

Effect of Advanced Platelet-Rich Fibrin Injection on Transforming Growth Factor- β 1 Level during Orthodontic Tooth Movement in Rabbits

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Abstract

Accelerated orthodontic tooth movement (OTM) has been widely desired because of its potential advantages, which include reduced treatment duration, enhanced posttreatment stability and reduction of adverse effects commonly associated with extended treatment periods. Recently, platelet-rich fibrin (PRF) and advanced platelet-rich fibrin (A-PRF) had potential functions in accelerating OTM and shortening treatment duration. This study was designed to examine the potency of intrasulcular injection of PRF and A-PRF in inducing transforming growth factor- β 1 (TGF- β 1) during OTM in rabbits. The study examined 45 rabbits (*Oryctolagus cuniculus*) that were randomly divided into 3 groups, each consisting of 15 animals: The untreated group (control), intrasulcular PRF injection group and A-PRF injection group. Each group was randomly divided into 5 subgroups according to the day of observation (days 0, 3, 7, 14 and 21). TGF- β 1 levels during OTM were determined by enzyme-linked immunosorbent assay (ELISA). Data gathered were analyzed using the 2-way analysis of variance and post hoc Tukey's honest significant difference ($p < 0.05$). The TGF- β 1 levels were significantly different ($p < 0.05$) on the compression side. The TGF- β 1 level in the A-PRF group was significantly lower than those in the PRF and control groups on days 7, 14 and 21 ($p < 0.05$). Decreasing TGF- β 1 levels can affect the balance of osteoclastogenesis, thereby altering osteoclast production and function. PRF and A-PRF can help create a favorable environment for bone regrowth, which can help preserve bone health and accelerate OTM.

Keywords: A-PRF, Orthodontic tooth movement, PRF, TGF- β 1

Introduction

The duration of orthodontic treatment, which is a significant issue for many patients, typically exceeds 3 years [1]. Prolonged treatment duration is linked to increased vulnerability to iatrogenic, which is associated with orthodontic appliances. This event involves root resorption, white patches, carious lesions and gingival irritation [2]. Reducing the treatment duration is advantageous for both orthodontists and patients. Thus, any treatments, techniques, or appliances that aim to reduce treatment duration are acceptable as long as they are supported by sufficient evidence demonstrating their efficacy and safety [1]. Surgical and nonsurgical methods have been proposed as potentially effective strategies for reducing treatment duration. However, procedures involving surgery, such as piezocision, micro-osteoperforation and corticotomy, are invasive and require complex methodologies [3]. Regarding the acceleration of tooth movement, nonsurgical techniques yield inconsistent outcomes. Moreover, biological substances such as prostaglandin and parathyroid hormone can cause irreparable systemic problems [4].

OTM refers to tooth movements in patients with orthodontic problems. It is caused by remodeling of the alveolar bone and periodontal ligament because of orthodontic force generated by orthodontic tools such as archwires and springs. Thus, platelet-rich concentrates may have a future in orthodontic practice [5]. Platelet-rich plasma refers to autologous platelet concentrations in a small amount of plasma, representing the initial generation of this technique. PRF, a 2nd-generation treatment, has the benefits of simpler preparation and longer lasting effects [6]. In the context of bone cell prevascularization, the development of autologous PRF, which may be created via a minimally invasive process, may meet the need for therapeutically usable cell-based tissue engineering methodologies. As a source of proteins essential for bone regeneration, such as TGF- β 1, insulin-like growth factor and platelet-derived growth

factor, PRF exhibits exceptional biological capability [7]. A-PRF is a modified form of PRF that is created by decreasing the centrifugation speed and increasing the centrifugation duration for the original fibrin. This modification increases platelet concentrations by reducing the number of cells that settle at the bottom of the tubes during centrifugation. Consequently, numerous proteins, including platelets, remain in the upper portion of the tubes where the clot is extracted [8,9].

The efficacy of orthodontic treatment is contingent on the remodeling of periodontal tissue and bone remodeling. Apposition and resorption comprise bone remodeling, which is highly dependent on the number of osteoclasts and osteoblasts [10]. TGF- β 1 is widely recognized as a regulator of osteoblast differentiation and proliferation. TGF- β 1 has various functions in promoting osteoblast growth, such as attracting osteoblast precursors or matrix-producing osteoblasts by chemotaxis and inhibiting osteoblast cell death [11]. TGF- β 1 is present in normal periodontal tissue, and its level increases in the periodontium during OTM. The level at the tension site was substantially higher than that at the pressure site, starting from 5 to 10 days after applying stress. Alterations in TGF- β 1 concentrations have a significant effect on the restructuring of periodontal tissue during OTM and can greatly enhance bone generation [12].

Rabbits have been used as subjects for scientific investigations because of their docile nature, lack of aggression, manageability, observability, cost-effectiveness compared to larger animals and brief life cycles encompassing pregnancy, lactation and maturation [13]. Male rabbits were selected for this study because of their immunity to estrogen fluctuations [14]. Thus, this study aimed to evaluate the effect of intrasulcular injection of PRF and A-PRF on the TGF- β 1 level during experimental OTM in rabbits, specifically on the pressure side.

Materials and methods

The research employed a laboratory experimental method with a quasi-experimental laboratory research design. The Ethics Committee of the Faculty of Dentistry and Prof. Soedomo Dental Hospital, Universitas Gadjah Mada, approved the study on August 6, 2023 (Letter no. 153/UNI/KEP/FKG-RSGM/EC/2023). The study sample consisted of 45 male rabbits (*Oryctolagus cuniculus*), aged 12 - 15 weeks, exhibiting a body mass of approximately 3000 - 3500 g. The experimental animals were allocated into 3 groups using randomization, with each group including 15 animals. The study comprised 3 groups: The group without treatment (control group), group that received intrasulcular PRF injections (PRF group), and group that received A-PRF intrasulcular injections (A-PRF group). The groups were randomly divided into 5 subgroups based on the day of observation (days 0, 3, 7, 14 and 21). Each subgroup comprised 3 rabbits. The experimental treatments to the animals were performed in accordance with the authorization granted by the Research Ethics Commission.

For anesthesia, the rabbits were administered ketamine at a dosage of 35 mg/kg body weight and xylazine at 5 mg/kg BW via intramuscular injection into the gluteal muscles. In the treatment group, 10 mL of rabbit blood was extracted using a hematocrit syringe from either the central artery or marginal vein in the rabbit's ear. To prepare for PRF, a tube containing 10 mL of rabbit blood was centrifuged at a speed of 2700 revolutions per minute (rpm) for 12 min. For A-PRF preparations, 10 mL of rabbit blood was centrifuged in a tube at a speed of 1500 rpm for 14 min. The intermediate layer within each PRF and A-PRF tube (0.6 - 0.8 mL) was isolated from the 2 layers using tweezers and sterile scissors or punch biopsies. Subsequently, the PRF and A-PRF fibrin clots were crushed for 10 min to create a membrane. PRF and A-PRF refer to the serum fluids obtained by compressing the membrane left at the bottom of the PRF and A-PRF boxes.

The rabbits were administered ketamine and xylazine intramuscularly (ketamine, 35 mg/kg BW; xylazine, 5 mg/kg BW) to induce anesthesia. An elastic separator was then inserted between the 2 incisors to facilitate the installation of brackets. A stainless steel square wire with a diameter of 0.016 in was connected to the bracket slot to position the open coil spring between the 2 brackets. The NiTi open coil spring has dimensions of 0.010"×0.030" and a length of 5.3 mm. When compressed to 2.1 mm, it exerts a force of 50 g (50 cN) for tooth movement. The force was tested using a dynamometer tension gauge (Medkraft Orthodontics, USA). The activation of open coil springs was performed for 21 days to induce OTM in rabbits. In the treatment groups, PRF and A-PRF were administered on the 1st day following the activation of the open coil spring, which serves as an indication of OTM.

After collecting samples of gingival crevicular fluid (GCF), the gingival sulcus of each rabbit was dried using a gentle stream of air. GCF samples were obtained from all groups on days 0, 3, 7, 14 and 21 alternately during relapse movement. Paper points were used for collection. The paper point was delicately placed approximately 1 mm into the mesial side of the gingival sulcus of the incisor and left in place for 60 s; the collecting process was repeated twice. Subsequently, 2 paper points were immersed in

a 1.5-mL Eppendorf tube filled with 350 μ L of physiological saline solution. Subsequently, the tube was centrifuged at 2000 rpm and 4 $^{\circ}$ C for 5 min. An ELISA was performed to evaluate the TGF- β 1 levels during OTM (**Figure 1**).

Analyzed data were obtained using a quantitative sandwich Rabbit ELISA kit specific for each protein (BT Lab, Shanghai Korain Biotech Co., Ltd., China). Each protein was quantified using its respective standard curves. The optical densities were quantified at a wavelength of 450 nm using a microplate reader (Bio-Rad Laboratories Inc., USA). The overall expression levels of TGF- β 1 were reported in picograms per milliliter (pg/mL).

The acquired data were organized into tables and analyzed statistically. The Kolmogorov-Smirnov test was conducted to determine whether the obtained data followed a normal distribution, whereas a test of homogeneity of variances was conducted to determine whether the obtained data were homogeneous. A 2-way analysis of variance test was performed to determine any disparities between the groups and interactions among groups. Subsequently, multiple-comparison Tukey's honest significant difference (HSD) test was conducted to identify the groups that had noteworthy differences.

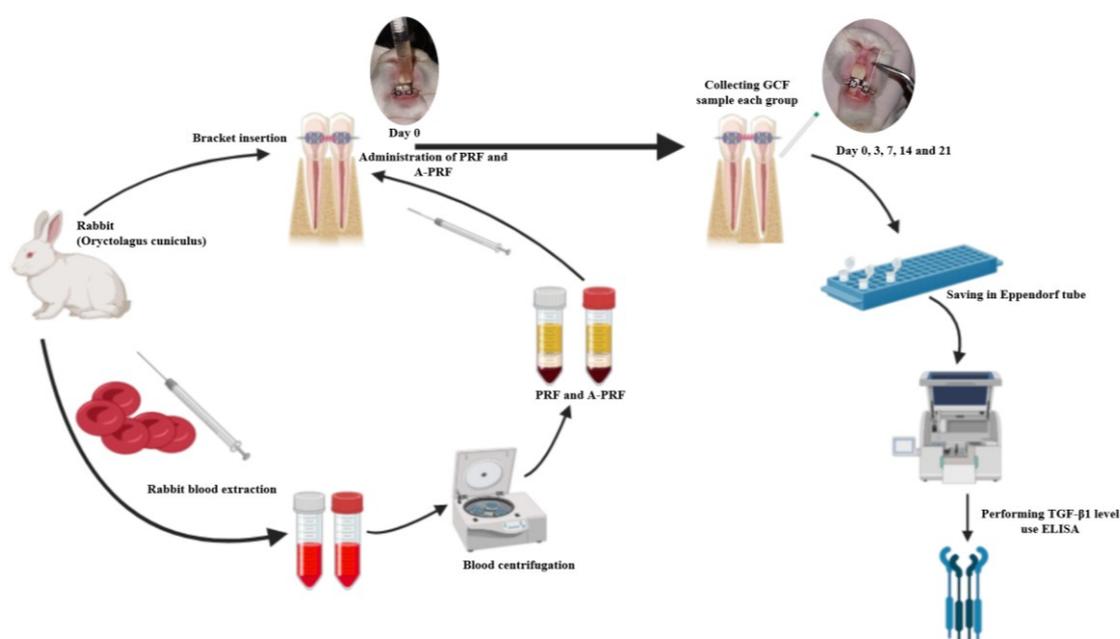


Figure 1 Schematic representation of the research methods.

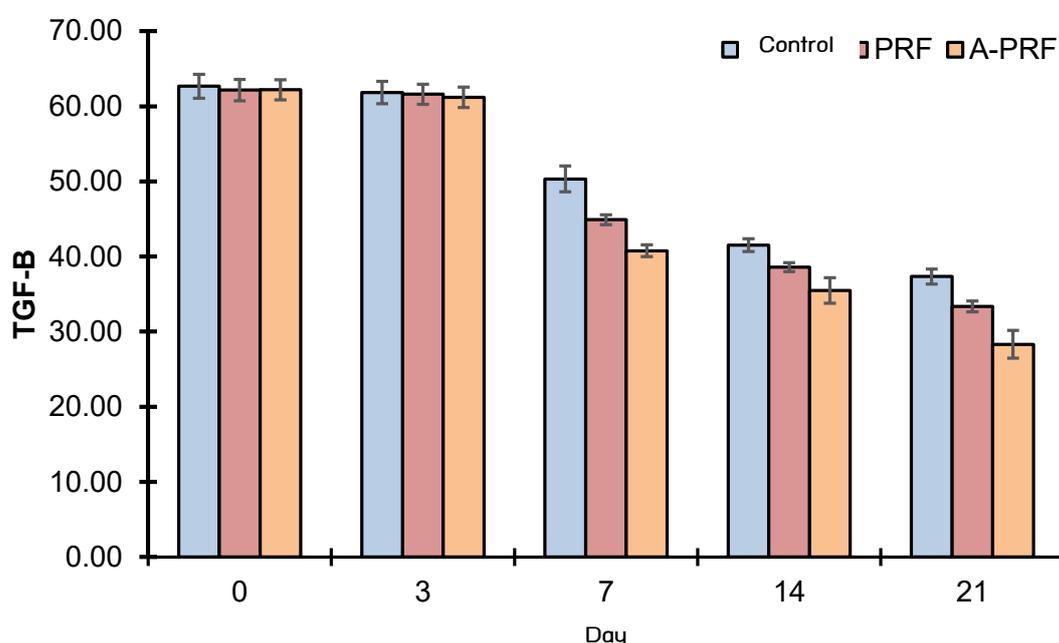
Results and discussion

The means and standard deviations of the total TGF- β 1 level of the 3 groups are summarized in **Table 1**. The Shapiro-Wilk normality tests and Lavene's test homogeneity for TGF- β 1 level confirmed our assumption of normality. In general, the numbers of TGF- β 1 in the A-PRF group was lower than that in the other groups. On day 0 after bonding orthodontic braces, a high TGF- β 1 level was detected in each group. TGF- β 1 levels were the lowest in the A-PRF group 21 days after bonding, and the changes exhibited statistical significance. TGF- β 1 level began to decrease on day 3 during OTM. Compared with the levels observed on days 0, 3, 7, 14 and 21, the mean TGF- β 1 level in the A-PRF group was lower than those in the control and PRF groups. The expression level of TGF- β 1 in the A-PRF group was significantly higher than those in the other groups on days 0, 3, 7, 14 and 21 after bonding ($p < 0.05$).

Table 1 Means and standard deviations of TGF- β 1 level (pg/mL) between the 2 groups on days 0, 3, 7, 14 and 21.

Observation time (Day)	Control group (A)	PRF group (B)	A-PRF group (C)	<i>p</i> -value	Post hoc comparison
0	62.67 \pm 1.6	62.19 \pm 1.41	62.15 \pm 1.33	0.445	NS
3	61.83 \pm 1.48	61.61 \pm 1.33	61.17 \pm 1.36	0.686	NS
7	50.33 \pm 1.71	44.91 \pm 0.66	40.76 \pm 0.78	0.000*	A < B < C
14	41.51 \pm 0.84	38.59 \pm 0.59	35.48 \pm 1.69	0.000*	A < B < C
21	37.33 \pm 0.99	33.35 \pm 0.74	28.32 \pm 1.84	0.000*	A < B < C

The values are displayed using the mean along with the standard deviation. Tested by 2-way ANOVA and post hoc Tukey's HSD test; A, untreated group (control); B, PRF injection group; C, A-PRF injection group. **p* < 0.05, significant difference between groups; NS, not significant.

**Figure 2** Comparison of TGF- β 1 levels in the 3 groups: Control, PRF and A-PRF groups from days 0 to 21 during OTM.

Our results demonstrated that A-PRF injection was effective in decreasing TGF- β 1 levels, as indicated by the significant difference in TGF- β 1 levels between the A-PRF, PRF and control groups on days 0, 3, 7, 14 and 21. TGF- β 1 functions as an immunoregulatory cytokine by controlling the proliferation, survival, differentiation and migration of immune cells. Osteoimmunology, the study of the close relationship between the immune and skeletal systems, has been supported by substantial evidence, including the indispensable function of TGF-1 in the development and maintenance of skeletal tissue. TGF-1 has 5 main functions in osteoimmunity: 1) TGF-1 promotes the differentiation of mesenchymal stem cells into chondrocytes and stimulates their proliferation, 2) TGF-1 stimulates the differentiation of osteoblast progenitor into osteoblast, 3) An increased level of TGF- β 1 stimulates the proliferation of osteoblasts while simultaneously inhibiting the expression of receptor activator of nuclear factor kappa B ligand (RANKL) in osteoblasts, 4) A low TGF- β 1 expression level stimulated the maturation of osteoclasts and 5) Hematopoietic stem cells remain in a dormant state because of TGF- β . TGF- β 1 is widely recognized as a regulator of osteoblast differentiation and proliferation. TGF- β 1 has multiple functions in promoting the growth of osteoblasts, such as attracting osteoblast precursors or matrix-producing osteoblasts by chemotaxis and inhibiting osteoblast cell death [11]. TGF-1 promotes bone formation by increasing osteoblast proliferation, differentiation and chemotactic attraction, as well as chondrocyte precursor cell extracellular matrix and proteoglycan synthesis [15,16]. Osteoclast activation was reduced, particularly when induced with high concentrations of TGF- β 1, whereas low TGF- β 1

concentrations facilitated osteoclast maturation [17]. TGF- β 1 induces osteoclast maturation in a biphasic pattern. TGF- β 1 stimulates osteoclastogenesis of various osteoclastic precursors, including hematopoietic precursors, when combined with RANKL and macrophage colony-stimulating factor (M-CSF) in a culture [18].

RANKL and OPG levels play crucial roles in the regulation of osteoclastogenesis. An increase in RANKL levels and a decrease in OPG levels will increase the RANKL:OPG ratio, which will promote osteoclast differentiation and activation, ultimately leading to increased bone resorption [19]. TGF-beta can influence the expression levels of RANKL and OPG, which are crucial elements in controlling osteoclast development and activation. A reduction in TGF- β 1 levels can affect the equilibrium between RANKL and OPG, thus influencing the production and activity of osteoclasts. Several investigations have demonstrated that a reduction in TGF- β 1 levels can increase the expression level of RANKL and reduce that of OPG. A study demonstrated that a reduction in TGF- β 1 levels within the bone can enhance the expression of RANKL and diminish the expression of OPG, resulting in the augmentation of osteoclast development and reduction in bone mass [11]. Enhanced osteoclastogenesis on the pressure side of orthodontic tooth movement leads to accelerated OTM [14].

The results showed that the TGF- β 1 level in the A-PRF group was significantly lower than those in the PRF and control groups on days 7, 14 and 21. As a constituent of the TGF-beta superfamily of cytokines, TGF-beta controls many cellular processes, including apoptosis, differentiation, migration and proliferation. The equilibrium between bone-forming cells (osteoblasts) and bone-resorbing cells (osteoclasts) is significantly influenced by TGF-beta during bone formation and remodeling [20]. TGF-beta promotes the development and activity of osteoblasts by activating both canonical and noncanonical signaling pathways. In addition, it enhances the expression of osteogenic genes such as Runx2, osterix, and collagen I in osteoblasts. In contrast, TGF-beta hinders osteoclast formation and function by regulating interactions between osteoblasts and osteoclasts through OPG/RANKL, BMPs and Wnt proteins. Consequently, it affects both stages of the bone remodeling process [21].

PRF is extensively used in the fields of regenerative medicine and dentistry. PRF has been thoroughly investigated because of its ample presence of growth factors and cytokines, together with its capacity to serve as a structural scaffold, facilitating the repair and rejuvenation of injured tissues. The development of PRF has advanced from laboratory tests conducted in a controlled environment to studies involving animals and finally to formal applications in clinical settings. PRF is extensively employed in various domains of stomatology, including implants, periodontics, surgery and orthodontics [22].

PRF is essential for reducing bone resorption on the pressure side, particularly when TGF- β 1 levels are low. PRF contains numerous growth factors and cytokines that can promote the multiplication and specialization of osteoblasts while hindering the activity of osteoclasts, which are responsible for bone tissue breakdown. When TGF-beta levels are insufficient, PRF can provide a large number of these growth factors to the surrounding area. This helps compensate for the shortage and stimulates bone development and remodeling on the strained side. Moreover, the anti-inflammatory properties of PRF can assist in regulating the bone microenvironment, which may lead to a decrease in excessive bone resorption, even when TGF- β 1 levels are not ideal. Platelet-rich growth factor can help create a favorable environment for bone regrowth, which can help preserve bone health and reduce the negative effect of bone loss [23,24].

We may assume that PRF application leads to an initial increase in cells and cytokines, which in turn promotes bone remodeling and speeds up OTM [25]. However, as time passes and PRF degrades, the levels of exogenous growth factors and cytokines decrease, potentially causing a decrease in OTM through a negative feedback mechanism involving autogenous growth factors and cytokines [26]. In contrast, a more comprehensive understanding of the precise effects and underlying processes of PRF necessitates meticulously planned investigations employing established methodologies and standardized PRF parameters.

Collectively, the findings of this preclinical investigation indicate that reducing the TGF- β 1 level on the pressure side by administering PRF and A-PRF injections can enhance the acceleration of OTM by promoting indirect osteoclastogenesis. Nevertheless, this study has several limitations, such as the absence of an A-PRF stock that must be utilized promptly after its preparation. Consequently, additional research is necessary to determine the applicability of these findings in a clinical context. Our future research will focus on creating an appropriate formulation material for clinical use in orthodontics. For instance, the freeze-dried version of A-PRF is used to ensure the long-term stability of the substance. Furthermore, the dosage of substances used in animal experiments must be adjusted when extrapolated to human subjects.

Conclusions

The TGF- β 1 level in the A-PRF group was significantly lower than that in the PRF and control groups on days 7, 14 and 21. Decreasing TGF- β 1 levels can affect the balance between RANKL and OPG, thereby altering osteoclast production and function. PRF and A-PRF can help create a favorable environment for bone regrowth, which can help preserve bone health and accelerate OTM.

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