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Original Article

Effect of local injection of injectable platelet-rich fibrin on bone remodeling during orthodontic tooth movement in dogs

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ABSTRACT

Background: This study aimed to assess the effect of local injection of injectable platelet-rich fibrin (i-PRF) on bone remodeling during orthodontic tooth movement in dogs.

Materials and Methods: In this animal study, the maxillary first premolars of four adult male mixed-breed dogs were bilaterally extracted, and a nickel–titanium closed coil spring with 150 g force was placed between the canine and second premolar teeth. One quadrant of the maxilla was randomly selected as the test quadrant, and 0.5 cc i-PRF was injected into the periodontal ligament (PDL) around the second premolar at 1, 21, and 42 days. The other quadrant served as the control group and received saline injections. The dogs were sacrificed after 63 days, histological sections were prepared, and changes in bone remodeling were assessed by comparing the percentage of osteogenesis and number of osteoblasts and osteoclasts between the two groups by the Wilcoxon and Mann–Whitney *U*-tests ($\alpha = 0.05$).

Results: The percentage of osteogenesis (16.0% ± 4.96% in i-PRF and 13.5% ± 4.43% in the control), the percentage of newly formed lamellar bone (10.25% ± 2.87% in i-PRF and 8.75% ± 2.36% in the control), the percentage of woven bone (5.75% ± 2.21% in i-PRF and 4.75% ± 2.36% in the control), the number of osteoblasts (15.0 ± 3.46 in i-PRF and 11.75 ± 2.36 in the control), and the number of osteoclasts (11.25 ± 4.34 in i-PRF and 6.25 ± 2.62 in the control) were not significantly different between the two groups ($P > 0.05$).

Conclusion: PDL injection of i-PRF around the second premolars of dogs under orthodontic force had no significant effect on bone remodeling.

Key Words: Bone remodeling, platelet-rich fibrin, tooth movement techniques

INTRODUCTION

Orthodontic treatments are based on the principles of biomechanics, and it is, in fact, the biological response to the applied forces that cause orthodontic tooth movement (OTM).[1] Load application to the teeth causes bone remodeling, which includes periods of bone resorption and bone formation at the pressure and tension sides around each tooth, eliciting an acute inflammatory response in periodontal tissues.[2]

OTM in the presence of a mechanical stimulus causes remodeling of the alveolar bone and periodontal

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ligament (PDL).[3] Bone remodeling involves bone resorption at the pressure site and osteogenesis at the tension site.[4] The magnitude of OTM can be controlled by the magnitude of applied force and biological responses of the PDL. The forces applied to the teeth cause some changes in the PDL, alter the blood flow, and induce the release of several inflammatory markers such as cytokines, growth factors, neurotransmitters, and arachidonic acid metabolites, which lead to bone remodeling.[5,6] OTM includes three phases: the first phase is characterized by fast OTM following load application, the second phase is a delayed phase with insignificant or no OTM, and the third phase is characterized by a gradual or sudden increase in OTM.

The application of platelet-rich plasma (PRP) for bone regeneration was introduced in the late 1990s. It was later used for orthopedic and oral surgical procedures. However, controversy still exists regarding the positive effects of PRP on osteogenesis.[7] PRP contains high concentrations of autologous platelets in a small volume of autologous plasma (minimum of 1,000,000 platelets/μL in 5 mL of plasma). The platelets present in this autologous plasma concentrate release alpha granules after coagulation at the wound site. Alpha granules contain a group of growth factors that cause cell proliferation and differentiation and are imperative for osteogenesis. Thus, in addition to coagulative effects, PRP is a rich source of growth factors that play a role in the enhancement of wound healing, bone regeneration, and proliferation of fibroblasts. The PRP gel is prepared by mixing PRP (obtained by centrifugation of autologous whole blood) with thrombin and calcium chloride. The addition of thrombin and calcium chloride to PRP automatically induces the alpha granules to release biological growth factors such as platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), and epidermal growth factor.[8]

Platelet-rich fibrin (PRF) is a modified form of PRP. PRF is the second-generation platelet concentrate that was first introduced by Choukroun and Ghanaati,[9] in France. It is an autogenous fibrin matrix that contains PDGF, leukocytes, and cytokines. The mechanism of action of PRF is through induction of proliferation of residual cells and bone regeneration. PRF has increased alkaline phosphatase activity *in vitro*. The release pattern of PDGF and TGF-β varies between PRP and PRF. In PRP, the release of such factors significantly decreases after the 1st day; however, PRF continues to release such factors in considerable amounts for up to 2 weeks.[10] Dohan Ehrenfest *et al*.[11] confirmed a difference in the release profile of VEGF from leukocytes in the use of PRP compared with PRF. In total, it appears that the PRF membrane can release higher amounts of growth factors over longer periods of time.[12] The injectable PRF (i-PRF) is the liquid form of PRF, which is obtained by low-speed centrifugation. I-PRF has advantages such as a high number of regenerative cells and high amounts of growth factors.[9]

Orthodontic treatments depend on OTM, and injection of PRP and PRF is believed to enhance OTM. The advantages of PRF compared with PRP include a one-step preparation process, the addition of no chemicals like anticoagulants, faster preparation, and simpler application.[13]

On the other hand, the duration of orthodontic treatment plays an important role in patient cooperation and periodontal status. Thus, attempts are ongoing to shorten the course of orthodontic treatment by acceleration of OTM. The application of PRF is a relatively novel modality for the acceleration of OTM. Thus, this study aimed to assess the effect of local injection of i-PRF on bone remodeling during OTM in dogs.

MATERIALS AND METHODS

This animal study was conducted on four adult male mixed-breed dogs between 10 and 12 months of age. The study was conducted in accordance with the guidelines for the care and use of laboratory animals. The study protocol was approved by the Ethics Committee of the School of Dentistry, Islamic Azad University, Khorasgan Branch (IR.IAU.YAZD.REC.1400.168).

Sample size

The sample size was calculated to be four in each group according to a previous study,[14] assuming $\alpha = 0.05$, $\beta = 0.2$, and a study power of 80%.

Eligibility criteria

Adult male mixed-breed dogs between 10 and 12 months of age and 15–20 kg weight were included.

Intervention

After the induction of general and local anesthesia, the maxillary first premolars of the dogs were bilaterally extracted, and nickel–titanium closed coil

springs (G&H Wire Co.) were used to connect the canine to the second premolar. To determine the proper length of the spring, the load of the spring was measured by a force meter (Dentaurum, Germany) to ensure the application of a 150 g load in this distance. Periapical radiographs were obtained from the teeth to ensure healthy PDL. Next, one quadrant of the maxilla was randomly selected as the test group, and 0.5 cc of i-PRF was injected at 8 points of mid-buccal, mid- lingual, distobuccal, distolingual, mid-distal, mesiolingual, mesiobuccal, and mid-mesial into the PDL around the second premolar. The other quadrant of the maxilla served as the control group and received 0.5 cc saline injections. Injections were performed at 1, 21, and 42 days after the placement of closed coil springs. The dogs were fed soft food during the study.

Preparation of injectable platelet-rich fibrin

Blood samples were obtained from the cephalic vein and collected in 10 cc test tubes without an anticoagulant. They were centrifuged (IntraSpin) at 700 rpm for 3 min with 60 g RCF. Next, 0.5 cc of the supernatant was collected by a syringe.

After 63 days, the dogs were sacrificed, the maxillae were resected, fixed in 10% formalin (Merck, Germany) for 48 h, and were then placed in 10% formic acid for 1 week. They were then dehydrated using graded concentrations of alcohol, embedded in paraffin, and sectioned by a microtome (Leica, RM 2035, Germany) into 5-µm slices. Five discs were obtained from the apical third of each premolar tooth (20 test and 20 control specimens in total). The slides were stained with hematoxylin and eosin (Merck, Germany) and observed under a light microscope (BX; Olympus) at ×100 and ×400. The changes in bone were assessed. The percentage of osteogenesis, the percentage of lamellar and woven bones, and the number of osteoblasts and osteoclasts were measured on five slides of each premolar tooth using Adobe Photoshop 7 software (San Jose, CA, USA), and the mean values were calculated and reported.

Statistical analysis

Data were analyzed by SPSS version 26 (SPSS Inc., IL, USA). Due to the small sample size, the normality of data distribution was analyzed by the evaluation of skewness and kurtosis, which showed nonnormal data distribution. Thus, the two groups were compared regarding the percentage of osteogenesis, percentage of lamellar and woven bones, and number of osteoblasts and osteoclasts by the Wilcoxon and Mann–Whitney *U* nonparametric tests at a 0.05 level of significance.

RESULTS

Figures 1-3 show osteogenesis, osteoblasts, and osteoclasts in the two groups. Table 1 presents the percentage of bone formation, lamellar bone and woven bone, and the number of osteoblasts and osteoclasts in the two groups.

The percentage of osteogenesis ($P = 0.581$), the percentage of newly formed lamellar bone ($P = 0.461$), the percentage of woven bone ($P = 0.593$), the number of osteoblasts ($P = 0.109$), and the number of osteoclasts ($P = 0.068$) were not significantly different between the two groups.

DISCUSSION

This study assessed the effect of local injection of i-PRF on bone remodeling during OTM in dogs. The

Table 1: Percentage of bone formation, lamellar bone and woven bone, and the number of osteoblasts and osteoclasts in the two groups (*n*=4)

Variable	Group	Minimum	Maximum	Mean±SD	Statistic	*P*
Bone formation (%)	i-PRF	10.0	22.0	16.00±4.96	−0.552	0.581
	Control	10.0	20.0	13.50±4.43		
Lamellar bone (%)	i-PRF	7.0	14.0	10.25±2.87	−0.736	0.461
	Control	7.0	12.0	8.75±2.36		
Woven bone (%)	i-PRF	3.0	8.0	5.75±2.21	−0.535	0.593
	Control	3.0	8.0	4.75±2.36		
Number of osteoblasts	i-PRF	10.0	18.0	15.00±3.46	−1.604	0.109
	Control	10.0	15.0	11.75±2.36		
Number of osteoclasts	i-PRF	5.0	15.0	11.25±4.34	−1.826	0.068
	Control	4.0	10.0	6.25±2.62		

i-PRF: Injectable platelet-rich fibrin, SD: Standard deviation

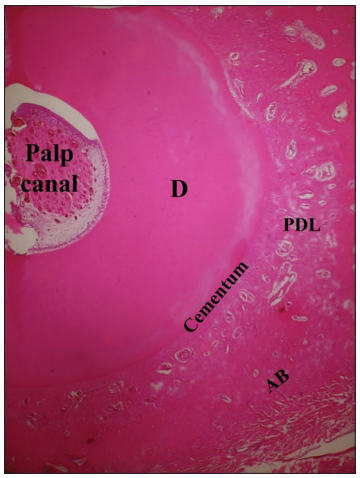

Figure 1: Photomicrograph of a transverse tooth section, indicating normal cementum, dentin, periodontal ligament, and alveolar bone in the control group (H and E, ×100). PDL: Periodontal ligament, D: Dentin, AB: Alveolar bone.

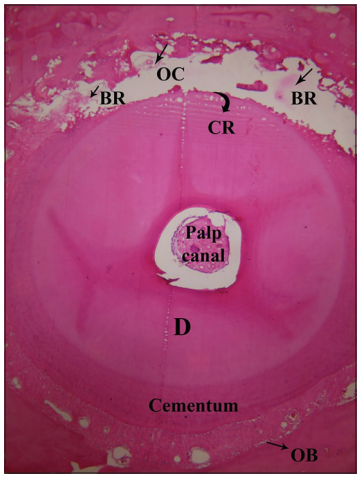

Figure 2: Photomicrograph of a transverse tooth section, indicating dentin, osteoclasts, osteoblasts, bone resorption, cementum resorption, and alveolar bone in the PR group (H and E, ×100). D: Dentin, OC: Osteoclasts, OB: Osteoblasts, BR: Bone resorption, CR: Cementum resorption.

results showed no significant difference between the two groups in any of the variables related to bone remodeling.

I-PRF has high concentrations of growth factors that may enhance tissue regeneration. TGF-β and PDGF have a direct correlation with the platelet content, while VEGF and fibroblast growth factor-2 have a poor correlation with the platelet content.[15] Furthermore, the expression of collagen genes, platelets, and lymphocytes increases in the application of i-PRF.[16] I-PRF has the highest concentration of platelets compared with other platelet concentrates,[9] but it may not have adequate amounts of leukocytes to induce the release of growth factors and cytokines from the platelet concentrates and significantly affect the regenerative process.

Zeitounlouian *et al*.[17] evaluated the efficacy of i-PRF for the preservation of bone and prevention of root resorption and showed that it had no significant effect on bone quality during canine retraction or the prevention of canine root resorption. Their results were in line with the present findings. Mu *et al*.[18] evaluated the effects of PRF in combination with deproteinized bovine bone mineral on bone remodeling in sinus floor augmentation in rabbits. They reported a higher percentage of newly formed bone in the intervention group; however, the overall bone volume was the same in the two groups. They concluded that the application of i-PRF in combination with deproteinized bovine bone mineral accelerated angiogenesis, bone remodeling, and substitution of graft material. The difference between their results and the present findings may be due to the evaluation of rabbits in their study versus dogs in the present study. Cömert Kılıç *et al*.[19] showed that the application of P-PRP and PRF along with bone grafting had no significant effect on bone formation and regeneration. Their results were in line with the present findings despite the use of different products and protocols.

Variations in the results can be due to differences in animal models, assessment periods, and biomaterial types. In the process of bone regeneration, biological events, including blood clotting, inflammatory reactions, and angiogenesis, occur along with cellular differentiation and mineralization.[19] Furthermore, the presence of blood clots is important for postoperative healing since they are replaced with connective tissue and are responsible for dynamic bone remodeling in subsequent phases.[20] In the first 2 weeks, the blood clots are still present, and then fibrous granulation tissue is formed, the number of inflammatory cells decreases, and osteoclastic activity increases.[20] By the progression of angiogenesis, mesenchymal cells are differentiated to osteogenic cells that differentiate to osteoblasts. Finally, bone remodeling leads to new bone formation even in the short term.

Erdur *et al*.[21] reported that the application of i-PRF induced the expression of pro-inflammatory cytokines, osteoclastic activity, and enhancement of OTM. An increase in the number of osteoclasts was also noted in the test group in the present study; however, it was

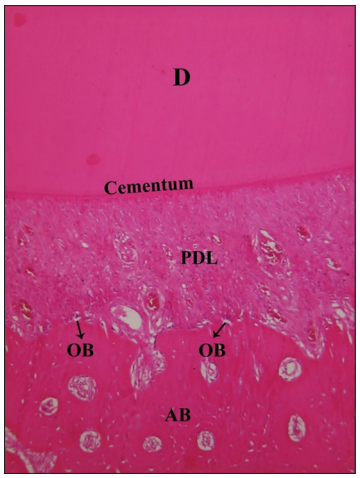

Figure 3: Photomicrograph of a transverse tooth section, indicating alveolar bone, periodontal ligament, osteoblasts, and dentin in the PRF group (H and E, ×100). PDL: Periodontal ligament, D: Dentin, OB: Osteoblasts, AB: Alveolar bone.

not statistically significant. Kizildağ *et al*.[22] evaluated new bone formation following autogenous bone grafting in combination with PRF in rabbit calvaria. They showed greater new bone formation and higher bone density in the autogenous bone + PRF group, compared with autogenous bone alone and the control group. Alhasyimi *et al*.[23] evaluated the effects of A-PRF containing hydroxyapatite carbonate on bone remodeling and relapse in rabbits and reported an increase in the number of osteoblasts and a reduction in osteoclastic activity in the PRF group containing hydroxyapatite carbonate. The difference between their results and the present findings can be due to using different animals and also differences between A-PRF and i-PRF in levels and types of cytokines. Yashwant *et al*.[24] showed that the application of PRF as a graft material or membrane at the decortication site enhanced wound healing. Miron *et al*.[25] demonstrated that i-PRF was capable of releasing higher concentrations of growth factors, greater induction of fibroblasts, and higher production of PDGF, TGF-B, and type I collagen, compared with PRP. Rashid *et al*.[26] evaluated the effects of PRP on the speed of OTM in dogs and showed a higher number of osteoclasts at the resorption site in the test group, indicating a higher speed of OTM in the PRP group. Furthermore, greater osteogenesis was noted in the PRP group. Their results were different from the present findings probably due to differences in sample size. Güleç *et al*.[27] evaluated the effects of different concentrations of PRP on alveolar bone density and speed of OTM in rats and showed that injection of moderate and high concentrations of PRP decreased alveolar bone density in paradental tissues, increased osteoclastic activity, and enhanced OTM. PRF releases several growth factors such as PDGF, fibroblast growth factor, and VEGF. PDGF stimulates cell proliferation, and VEGF induces angiogenesis. Since OTM is an inflammatory process, the presence of leukocytes in PRF can enhance OTM. Cytokines, interleukins, and tissue necrosis factors that are also present in PRF regulate the immunological reactions involved in OTM as well. Nonetheless, platelet concentrates can have several biological effects such that a previous study showed that growth factors may inhibit the release of cytokines, suppress inflammation, and induce tissue regeneration.[28] To further clarify the role of i-PRF in bone remodeling, the degree of inflammation should be assessed at both the injection and control sides. Che *et al*.,[29] in a review study, reported that the application of PRF along with bone graft materials increases osteogenesis; however, some concerns still exist with respect to the acceleration of OTM.

The small sample size and evaluation of histological sections only at 1 time-point (63 days) due to the design of the study were among the limitations of this study. Future studies are recommended to immunohistochemically and radiographically assess the effects of platelet concentrates such as i-PRF and L-PRF on OTM, bone remodeling, and root resorption in a larger sample size.

CONCLUSION

PDL injection of i-PRF around the second premolars of dogs under orthodontic force had no significant effect on bone remodeling.

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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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