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Research Paper
Bone Biology

Histological evaluation of combined platelet-rich fibrin membrane and piezo-incision application in orthodontic tooth movement

C. Sar[1], S. S. Akdeniz[2], A. Arman Ozcirpici[3], F. Helvacioglu[4], D. Bacanlı[5]

[1]Department of Orthodontics, Faculty of Dentistry, Istanbul Okan University, Istanbul, Turkey; [2]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Baskent University, Ankara, Turkey; [3]Department of Orthodontics, Faculty of Dentistry, Baskent University, Ankara, Turkey; [4]Department of Histology, Faculty of Medicine, Baskent University, Ankara, Turkey; [5]Animal Research and Producing Centre, Baskent University, Ankara, Turkey

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Abstract. The aim of this study was to evaluate the effect of platelet-rich fibrin (PRF) membrane on tooth movement in comparison with shunt control and piezoelectric surgery. Sixteen White Vienna rabbits were included in the study and divided into two groups. Reciprocal forces (20 g) were applied on the maxillary incisors by an orthodontic appliance. In group 1, PRF membrane was placed subperiosteally on the distal alveolar bone surface of the right central incisors and the left side was kept as control. In group 2, piezo-incisions 3 mm in depth were performed and combined with PRF membrane on the distal alveolar bone surface of the right central incisors, while the left side was kept as control. All rabbits were euthanized on day 21 and bilateral alveolar bone segments from the distal regions were removed for histological evaluation. Isolated PRF membrane application increased the blood vessel (8.3 ± 1.0; $P = 0.026$), osteoblast (6 ± 0.8; $P = 0.027$), and osteoclast (2.3 ± 0.8; $P = 0.026$) counts significantly compared to shunt control. Combined application of PRF membrane + piezo-incision increased the blood vessel (15.3 ± 0.8; $P = 0.027$), osteoblast (9.8 ± 1.4; $P = 0.026$), and osteoclast (3.3 ± 0.8; $P = 0.024$) counts significantly compared to shunt control. The increases in blood vessel count and osteoblast cell count were more evident in the combined application group (both $P = 0.002$). PRF membrane application significantly increased bone turnover, and the combined application of PRF membrane + piezo-incision was found to be the best method for increasing bone turnover.

Key words: accelerated tooth movement; platelet-rich fibrin; PRF; PRF membrane; piezoelectric surgery; piezo-incision.

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Adults comprise 20% of all orthodontic patients, and this ratio has been increasing substantially[1]. The length of orthodontic treatment is a major problem particularly among adult patients; therefore, accelerating the tooth movement and reducing the total treatment time are crucial for clinicians. Adult patients have slower cell mobilization rates, irregular orientation of collagen fibres, and an increased risk of periodontal disease when compared to children[2]. These biological circumstances increase the total treatment time and require the application of higher orthodontic forces, which are related to a higher risk of periodontal complications.

The techniques of low-intensity laser therapy, photobiomodulation, pulsed electromagnetic fields, electric currents, pharmacological agents, corticotomies, periodontal ligament distraction, corticision, and selective decortication of bone followed by grafting (Wilckodontics, or periodontally accelerated osteogenic orthodontics (PAOO)) have previously been recommended for accelerating tooth movement[3–12]. The regional acceleratory phenomenon (RAP), first described by Frost[13] in 1983, includes alveolar bone incision at a cortical level. Cortical bone plates are accepted as the main resistance for tooth movement. Cortical incisions lead to mechanical damage to the alveolar bone, stimulate bone remodelling, and activate osteoblasts and osteoclast cells. This process promotes a reduction in bone density, which causes lesser resistance to tooth movement[13].

Platelet-rich fibrin (PRF) is characterized by a dense fibrin mesh (similar to natural extracellular matrix) and rich platelet, leukocyte, growth factor, and stem cell content. PRF membrane actively produces and releases growth factors and cytokines for up to 28 days. It increases the levels of angiogenesis and bone turnover in the alveolar bone, and stimulates osteoinductive effects over osteoblasts, periodontal ligament cells and bone marrow mesenchymal stem cells[14–18].

The application of platelet concentrates following PAOO has been described previously in two studies. Muñoz et al. combined traditional bone grafting with PRF membrane following corticotomy and evaluated levels of postoperative inflammation, infection, and pain[19]. Murphy et al. used platelet-rich plasma (PRP) to increase bone graft stability without any further determination of the intra- and postoperative clinical effects[20]. However the exact effects of other platelet concentrates and PRF membranes on the rate of orthodontic tooth movement are not well documented. The aim of this study was therefore to evaluate the isolated effect of PRF membrane on tooth movement in comparison with combined piezo-incision and PRF membrane application in a rabbit model.

Materials and methods

This study was approved by the Institutional Review Board and Animal Ethics Committee of Baskent University and was supported by the Baskent University Research Fund. Sixteen young adult male White Vienna rabbits bred in the Baskent University Animal Research and Production Centre were included in the study. The rabbits were kept under a 12-h day/night cycle and were supplied with a standard laboratory diet and water ad libitum.

All rabbits were anaesthetized by intraperitoneal injection of ketamine (45 mg/kg) and xylazine (7 mg/kg) combination. Preoperative enrofloxacin (10 mg/kg intramuscular) and fentanyl (0.02 mg/kg subcutaneous) were given to each rabbit. First, PRF membrane was generated using 8 ml of autologous blood from the ear vein of all included rabbits and an orthodontic appliance was applied under general anaesthesia. Reciprocal forces of 20 g were applied on the maxillary incisors by orthodontic springs (Fig. 1). The 16 animals were then divided randomly into two equal groups of eight rabbits. A split-mouth design was used; one side was set as the study group, while the other side was set as the control group.

Bilateral mucoperiosteal flaps were released. In group 1, PRF membrane was placed sub-periosteally onto the distal alveolar bone surface of the right central incisors (Fig. 2). In group 2, piezo-incisions of 3 mm in depth, 0.5 mm away from the corresponding tooth surface were made at an inclination of 90° to the distal alveolar bone surface of the right central incisors using a piezoelectric surgery device (VarioSurg 50/60 Hz; NSK Nakanishi Inc., Kanuma, Japan) (Fig. 3) and these cuts were combined with PRF membrane. The left side was kept as shunt control in both groups. The mucoperiosteal flaps were repositioned and closed using tension-free resorbable sutures. All rabbits were euthanized on day 21 by overdose of general anaesthesia, and the bilateral alveolar bone segments from the distal surface of the central incisors were then resected for histological evaluation.

Histological evaluations

The alveolar bone segments were fixed with formalin solution. Bone segments were decalcified using formic acid solution, and haematoxylin and eosin staining was performed. Every alveolar bone sample was sliced into six pieces of 50 μm in width. Blind histological examinations were performed on all six pieces by the same researcher and the mean counts from these examinations were used for the statistical evaluations. The blood vessel count was calculated by counting the veining. Bone turnover was interpreted by scoring the orientation of the collagen fibres in the periodontal ligament layer (regular = 1; irregular = 0) and counting the osteoblast and osteoclast cells. In particular, the number of osteoblast cells with wide nuclei, showing

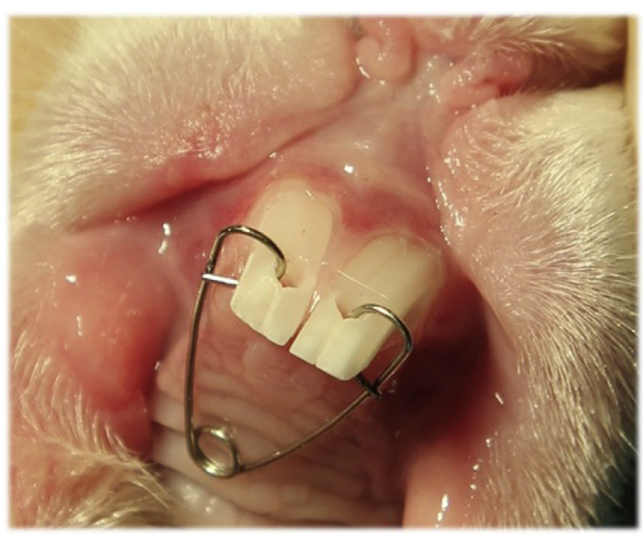

Fig. 1. The pre-prepared orthodontic appliance was placed on the upper central incisors and a 20 g standard orthodontic force was applied.

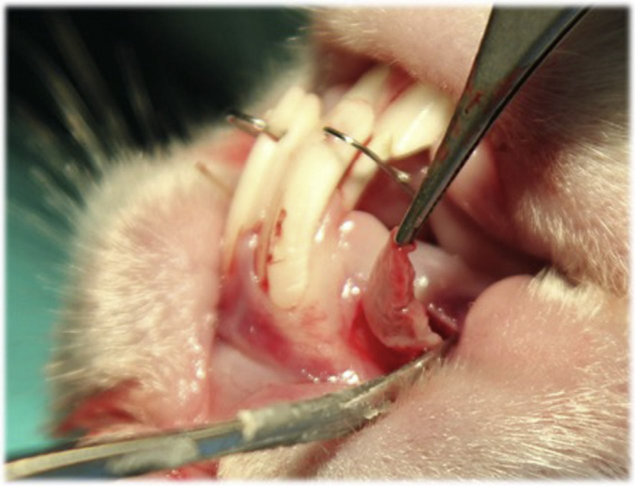

Fig. 2. PRF membrane was generated from 8 ml of autologous blood and was placed subperiosteally onto the distal surface of the right central incisor.

Fig. 3. A 2-mm deep vertical cortical bone cut was made along the distal bone surface of the right central incisors using a piezoelectric surgical device.

active bone synthesis, and the number of osteoclast cells attached to the trabecular bone surface in the region of resorption were calculated.

Statistical analysis

A paired study design was used to compare the interventions considered. The sample size was determined on the basis of the appliance group (mean 5.544 ± 251.1) in the study of Caglaroglu and Abdulvahit[21]. A sample size of eight would give 90% power to detect a significant difference at the 0.05 level. The histological parameters evaluated – osteoblast, osteoclast, and blood vessel counts and the periodontal ligament permutation score – were compared statistically between the subgroups using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). The measurement data were recorded as the mean ± standard deviation (SD). According to normality testing, a non-parametric distribution of outcomes was detected. Consequently, the two sides of each maxilla in each animal were compared statistically using the Wilcoxon signed rank test with respect to all outcomes considered. A P-value of ≤ 0.05 was considered to indicate a statistically significant difference.

Results

No obvious signs of orthodontic appliance-related discomfort were detected and no surgical intervention-related infection was noticed at the distal surfaces of the right or left central incisors in any of the animals.

In group 1, the differences in osteoblast, osteoclast, and blood vessel counts between the PRF membrane and shunt control subgroups were statistically significant (all $P < 0.05$). Blood vessel and osteoblast cell counts were distinctly higher in the PRF membrane subgroup (Fig. 4), while the osteoclast cell count showed a slight difference (Table 1).

In group 2, the differences in osteoblast, osteoclast, and blood vessel counts between the control subgroup and the combined PRF membrane + piezo-incision subgroup were statistically significant (all $P < 0.05$). The osteoblast cell count was distinctly higher in the combined PRF membrane + piezo-incision subgroup, while the blood vessel and osteoclast cell counts showed a slight increase (Fig. 5) (Table 2).

When the study groups (isolated PRF membrane application vs. combined PRF membrane + piezo-incision) were statisti-

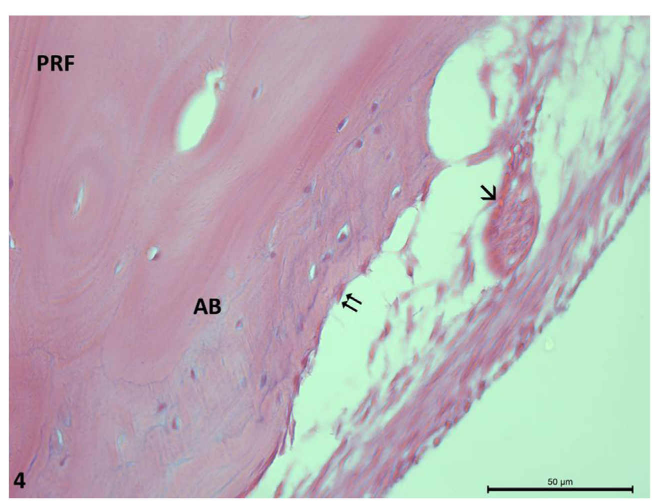

Fig. 4. Distal surface of the right central incisor in group 1 (PRF membrane subgroup). PRF, platelet-rich fibrin membrane; AB, alveolar bone; →, capillary blood vessel; ⇉osteoblast cell with tabloid nuclei (haematoxylin and eosin, ×400).

Table 1. Comparison of the PRF membrane and shunt control subgroups; mean ± SD values, Wilcoxon signed rank test.

Group 1, $n = 8$	PRF membrane subgroup	Shunt control subgroup	Z	Sig.
Osteoblast count	6 ± 0.8	1.8 ± 0.8	−2.214[a]	0.027*
Osteoclast count	2.3 ± 0.8	0.1 ± 0.4	−2.232[a]	0.026*
Blood vessel count	8.3 ± 1.0	3.3 ± 0.5	−2.232[a]	0.026*

PRF, platelet-rich fibrin; SD, standard deviation.
* Statistically significant difference, $P < 0.05$.

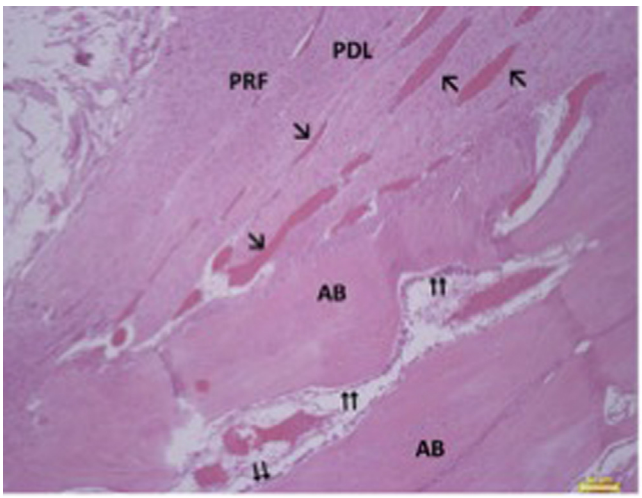

Fig. 5. Distal surface of the right central incisor in group 2 (combined PRF membrane and piezo-incision subgroup). PRF, platelet-rich fibrin membrane; AB, alveolar bone; PDL, periodontal ligament; →, capillary blood vessel; ⇉ active osteoblast cell (haematoxylin and eosin ×200).

Table 2. Comparison of the combined PRF membrane + piezo-incision and shunt control subgroups; mean ± SD values, Wilcoxon signed rank test.

Group 2, $n = 8$	PRF membrane + piezo-incision subgroup	Shunt control subgroup	Z	Sig.
Osteoblast count	9.8 ± 1.4	2 ± 1.2	−2.232[a]	0.026*
Osteoclast count	3.3 ± 0.8	0.3 ± 0.7	−2.251[a]	0.024*
Blood vessel count	15.3 ± 0.8	4 ± 1.2	−2.214[a]	0.027*

PRF, platelet-rich fibrin; SD, standard deviation.
[a] Based on negative rank.
* Statistically significant difference, $P < 0.05$.

cally compared with each other, significant differences were found for the osteoblast and blood vessel counts (both $P < 0.05$), whereas there was no statistically significant difference in osteoclast count between the groups ($P > 0.05$) (Table 3).

A regular periodontal ligament permutation was seen in 87.5% of the samples in the PRF membrane subgroup and in 100% of the samples in the combined PRF membrane + piezo-incision subgroup. The difference in periodontal ligament permutation between the two groups was not statistically significant ($P > 0.05$).

Table 3. Comparison of PRF membrane application and combined PRF membrane + piezo-incision subgroups; mean ± SD values.

	PRF membrane subgroup ($n = 8$)	PRF membrane + piezo-incision subgroup ($n = 8$)	Sig.
Osteoblast count	6 ± 0.8	9.8 ± 1.4	0.002*
Osteoclast count	2.3 ± 0.8	3.3 ± 0.8	0.093
Blood vessel count	8.3 ± 1.0	15.3 ± 0.8	0.002*

PRF, platelet-rich fibrin; SD, standard deviation.
* Statistically significant difference, $P < 0.05$.

Discussion

The procedure for generating PRF membrane is easy, repeatable, cheap, and without complications; therefore, PRF membrane has been used increasingly in the field of dentistry since 2011[14]. It can be obtained from as little as 8–10 ml of autologous blood without the need for any heterogeneous agent, in contrast to the preparation of PRP. Furthermore, PRF can release seven times the level of growth factor as compared to PRP[14]. Additionally, PRF membrane accelerates mucoperiosteal flap healing, increases the long-term post-treatment stability of PAOO, and supports osteogenic properties[19], and releases platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), and vascular endothelial growth factor (VEGF) for approximately 1 month after application, factors that are important for prominent neovascularization and increased collagen formation and bone turnover[17,18]. Furthermore, TGF-β regulates the proliferation of osteoblast cells with a series of other cytokines and bone morphogenetic proteins (BMPs)[22]. These favourable effects of PRF membrane led us to hypothesize that the increased turnover rates related to PRF membrane treatment would help to accelerate orthodontic tooth movement. It is well known that the use of PRF as a covering membrane provides a growth factor-rich micro-environment and stimulates the processes of both soft and hard tissue remodelling[23].

The histological evaluation performed in this study, investigating the effect of PRF membrane on orthodontic tooth movement, is novel. The isolated effect of PRF membrane and the combined effect with piezo-incision were evaluated separately to understand the contribution of PRF in tooth movement. Also, incisions were performed on the control sides and the flaps were closed with sutures in order to eliminate the effect of surgery itself on tooth movement and to evaluate the exact effect of PRF membrane in both groups. The novel findings of this study demonstrated that the application of isolated PRF membrane could significantly increase the alveolar bone turnover and that when combined with piezo-incisions, the results were better.

The use of PRF membrane alone led to an almost three times higher osteoblast cell count and almost 2.5 times higher blood vessel count when compared to the untreated control subgroup. The combined use of PRF and piezo-incision led to an almost five times higher osteoblast cell count and almost 3.5 times higher blood vessel count when compared to the control subgroup. Although the difference in osteoclast counts between the subgroups was not as distinct as the difference in osteoblast counts, the difference was also found to be statistically significant. The increased blood vessel, osteoblast, and osteoclast counts showed the significant increase in alveolar bone turnover rate, demonstrating accelerated tooth movement.

The periodontal ligament is a thin layer of dense connective tissue between the alveolar bone and cementum[24]. Tension and compression zones occur in the periodontal ligament layer of the tooth under orthodontic forces. The tension zone promotes new bone formation, while the compression zone activates osteoclast cells and eventually promotes bone resorption[25]. A regular collagen fibre permutation and density are important for ideal balance between bone anabolism and catabolism. No significant difference in collagen fibre permutation was detected between any of the subgroups in the current study at the end of day 21 postoperative.

It has been shown that corticotomy via piezo-incisions increases the release of BMP-2 and this protein plays an important role in the remodelling of alveolar bone[26,27]. Corticotomy can successfully accelerate orthodontic tooth movement by reducing cortical bone resistance and increasing particular cytokines for bone remodelling. However, it should be kept in mind that the application of these cortical incisions is considered a relatively invasive procedure and carries the risk of postoperative alveolar bone resorption and gingival recession[28]. In order to avoid such complications, Kim et al. used a corticision technique that does not require full flap reflection to access the cortical bone[10,11]. Corticisions are minimally invasive bone cuts that are performed with a reinforced scalpel and a thin chisel to separate the interproximal cortices transmucosally. Peron et al. compared the effect of corticotomy and corticisions in rats[28]. They found that corticotomy increased bone resorption in the early stages of tooth movement, while corticisions could not provide this resorption. When the aim is to provide early stage acceleration of orthodontic tooth movement, corticotomies could be considered more favourable than corticisions.

Baloul et al. reported that tooth movement was significantly enhanced only during the first week following selective alveolar decortication[29]. Furthermore, Kim et al. indicated that after the application of corticision, most tooth movement occurs during the first 2 weeks[10]. PRF membrane can release growth factors for 28 days and this may help provide longer acceleration of tooth movement during orthodontic treatment. The application of PRF membrane is safe, periodically repeatable, and well-tolerated by the patient.

Heavy orthodontic forces (>50 g) can reduce the total treatment time; however, the level of force should be as light as possible to minimize complications such as hyalinization, irreversible root resorption, and cessation of tooth movement[30–32]. Methods of accelerated tooth movement without increasing the orthodontic force can be divided into pharmacological and physical. Pharmacological methods are not generally preferred due to side effects of systemic usage. Therefore, new studies on physical methods are being published[26,28]. The day 21 postoperative results for small orthodontic forces (20 g) were demonstrated in the present study. Well-conducted, prospective clinical and/or experimental trials evaluating the effect on bone turnover of the application of different platelet concentrates at different time points are needed as further investigations.

PRF membrane can significantly increase bone turnover and this may help accelerate tooth movement during orthodontic treatment. The application of PRF membrane is an easily acceptable and repeatable method for use during the orthodontic treatment of adult patients. According to the results of this study, it can be concluded that both methods presented promising results, but that the combined application of PRF membrane and piezo-incisions proved to be more effective.

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Competing interests

None declared.

Ethical approval

This study was approved by the Institutional Review Board and Animal Ethics Committee of Baskent University (project number D-DA13/05).

Patient consent

Not required.

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Address:
Sıdıka Sinem Akdeniz
Department of Oral and Maxillofacial Surgery
Faculty of Dentistry
Baskent University
1 Cadde 11
Sokak no: 26 Bahçelievler
06490
Çankaya
Ankara
Turkey
Tel.: +90 3122151336-200
E-mail: sdksoydan@yahoo.com