM group, these particles were observed at varying dimensions and were surrounded by collagen fibers capsules (Figure 3). In addition,, polymorphonuclear (PMN) cells were seen in the presence of these particles. In socket preservation sites, mononuclear and polynuclear inflammatory cells were observed more frequency than in the control group.

The mean bone formation values were 41.72±8.60%, 46.80±6.75%, and 52.02±10.43% in DBBM, BDDM+PRGF, and control groups, respectively, without any significant differences (p=0.15). The buccal and lingual walls of the socket enclosed a large central area which was occupied by adipocyte cells, inflammatory cells, bone marrow and vascular structures. The bone marrow rate in the DBBM group was less and the majority of bone which had formed around the DBBM particles was woven bone.

Table 1. Socket buccolingual dimension data in mm immediately after extraction and at 3-month follow-up (Mean ± SD in mm)

Intervention	BL* width at baseline	BL width 3 months postoperatively	P
DBBM	5.5±0.83	3.5±0.83	0.039
DBBM+PRGF	5±0.63	3±0.63	0.038
Clot	5±0.63	2.33±1.53	0.038

BL* = buccolingual

Table 2. Socket depth dimensions immediately after extraction and 3 months postoperatively (Mean ± SD in mm)

Intervention	Socket depth at baseline	Socket depth 3 months postoperatively	P
DBBM	8.0±2.19	1.75±0.75	0.028
DBBM+PRGF	8.5±2.34	1.66±1.03	0.027
Clot	6.16±1.16	2.33±1.16	0.027

Discussion

The main aim of socket preservation is preserving the extraction socket dimensions, thereby preventing ridge resorption, particularly if the implant placement is intended in a period of 6 months.[25]

In this study, to obtain the growth factors the standard protocol of Anitua et al[27] was used. According to this protocol PRGF was used as platelet-rich product which has many advantages compared to PRP. Some of these advantages include its preparation by a centrifuge, the use of calcium chloride activator which reduces the risk of immune response and disease transmission compared to bovine thrombin in PRP, less blood volume which is needed for preparation of growth factors, platelet content survey possibility, growth factor concentration, stimulation of fibroblast migration, increase in the fibroblast migration, angiogenesis and intercellular matrix deposition which makes wound healing faster.

In this study, DBBM was used in one of the sockets for socket preservation in a period of 3 months. This period of time for evaluation was based on Cardaropoli et al,[31] Cardaropoli et al,[32] and Araujo et al[26] and it was shown that the change rate in socket dimensions at 3– 6-month intervals in the dog model is very negligible.

In a study by Araujo et al (2009), there was no difference in the change rate of lower thirds and middle thirds of socket dimensions between DBBM and control groups. However, in the coronal thirds these changes were on average 12% less in the DBBM group. However, the rate of bone resorption in both DBBM and control groups was the same.[(26)] In this study the bone resorption rate for BL and AC aspects in the DBBM group was less than the control group without any significant differences between the groups.

Araujo et al[33] considered DBBM+collagen as more effective in socket preservation in comparison to blood clot and claimed that DBBM+collagen did not prevent the socket remodeling process and preserved hard tissue dimensions.

Conversely, Heberer et al[34] in their study compared the DBBM+collagen sockets with blood clot and reported 20% more bone resorption rate in non-augmented areas. In other words, DBBM prevents remodeling. From this viewpoint the study is consistent with the present study as it was observed that bone marrow was more prominent in the control group than in the DBBM group. The majority of DBBM particles were in the lower part of the sockets and small amounts of woven bone had surrounded the particles.

In a study by Nevins et al,[35] DBBM was also used for socket preservation. Although socket dimensions in the DBBM group were preserved better than the blood clot group but a large amount of vertical bone resorption occurred in the DBBM group. This resorption indicated that use of DBBM will not necessarily prevent resorption of the socket, and socket preservation rate with and without the use of DBBM will occur. The results of this study are also consistent with the present study.

According to the histological results of the present study, the preserved sockets were occupied mainly with immature woven bone even after 3 months of healing. This outcome is consistent with the results of studies carried out by Araujo et al (2009) and Araujo et al (2008), in which the amount of immature bone were reported to be 15% and 20%, respectively.[33-36]

The woven bone presence raises a concern whether other dimensional changes in socket dimensions might arise or not during bone maturing.

In this study, DBBM particles placed in grafted sockets remained as virtually without resorption, might indicate that DBBM in the graft is inert and its absorption process is very slow. These findings are consistent with the results of studies carried out by Traini et al[38] and Artzi et al.[37]

The apical parts of preserved socket with and without DBBM contained a similar amount of mature bone. However, in the coronal parts of preserved socket the osseous tissues were less mature. This finding was confirmed in 2009 by Araujo et al[36] They attributed this event to the bloodstream inside the socket, which during the socket preservation process, carried DBBM particles into the coronal sites. Since these particles are less affected by remodeling process, there are lower amounts of bone formation.

The presence of multinuclear cells on the surface of DBBM particles was seen in this study, which has also been previously reported by Houshmand et al[39] and Simon et al.[40] In those studies they recognized these cells as osteoclasts and considered they were related to progressive absorption and disposal of graft materials. However, a number of researchers identified multinuclear cells as active osteoclasts (Tapety et al[41]) and some others as inactive osteoclasts (Taylor et al[42]).

Application of growth factors which are biological mediators in tissue repair and reconstruction process associated with the use of graft materials has been reported for socket preservation. In fact, different and even contradictory effects of PRGF on the rate of bone formation have been reported in various studies. In addition, in this study the combined use of DBBM+PRGF showed maximum reduction in BL dimensions than the blood clot group (control group) and DBBM group but these differences were not statistically significant.

Fuerst et al[43] in comparison of the differences between the collagen and collagen+PRGF in the reconstruction of jaw defects in pigs concluded that PRGF reduced the rate of bone formation and there was also no evidence of an increase in osteogenesis rate in the study. The results of this study are consistent with the findings of the present study. They reported that possible cause of this phenomenon can be PRGF ability for recruiting inflammatory cells into the graft area. In addition, an increase in the long-term activity of macrophages used at PRGF site was seen. In the present study the increased level of mononuclear and multinuclear inflammatory cells at the socket preservation sites were also observed more frequently than the blood clot sites. Also the impact of PRGF is discussed more on the epithelial tissue repair. Therefore expectation for significant effect on bone healing process is weak according to current evidence.

Furthermore, Suba et al[44] in a canine study demonstrated an increased bone formation rate after 3 months of socket preservation by adding growth factors to the synthetic materials, claimed that β-tricalcium phosphate in combination with PRP leads to more intensive bone regeneration in comparison to β-tricalcium phosphate alone. However, these differences were not significant at 6-month post-extraction interval. In fact, PRGF is more involved in the early stages of healing. Since these processes have little effect on the general outcome of the socket and its dimensions, in the present study 3-month fellow-up periods were considered as reasonable periods for investigation of dimensional changes and extraction socket histology. This indicates that a shorter period of time for observing the socket healing may reveal more early effects of the PRGF.

Using PRGF in the present study leads to consistent results with the previous studies that claim the growth factors probably hasten wound healing phenomena without increasing the bone formation rate.[21,24,27] The presence of new bone formation density and blood vessels in DBBM+PRGF site in the current study is similar to Anitua et al[45] in which animals underwent bilateral open sinus lift operation and in one site only DBBM and in the other site DBBM+PRGF were used. In that experiment the amount of bone and blood vessels were also more in the DBB+PRGF group.

Various biological effects of growth factors have been attributed to the rapid degradation of polypeptide structures and low local bioavailability. To compensate those shortcomings the specific formulation of growth factors in combination with some biomaterials has resulted in encouraging results. For instance, a mixture of surgical-grade calcium sulfate and PRP leads to enhancement in bone regeneration in 2 weeks. However, these effects were not consistent in longer follow-ups.[48,49] The same reason would be attributed to the findings of the present study.

In the systematic review of Heggeler et al,[50] the effect of socket preservation on human non-molar teeth was evaluated and it was concluded that without the use of graft materials in socket preservation the socket dimension reduction observed were 2.6-4.56 mm and 0.4-3.9 mm, respectively. Therefore, socket preservation may decrease ridge dimension reduction but does not prevent it. However, socket dimension reduction was lower in studies in which growth factors were used and bone resorption was about 3.48 mm in width and 2.64 mm in height.

Using a membrane in socket preservation has been considered a controversial issue. The benefit of application of occlusive membrane in socket preservation was challenged by some recent studies[28,29,30] due to exposure problems of membranes, acceleration of the inflammatory cell response, time consumed and treatment costs. To prevent the above-mentioned complications we did not include the use of occlusive membranes in our study and a primary closure of the socket was applied instead.

Conclusion

Within the limitations of this study, the results show that there were no statistically significant differences in socket dimension preservation by using DBBM with and without PRGF. Although the standard preparation method of PRGF was used in this study, considering the absence of evidences on the optimum time of effectiveness and high sensitivity for formulation of polypeptide growth factors for clinical use, additional studies will be needed to confirm these results.

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