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Effects of Injectable platelet rich fibrin (i-PRF) on reduction of relapse after orthodontic tooth movement: Rabbits model study

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Abstract

OBJECTIVES: The objective of this study is to determine whether submucosal local injection of i-PRF may affect orthodontic relapse by increasing bone density, which further leads to reducing orthodontic relapse.

MATERIALS AND METHODS: Forty-five adult male albino rabbits were randomly divided into three groups: group I (control) with 15 rabbits injected with 200 μ l of phosphate-buffered saline (PBS), group II with 15 rabbits injected with 200 μ l of i-PRF, and group III of 15 rabbits inject with 400 μ l of i-PRF. The lower incisors of rabbits moved distally by a modified orthodontic appliance for 2 weeks; then, the appliance was maintained in position to retain the gaining space for 2 weeks. During the retention period, each group was injected with the specific drug every 7 days. After the retention period, teeth were allowed to relapse by removal of the orthodontic appliance. The results were evaluated by measuring the amount of orthodontic relapse and bone density. The statistical analysis performed by ANOVA and Duncan ($P < 0.05$ was considered significant).

RESULTS: I-PRF groups showed a significant reduction in the amount of relapse at 10, 13, 17, and 20 days compared to the control group, indicated by the highest percentage of relapse for the control group at the end of the study (20 days); it was (90.4%) in compared to lowest percentage of relapse for i-PRF groups—they were 61.2% and 59.9%, respectively.

CONCLUSION: Results indicated that i-PRF has the potential to enhance the stability of teeth after orthodontic tooth movement and could have the ability to reduce relapse, probably by increasing the alveolar bone density.

Keywords:

Alveolar bone remodeling, bone density, injectable platelet-rich-fibrin (i-PRF), orthodontic relapse

Introduction

One of the most hardships after orthodontic treatment is the relapse that may occur after treatment. Thus, relapse becomes the major concern for all orthodontics with respect to the goals of long-term maintenance of favorable aesthetics and ideal function of occlusion that have been achieved at the end of orthodontic

treatment.^[1] A previous study on relapse concluded that only 30%–50% of orthodontic patients maintained the post-treatment alignment over 10 years.^[2] Therefore, reduction of relapse after orthodontic tooth movement (OTM) is tried in several mechanical ways by using retainers. However, many problems were associated with the prolonged use of different types of retainers, including lost patient cooperation, the retainer getting lost or broken, and the tendency to cause periodontal problems.^[3] Studies recently

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suggested utilizing pharmacologic therapy, biomaterial, and the chewing force in an attempt to provide another mechanism to enhance the stability of teeth after orthodontic treatment.^[4-7]

The authors proposed a different type of pharmaceuticals for control of relapse. The drawback in the use of these drugs is due to their potential risks, side effect, and they are not 100% autologous. In addition to that, there was an argument about their effectiveness in mitigating relapse.^[8,9]

Scientists considered platelet-rich plasma (PRP) as a source of high concentration of autologous growth factors that could improve wound healing. However, later several limitations make its use in the medical and dental fields highly inefficient. These limitations include long preparation time, procedures needed to add anticoagulant factors, quickly releasing a growth factor, and no scaffold, and is therefore required to be used in combination with other biomaterials.^[10] Later, attempts were made to overcome the drawback in the PRP, leading to the discovery of the second generation of platelet concentration called platelet-rich fibrin (PRF),^[11] which offers many advantages in comparison to PRP^[12] and is a fully autologous system (no risks of immune rejection and pathogen transmission) free of any anticoagulant,^[10] and allows slow release of growth factors till 28 days after application.^[13] Thus, it showed many application in the dental and medical fields with a high rate of success but because of its solid nature, it is used in the orthodontic field remain as a subject of study.^[6]

To cater to the demand for the development of new PRF with fluid substance, scientists changed the procedure of centrifugation by using plastic tubes instead of glass tubes for blood collection and by reducing the revolution per minute (rpm) and centrifugation time, which led to the introduction of an injectable PRF (i-PRF).^[14] Recent reports have revealed that in addition to it is liquid nature, i-PRF shows many advantages in comparison to the other types of PRF. The primary one is its ability to form an autologous fibrin scaffold for up to 15 minutes (serves as a reservoir of natural growth factors); slow release of different types of growth factors; and a high number of platelets, leukocytes, and regenerative cells.^[6,14]

The effects of i-PRF on inhibition of relapse of teeth after OTM have not been investigated yet. We hypothesized that the utilization of submucosal local injection of i-PRF might have an impact on inhibition of the relapse after OTM because of the direct mode of action and their dual action in the enhancement of stability of teeth after OTM. The first action is the anabolic effect of i-PRF, which increases alveolar bone remodeling. The second one is the ant-catabolic effect by suppressing RANKL by stimulation of osteoprotegerin (OPG) expression.^[15]

Materials and Methods

Animal samples and experimental groups

The study included a total of 45 healthy adult male albino rabbits aged 5–8 months. The average weight of rabbits was 1720 g at the beginning of the experiment; they were housed in an animal house prepared for this experiment in a 12-h light/dark environment at the same conditions of good ventilation, temperature, and humidity. They had free access to tap water and an adequate stable diet (green vegetables, corn, and grains) throughout the experiment. The health status of each rabbit was evaluated by daily body weight monitoring for more than 1 week before the start of the experiment as well as during the time of the experiment.

The Research Ethics Committee of the Faculty of Dentistry, University of Mosul, Ministry of Higher Education and Scientific Research, Iraq approved all experiments, protocol, and guidelines for this study, with the approved REC reference number UoM. Dent/A.22/20 on December 8, 2020.

This experiment used a random design to reduce the inter-individual variation and ensure that each rabbit has an equal probability for selection for any group. Animals were randomly divided into three groups with 15 rabbits in each. Group I (control) of 15 rabbits was injected with a 200 µl of phosphate-buffered saline (PBS) (MyBiosource, sunny Southern California, San Diego, USA), PBS widely used as a vehicle during injection of pharmacologic drug for orthodontic relapse control as described in previous studies.^[16,17] Group II of 15 rabbits was injected with i-PRF 200 µl. Group III of 15 rabbits was injected with i-PRF 400 µl. Each group was further subdivided into three subgroups of five rabbits each according to the time of sacrifice (0, 10, and 20 days).

Orthodontic appliance insertion

A modified orthodontic appliance was used, with heavy Ni-Ti open coil spring (0.012" × 0.036" DB Orthodontics, West Yorkshire, United Kingdom) consisting of five circles and about 5.5 mm in length was inserted along the stain less steel rectangular wire 0.016" × 0.022 (Orthometric, Brasil). Bands were ligated together with 0.01-inch stainless steel ligature wire (Dentaurum, Germany) to close the space created from the open coil spring. Before insertion of the orthodontic appliance, all rabbits were anesthetized with a mixture of ketamine hydrochloride (50 mg/kg body weight) (Holden Netherland, India) and xylazine hydrochloride (10 mg/kg body weight) (Alfasan, Woerden-Holand) were injected intramuscular in the thigh muscle.^[18] Then, 15 min after anesthetic methods [Figure 1a], a fixed orthodontic appliance was banded to the lower central incisors with zinc polycarboxylate cement (DoriDent,

Austria) [Figure 1b]. The force exerted by spring was determined prior to insertion, with a stress and tension gauge (dynamometer, Hahnkolf, Stuttgart, Germany); it exerted about $50 \text{ g} \pm 2 \text{ g}$ of reciprocal force when activation at 2.4 mm. The force was exerted for 2 weeks and led to distal movement of the lower incisors distally as shown in [Figure 1c].

After 2 weeks, the springs were not activated and both incisors were retained in position for 2 weeks as a retention period. Then, the orthodontic appliances were removed to allow the lower incisors to return to the original position, and the distance between the tip of the mesial surface of the lower incisors was measured.^[1]

Preparation of i-PRF

Blood collection was performed using 10-ml plastic tubes (i-PRF, Choukron Plastic, Pan vacuum without any additive, USA) prepared briefly from 5 ml of rabbit blood. Blood was collected by using cardiac puncture.^[19] According to this method, blood is withdrawn slowly to prevent the heart from collapsing and then immediately centrifuged to prepare the i-PRF according to the low-speed centrifugation concept (LSCC).^[14] I-PRF was prepared according to Jasmine *et al.*^[20] technique in which 5 mL of collected blood without anticoagulant were placed in the horizontal centrifuge (PC-02 Centrifuge, Hettich Universal 320 Zentrifugen, Germany) as shown in Figure 2, with other tubes opposite to the main tube filled with 5 mL of tap water to maintain the balance during centrifuging for 5 min at 1000 rpm and at 21°C-24°C. At the end of centrifuging the upper plasma layer, an orange color area in the tube was collected and designated as i-PRF [Figure 2].

Method of administration and site of injection

The i-PRF and PBS were administered with the specified drug as specified by each group. Delivered locally into the submucosa by using a disposable 1-unit insulin syringe with a 25-G needle (China) inserted immediately and parallel to the mesial surface of the experimental side (right mandibular incisor) and adjacent to the mesial surface of mandibular right central incisors. Half of the dose was given into the labial and half into the lingual aspect of vestibular mucosa as shown in Figure 3, simulating the method of infiltrative local anesthesia injections according to a study done by Zeitounlouian *et al.*^[6] The injection was repeated every 7 days during the retention period (the first injection gave immediately at the 1st day of retention period while the second injection gave at 7th day of the retention period), according to Alhasyimi *et al.*^[1]

Measurement of tooth movement

The distance between the mandibular incisors at the level of the mesial tip of left and right lower incisor was measured by direct manual measurement with electrical digital vernier (Qingdao, China) accurate to 0.01 mm

to determine the amount of relapse [Figure 4]. Each measurement was repeated three times and the mean was recorded.

Measurements were taken on days 0, 2, 4, 7, 10, 13, 17, and 20 after removal of the orthodontic appliance. At the time of removing the orthodontic appliance the amount of space recorded between lower incisors teeth was considered as space 0 (sp0) and on the other day of measurement as space 1 (sp1), relapse distance (RD) was measured by subtracting sp1 from sp0 ($\text{RD} = \text{sp0} - \text{sp1}$). The RD was calculated and provided in percentage (%) by multiplying the RD by 100 then divided by sp0.^[1]

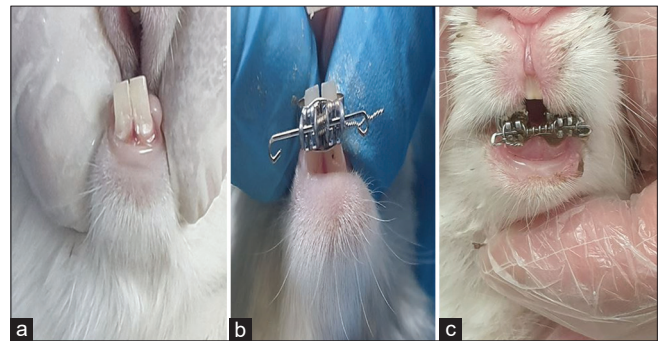


Figure 1: (a) Pre-orthodontic appliance insertion (b) at time of appliance insertion; (c) post orthodontic tooth movement (during retention period)



Figure 2: i-PRF obtained after centrifugation

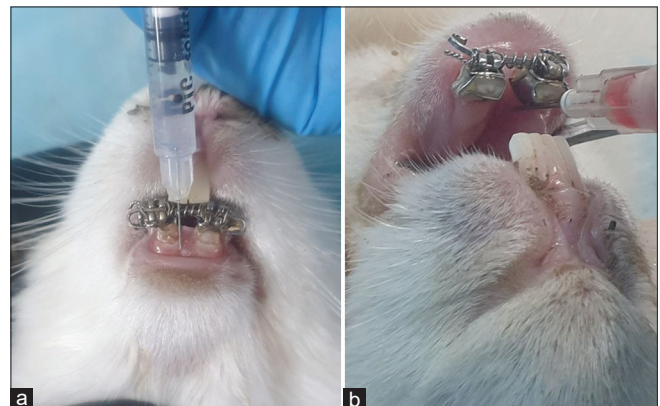


Figure 3: Local drug injections (a) labial injection (b) lingual injection

CBCT scanning procedures

After sacrificing rabbits under pentobarbital anesthesia in different periods of treatment at 0, 10, and 20 days, the mandibles of the rabbits were dissected carefully. Then, the mandible was stored in neutral 10% buffered formalin (Scharlan, Spain) till scanned by CBCT. Data for evaluating the cortical and cancellous bone density were collected by scan unit Gendex Dental system panoramic and cone beam system (Gendex 1910 North Penn Road, PA 19440, USA). All CBCT scans were taken under constant condition (10.0 mA and 90 kV) with 8.7 s, rotation of 3600, and were of 0.2 mm voxel size. Then, the commercial software (Blue sky plan version 4.8.2) was utilized to determine the Hounsfield Unite (HU) value for each CBCT image. The sagittal plane on the axial view was set at three levels by moving posteriorly from the crest of the alveolar bone. The first level (cervical) was 3 mm from the alveolar crest ridge, the second level (middle) was 6 mm from the alveolar crest ridge, and the third level (apical) was 9 mm from the alveolar crest ridge. Following that, we switched to a sagittal view to identify the interesting area according to Campos *et al.*^[21] To measure the cortical bone density, the midpoint of the cortical bone thickness was selected to represent its density at each point on each level. Also, the density of the cancellous bone was measured at the midpoint of cancellous bone thickness at each point on each level.

To determine the experimental error and intra-examiner calibration, scans were randomly selected for 10 animals and were measured twice by the examiner, with an interval of 1 month between measurements of HU for both cortical and cancellous bone density. These measurements were then compared by paired *t*-test; $P < 0.05$ was considered significant, which showed no significant difference for any of the study variables.

Statistical methodology

The statistical analysis of the data for relapse distance and bone density among different groups was performed by ANOVA and Duncan. $P < 0.05$ was considered significant. Statistical analysis was performed using IBM SPSS Statistics, Version 25 (IBM Corporation, USA). It was used to determine the amount and percentage of the variable.

Results

Regarding the animal status, there was no weight loss in all groups throughout the study and there were no significant differences in weight gain among groups. The mean of the initial weight of animals was 1720 g and at the time of orthodontic appliance removal was 1840 g; there were no significant differences in weight gain among experimental groups during the stages of

the experiment. Weight gain for group I was 252.6 g, for group II was 247 g, and for group III was 244.8 g. These results suggest that appliance placement and injection procedure did not have an effect on the overall animal's health.

There was no significant difference in the amount of space gained among all experimental groups at the time of appliance removal, as shown in Table 1.

Statistical analysis among experimental groups [Table 2] showed that although the mean of RD for the control group on days 2, 4, and 7 had higher RD than the i-PRF groups, there was no significant difference among all groups. However, a significant difference in RD for group I was found on days 10, 13, 17, and 20 compared to i-PRF groups. Furthermore, no significant difference in the amount of RD was found between i-PRF groups on days 2, 4, 7, 10, 13, 17, and 20 [Figures 5 and 6].

Comparison of the cortical and cancellous bone density among different groups

The statistical analysis for cortical and cancellous bone density showed that the control group had the significantly lowest cancellous bone density than the i-PRF groups only in the cervical and middle regions at

Table 1: Comparison of the mean of space gaining among different groups

Groups	Sub G.	Mean●	SD*	Duncan**	P***
I	0 days	3.25	0.395	A	0.967
II		3.18	0.363	A	
III		3.31	0.427	A	
I	10 days	3.12	0.279	A	
II		3.05	0.155	A	
III		3.29	0.319	A	
I	20 days	3.17	0.248	A	
II		3.18	0.408	A	
III		3.15	0.157	A	

*Standard deviation ** Different letters vertically mean a significant difference
 ***A significant difference existed at $P < 0.05$ ●mean expressed in mm



Figure 4: Direct manual intra oral measurement of interproximal space with digital vernier

Table 2: Comparison of the mean of relapse distance among different groups for each day

Group	Days	Mean●	Duncan**	P	Days	Mean	Duncan**	P
I	0	0.00	-	-	10	2.18	A	0.001*
II		0.00	-			1.59	B	
III		0.00	-			1.65	B	
I	2	1.04	A	0.150	13	2.53	A	0.000*
II		1.00	A			1.70	B	
III		1.08	A			1.74	B	
I	4	1.39	A	0.344	17	2.73	A	0.000*
II		1.16	A			1.81	B	
III		1.36	A			1.89	B	
I	7	1.62	A	0.128	20	2.86	A	0.000*
II		1.33	A			1.91	B	
III		1.50	A			1.95	B	

●mean expressed in mm **A significant difference existed at $P < 0.05$ -P cannot be computed because the standard deviation of all groups are 0 *Different letters vertically mean a significant difference

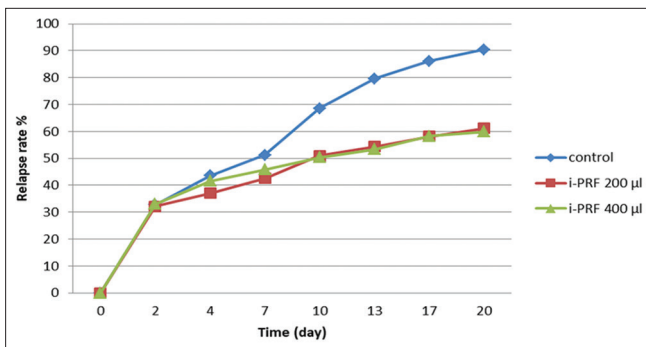


Figure 5: Comparison of the relapse rate percentage among different days for each group

the three time intervals. For cortical bone, there were no significant differences in all experimental groups at the three time intervals. In addition, there were no significant differences in the amount of cortical and cancellous bone density between i-PRF groups in all groups at the three time intervals [Tables 3 and 4].

Discussion

The control group showed a significant ($P < 0.05$) increase in the amount of relapse in comparison to the i-PRF groups at days 10, 13, 17, and 20 after removal of orthodontic appliance [Figures 5 and 6]. No other study has examined i-PRF in the inhibition of relapse. The results of the present study confirm the findings of Alhasyimi *et al.*^[1] However, Alhasyimi *et al.*^[1] used carbonated hydroxy advance PRF (a-PRF) in inhibition of orthodontic relapse; the authors pressed the a-PRF to obtain their release and then incorporated it with hydrogel as scaffold because it lost natural scaffold during preparation procedure. Thus, they ignored the loss of natural scaffold that can be obtained by using i-PRF (100% autologous), which is the hallmark of i-PRF that can retain growth factors until they reach the target area. Then, they start to slowly degrade and release different types of growth factors. Furthermore, there was

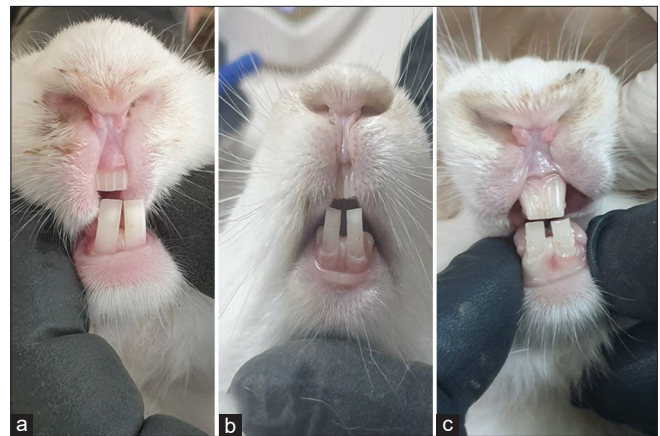


Figure 6: At day 20 (a) Group I (control); (b) Group II (i-PRF 200 µl); (c) Group III (i-PRF 400 µl)

no significant difference in relapse between i-PRF groups because difference in dose between the i-PRF groups may not be enough to make a significant difference.

Relapse occurred in both the control group and i-PRF groups. Generally, cumulative relapse increased with time after orthodontic appliance removal, with a greater amount of relapse at the end of the study [Figure 5]. This is due to the nature of the tooth movement after relapse as the tooth has a tendency to retain its original position before movement after orthodontic appliance removal. Moreover, the study showed that there was no significant difference in the amount of space gained among all experimental groups at the time of appliance removal, as shown in Table 1. The reason for this limited insignificant difference in space gaining between lower incisors in all experimental groups was due to no activation of open coil after orthodontic appliance insertion (cessation of orthodontic force due to loss of spring action) and the standard length of coil spring.

At days 2, 4, and 7 after the appliance was removed and the tooth began to relapse, no significant ($P < 0.05$)

Table 3: Comparisons of Hounsfield Unit mean of cortical and cancellous bone at cervical and middle regions for all experimental groups

Groups	Sub G.	Cortical bone			Cancellous bone		
		Cervical			Cervical		
		Mean	Duncan**	P	Mean	Duncan**	P
I	0 day	2025	A	0.086	1042	A	0.010*
		2155	A		1494	B	
		2203	A		1466	B	
II	10 days	1940	A	0.069	969	A	0.000*
		2022	A		1266	B	
		2080	A		1282	B	
III	20 days	2021	A	0.316	984	A	0.000*
		2084	A		1334	B	
		2132	A		1408	B	
		Middle			Middle		
I	0 day	2375	A	0.905	649	A	0.006*
		2400	A		935	B	
		2392	A		941	B	
II	10 days	2222	A	0.534	530	A	0.009*
		2278	A		764	B	
		2255	A		782	B	
III	20 days	2587	A	0.864	578	A	0.001*
		2578	A		836	B	
		2560	A		834	B	

●mean expressed as Hounsfield Unit (HU) *A significant difference existed at P<0.05 **Different letters vertically mean a significant difference

Table 4: Comparison of Hounsfield Unit mean of cortical and cancellous bone at the apical region for all experimental groups

Groups	Sub G.	Cortical bone			Cancellous bone		
		Apical			Apical		
		Mean	Duncan**	P	Mean	Duncan**	P
I	0 day	2650	A	0.486	641	A	0.410
		2675	A		699	A	
		2665	A		698	A	
II	10 days	2313	A	0.068	583	A	0.076
		2330	A		654	A	
		2393	A		671	A	
III	20 days	2674	A	0.086	593	A	0.593
		2685	A		658	A	
		2680	A		662	A	

●mean expressed as Hounsfield Unit (HU) *A significant difference existed at P<0.05 **Different letters vertically mean a significant difference

difference was found among all groups despite the fact that the i-PRF groups had lower mean relapse values than the control group. It is interesting to note that the percentage of relapse rate occurred quickly during the initial 2 days in all groups [Figure 5] and accounted for the majority (36.2%, 52.4%, and 55%) of total relapse in groups I, II, and III, respectively. According to Dunn *et al.*^[22] and Kiliç *et al.*^[23] the reason for this higher relapse during the initial 2 days after appliance removal is related to the displacement phase (initial phase) and not bone remodeling. In the displacement phase, the initial tooth movement occurs inside the socket of the

alveolar bone by physical compression of the viscoelastic periodontal ligament and bending of the alveolar bone.

Measurement of bone density

Changes in the alveolar bone density occur due to stimulation of the active bone remodeling during tooth movement, and the rate of tooth movement is inversely related to the bone density.^[24] The alteration in the bone density occurs as a result of resorption of preexisting bone and the formation of new bone during the remodeling process.^[25] One of the common characteristics of new bone is that it has less mineral content than preexisting bone, which leads to a distribution of the degree of bone mineralization (DBM) and thus inherent changes.^[26] Some researchers have demonstrated that CBCT is highly recommended for evaluating the alveolar bone density.^[21,25,27,28]

There were no significant differences in the cortical bone density among experimental groups in all regions. This might be due to the protocol of the study as all experimental groups were subjected to the same procedure of tooth movement and relapse. Furthermore, because cortical bone forms the external layer or cortex of alveolar bone, it has less contact with the root surface during tooth movement. Additionally, because the cortical bone is stronger and denser than cancellous bone, it has less metabolic activity.^[29] These findings are consistent with the findings of Zeitounlouian *et al.*^[6] who investigated the effect of i-PRF on alveolar bone remodeling after canine retraction in the extracted area of upper first premolars and found that i-PRF had no effect on alveolar bone remodeling.

In the experimental groups, the highest level of bone density was observed at the time 0. This can be attributed to the effect of the retention period, whereas there was no tooth movement, which allowed the bone to increase mineral density. The lowest level was observed at 10 days as a consequence of the increase in tooth movement in response to relapse. Then, an increase in the bone density was seen in all experimental groups from time day 10 till day 20. This finding is consistent with some previous studies that found a reduction in the alveolar bone density by orthodontic treatment.^[24,30] In contrast to other studies^[25,31] that found that density increases with tooth movement, Chun and Lim^[32] found no significant difference, implying that the presence of difference may be dependent on the specific sites in the mandible being examined.

It is interesting to note that as bone density increased significantly between the groups, the risk of relapse decreased significantly. However, these findings suggest the need for additional research to confirm the findings of this study, determine the most effective site for

administration of i-PRF, compare the effect of injections numbers during the retention period, and compare different centrifugation protocols.

A few limitations were observed in this study. The number of animals was small, which might have masked significant findings. In addition to that, there are differences in the rate of metabolism and remodeling of alveolar bone for animal models compared to those in humans; animal models have faster bone turnover than humans which may also have influenced the outcome of the study.

Conclusions

This study confirmed that submucosal local injection of i-PRF might reduce relapse after OTM, and there has been an increase in the bone density in the cervical and middle regions of alveolar bone after injection of i-PRF. Therefore, local injection of i-PRF might be a useful method for enhancement of stability after OTM. Thus, the alternative hypothesis presented in this study is acceptable.

Theoretically, there will be an increase in the inhibition of relapse with an increase in the i-PRF dosage, resulting from an increase in the number of growth factors that could improve the remodeling and regeneration process of new bone formation after OTM, which in fact did not occur in the present study.

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Conflicts of interest

There are no conflicts of interest.

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