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A New Approach for Root Surface Biomodification Using Injectable Platelet-Rich Fibrin (I-PRF)

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Literature Search F
Funds Collection G

EFG 1 **Bozan Serhat İzol**
ABDE 2 **Devrim Deniz Üner**

1 Department of Periodontology, Faculty of Dentistry, Bingöl University, Bingöl, Turkey
2 Department of Periodontology, Faculty of Dentistry, Harran University, Şanlıurfa, Turkey

Corresponding Author: Devrim Deniz Üner, e-mail: dvrmdnzn@gmail.com

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Background: This study investigated the potential effects of Injectable Platelet-Rich Fibrin (I-PRF) on root coverage of free gingival graft surgery.

Material/Method: A total of 40 patients with Miller class I or II gingival recession were included. The patients who participated in this study were randomly divided into 2 groups, including the control and experiment groups. The patients in the control group were treated only with free gingival graft (FGG). The patients in the experiment group were treated with free gingival graft and injected with I-PRF as a root surface biomodification agent (FGG+I-PRF). The patients were called back after 3 months, and the amount of exposed root surface was determined and compared to the preoperative findings.

Results: The mean initial exposed root surface was 4.7 ± 1.49 mm for the FGG+I-PRF group, 4.1 ± 1.07 mm for the FGG group, and 4.4 ± 1.31 mm for all subjects. Three months after the operation, the mean root surface coverage values of the 2 groups were 3.5 ± 1.05 and 3.9 ± 0.78 mm in the control and experiment groups, respectively.

Conclusions: The findings showed that the injection of Injectable Platelet-Rich Fibrin (I-PRF) had a positive effect on root coverage in free gingival graft surgery.

MeSH Keywords: **Gingiva • Immunologic Factors • Periodontal Cyst**

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Background

Gingival recession is defined as retraction of the gingival margin in the enamel-cement border towards the apex and subsequent exposure of the root surface [1–3]. The prevalence of gingival recession increases with old age, various periodontal diseases, and poor oral hygiene [4,5]. The incidence of gingival recession ranges from 50% to 100% among adults [6]. In gingival recession cases, the first mechanism responsible for causing apical gingival migration is loss of bone support offered by the alveolar bone crest [7]. Various etiological factors have been reported for gingival recession. The accumulation of dental plaque is the main etiological factor for the gingival recession. Other risk factors include developmental defects such as bone dehiscence, chronic trauma, frictional injury due to scratching of gingiva (tooth brushing), tooth malposition of teeth, gingival ablation, abnormal frenum attachment, aging, smoking, and iatrogenic dentistry injury [8]. There are several periodontal surgical methods that have been developed to close the exposed root surface that arises from gingival recession, such as free gingival graft (FGG) [9], coronary positioned flap, semilunar coronally positioned flap [10], subepithelial connective tissue graft (CTG) [11], pediculated soft tissue grafts [12], thrombocyte-rich Fibrin (TRF) [13], and acellular dermal matrix [14]. These operations aim to increase the amount of attached gingiva and stop gingival recession, subsequently decreasing the sensitivity of the root and improving plaque control and aesthetic appearance [15]. The free gingival graft method is a simple surgical treatment that is used to increase the amount of attached gingiva. In 1985, Miller proposed 4 classes of marginal tissue recessions based on the level of gingival margin with respect to the mucogingival junction (MGJ) and the underlying alveolar bone, as follow. Class I: Marginal tissue recession not extending to the MGJ, with no loss of interdental bone or soft tissue. Class II: Marginal recession extending to or beyond the MGJ, with no loss of interdental bone or soft tissue. Class III: Marginal tissue recession extends to or beyond the MGJ, and loss of interdental bone or soft tissue is apical to the cemento-enamel junction (CEJ) but coronal to the apical extent of the marginal tissue recession. Class IV: Marginal tissue recession extends to or beyond the MGJ, and loss of interdental bone extends to a level apical to the extent of the marginal tissue recession [16].

Platelet-Rich Fibrin (PRF) is a second-generation platelet concentrate that was introduced by Choukroun et al. in 2001. It is thought that PRF can improve tissue regeneration due to its effects on vascularization, capturing the circulating stem cells, immune control, and closure of the epithelium [17].

Among the several methods that have been developed to treat gingival recession, connective tissue graft application is accepted as the criterion standard. Researchers have

investigated the root coverage effects of various biomodification agents, including Emdogain, various root development regulators, laser treatments, growth factors, and thrombocyte-rich plasma [18]. However, several studies have found that RSB (root surface biomodification) methods negatively affected root surface coverage [19–21].

PRF has the capacity to conserve open wounds and improve healing due to its ability to attract epithelial cells and facilitate microvascularization. Thus, application of PRF (by itself or in combination with connective tissue or FGG) for root coverage has become increasingly popular for gingival recession treatments [22–27].

Injectable PRF (I-PRF) is the liquid form of PRF. I-PRF is a bioactive agent obtained by low-speed centrifugation, and it has the capacity to stimulate tissue regeneration. At high concentrations, PRF may stimulate the secretion of several growth factors and trigger fibroblast migration [28]. I-PRF is generally used in regenerative treatments, with good outcomes [29]. One of the components that make up I-PRF is fibronectin [30], which is an extracellular glycoprotein with a high molecular weight (approximately 440 kDa) [31]. Application of fibronectin to root surfaces improves cellular proliferation from the periodontal ligament towards the supracrestal parts [32]. Fibronectin is used as an RSB agent in periodontal surgery [33].

In our study, we applied I-PRF (together with FGG) to the exposed root surfaces of patients with Miller class I or II gingival recession.

Material and Methods

Subject selection

We included 40 subjects (22 female and 18 male patients) in the study. The subjects were chosen from among patients with gingival recession complaints who applied to the Periodontology Division of the Faculty of Dentistry at a public university in Turkey. The ages of the subjects ranged from 24 to 36 years. The exclusion criteria were: the presence of any systemic disease affecting the wound healing (e.g., uncontrolled diabetes mellitus), smoking, pregnancy, and poor oral hygiene. The selected patients were informed about oral hygiene management. Scaling and root planing (SRP) operations were performed to create healthy gingival tissues as needed before the operation. After SRP, patients were kept under observation for approximately 2 months to obtain gingival health necessary for periodontal surgery.

All subjects had Miller class I or II gingival recession. There was also no interdental bone or soft tissue degeneration, and

Figure 1. (A) I-PRF obtained. (B) Application of I-PRF to the root surface through with the injector.

the gingival pocket depth was not deeper than 3 mm. All subjects signed a written informed consent form to participate in this study.

Sample

The subjects were randomly divided into 2 groups of equal sizes. The patients in the control group were treated only with FG. The patients in the experiment group were treated with FG, and I-PRF was applied to the exposed root surfaces.

Surgical procedure

Aseptic and antiseptic techniques were used for every subject. The surgical site was locally anesthetized using 20 mg/mL of lidocaine HCl and 0.0125 mg/mL of epinephrine HCl (Jetokain®, Adeka, Turkey). The exposed root surfaces were smoothed with curettes (Hu Friedy®, USA). No material was applied to the exposed root surfaces of the patients in the control group. In the experiment group, 20 cc of blood was drawn from each subject, and the samples were centrifuged (700 rpm for 3 min) to obtain I-PRF. I-PRF was applied to the root surfaces for 5 min (Figure 1A, 1B). The exposed root surface was prepared with a no. 15 sharp curette. The enamel-cement border was horizontally incised in the mesial and distal directions, which was followed by vertical incisions to form trapezoidal flaps. The partial-thickness flap was elevated and removed with surgical scissors. It was ensured that the periosteum was intact and at least 3–5 mm away from the apex of the exposed root surface. The size of the recipient area was determined using an aluminum foil. The donor area was chosen as the area between the first and second premolars that did not contain rugae. The donor area was incised 2 mm away from the gingival border. The graft

Figure 2. Sutured the free gingival graft to recipient area with ePTFE sutures.

Figure 3. The appearance of the gingiva after the sutures are removed 10 days after the operation.

Figure 4. Measurement of new-formed gingival margin by using a periodontal probe 3 months after the operation for root coverage values.

Table 1. The amount of exposed root surface 3 months after the operation and percentage of root surface closure.

Patient no.	FGG			FGG+I-PRF		
	Basal (mm)	3 months after operation (mm)	Root coverage (%)	Basal (mm)	3 months after operation (mm)	Root coverage (%)
1	4	1	75.0	3	0	100.0
2	3	0	100.0	4	0	100.0
3	4	0	100.0	5	0	100.0
4	4	0	100.0	4	0	100.0
5	3	0	100.0	6	1	83.33
6	4	1	75.0	4	0	100.0
7	5	2	60.0	6	2	66.66
8	5	2	60.0	2	0	100.0
9	2	0	100.0	3	0	100.0
10	6	1	83.33	4	0	100.0
11	3	1	66.66	4	0	100.0
12	5	0	100.0	5	1	80.0
13	6	0	100.0	3	0	100.0
14	4	0	100.0	5	1	80.0
15	3	0	100.0	6	2	66.66
16	4	0	100.0	4	0	100.0
17	5	2	60.0	7	3	57.14
18	4	0	100.0	6	2	66.66
19	5	1	80.0	5	0	100.0
20	3	1	66.66	4	0	100.0
Mean			83.16±18.48			88.35±15.64%

was at least 1 mm wider than the size of the recipient area and at least 1.5–2-mm-thick. The graft was placed on a gauze (impregnated with isotonic solution), and the fatty tissue was removed. Polytetrafluoroethylene (ePTFE) suture 5-0 was used for suturing (Figure 2). The patients were discharged with recommendations. The sutures were removed 10 days after the operation (Figure 3).

Clinical assessment

The degree of gingival recession was measured using a William's probe and photographed. Ten days after the operation, the sutures and the post-operative changes were photographed. Three months after the operation, the subjects were called back, and the degree of gingival recession was measured using a William's probe and photographed (Figure 4). The pre- and

post-operative root exposures were determined using the Mac-OS Photos application.

Statistical analysis

Data were analyzed using SPSS v.21 software. The Shapiro-Wilk test was used to assess normal distribution of the data. Descriptive statistics were used to determine the frequency, percentage, mean, and standard deviation values. The differences between the male and female groups were analyzed by independent-samples *t* test. The differences in the root surface coverage values between the groups was determined by one-sample *t* test. $P<0.05$ was accepted as the level of statistical significance.

Results

We included 40 patients (22 female and 18 male patients) in the study. The mean age of the patients was 28.55 ± 6.03 (19–39). The mean root coverage value of all subjects was $87.76 \pm 17.10\%$. The mean root coverage was $83.16 \pm 18.48\%$ for the control group and $88.35 \pm 15.64\%$ for the experiment group. Complete root coverage was observed in 55% of the patients in the I-PRF group and 50% of the patients in the control group (Table 1).

Before the operation, the mean total exposure of root surfaces for the female and male participants were measured as 3.68 ± 0.94 mm and 3.72 ± 0.89 mm, respectively. Additionally, according to the results of the independent-samples *t* test, there was no statistically significant difference between the male and female patients before the operation regarding their exposed root surface values ($P=0.891$). According to the results of the examinations carried out 3 months after the operation, the mean total root surface closure in the female patients was determined to be 4.27 ± 1.42 mm, while in the male patients this value was 4.55 ± 1.19 mm. Additionally, according to the results of the statistical analysis, there was no statistically significant difference between the female and male patients in terms of their mean root surface coverage values.

The mean initial exposed root surface value was 4.7 ± 1.49 mm for the FGG+I-PRF group, 4.1 ± 1.07 mm for the FGG group, and 4.4 ± 1.31 mm overall for all subjects. Three months after the operation, the root surface coverage values of the 2 groups were determined as 3.5 ± 1.05 and 3.9 ± 0.78 mm in the control and experiment groups, respectively. The one-sample *t* test was performed to evaluate the effects of I-PRF on root surface coverage on FGG operations after the operation. The mean value of the control group was used in the test (3.5 mm). As a result of the one-simple *t* test, the *p*-value was determined as 0.022.

A paired-samples *t* test was performed to compare the means of GRH (gingival recession height) at the baseline and 3 months after the operation. In the FGG group, there was a significant difference between the GRH at baseline and the GRH at 3 months after the surgery. The mean GRH in the FGG group at baseline and the mean GRH at 3 months after the operation were 4.1 and 0.6 mm, respectively (Figure 5). Considering the results of the analysis, we determined that we would obtain the mean values of 3.5 and 3.9 mm for gingival height in the FGG and FGG I-PRF procedures, respectively.

According to the results of this study, it was concluded that the use of I-PRF as an RSB agent in FGG operations increases the amount of new gingival tissue.

Figure 5. Exposed root surface value at baseline and at 3 months after surgery.

Discussion

Various methods and materials are used to treat gingival recession, including free gingival graft, laterally positioned flap, pediculated soft tissue grafts, enamel matrix derivatives, and platelet-rich fibrin [23,34–38]. The free gingival graft method is the most commonly used method for treatment of gingival recession [39–41]. Reported root surface coverage range from 43% to 100% [42–44]. In our study, the mean root surface coverage was 87.76% (88.33% for the control group and 91.66% for the experiment group). Even though the root surface coverage was higher in the experiment group (FGG+I-PRF), the difference between the 2 groups was not statistically significant.

Several researchers reported applying various biomodification agents for root surface coverage; however, the effects of these agents on root surface coverage are still unclear [45]. Some researchers argued that application of chemical agents to the smoothened root surface improves the outcome [46]. These agents remove the smear layer, bring out the collagen fibers to the surface of the dentin matrix, and remove the cytopathic material that inhibits gingival fibroblast proliferation [47]. However, many studies revealed that RSB agents affect treatment outcomes negatively [19,21,45]. Sture Nyman, Lindhe, and Karring investigated the effects of citric acid on monkeys' teeth. They found that application of citric acid (which is used to demineralize the root surface) did not contribute to the outcome of the treatment [48]. There are various methods and materials that are used for root surface biomodification, such as chemical agents (e.g., citric acid, EDTA, tetracycline, fibronectin), enamel matrix proteins, platelet-rich plasma, recombinant human growth factors, hyaluronic acid, and lasers [33]. Bittencourt et al. used EDTA for semilunar repositioned flap operations. The root coverage was found to be 70.2% in the experiment (EDTA) group and 90.1% in the control (no EDTA) group [19]. Dilsiz et al. (2010) used the Nd: Yag laser method

for root surface biomodification. They found that the root surface coverage was 33% in the experiment group (laser application) and 77% in the control group [21]. Another study that administered citric acid on the root surface for biomodification reported that the coverage was 74% in the experiment (citric acid) group and 66% in the control group, but the difference between the 2 groups was found to be insignificant [49].

In FGG procedures immobilization of graft is essential for the success of the operation [50]. The development of autologous injectable platelet-rich fibrin (PRF), which can be generated from peripheral blood in a minimally invasive procedure, fulfils several requirements for clinically applicable cell-based tissue-engineering strategies. Using PRF containing blood plasma, platelets, and leukocytes for tissue-engineering purposes results in the initiation of wound healing processes and improvement of the process of angiogenesis [51]. I-PRF contains fibronectin [30], which is an adhesive glycoprotein [52]. Therefore, we speculated that it might have an adhesive effect on graft immobilization.

Our literature search failed to find any studies that applied I-PRF as an RSB agent. We decided to use I-PRF as an RSB agent given that it contains fibronectin, it is known to improve the healing process, and it has an adhesive effect.

Several studies have been conducted to improve the percentages of complete coverage with RSB [9,19,21]. Deepalakshmi et al. (2006) used the free gingival graft method and observed that 4 out of 10 subjects had complete root coverage [9]. Dilsiz et al. (2010) used Nd-YAG laser for RSB with CTG treatment. After the treatment, complete root coverage was observed in 18% of the subjects in the Nd-YAG group and in 65% in the control group [21]. Bittencourt et al. used EDTA as an RSB agent

in combination with the semilunar coronally repositioned flap method. After the treatment, complete root coverage was observed in 40% of the subjects in the EDTA group and in 66.7% of the patients in the control group [19].

In the literature, many studies have evaluated the decrease of the denuded root surface height after FGG operations [32,53–55]. Cafesse et al. reported that the use of CA as a root surface modification agent in FGG operations led a reduction in recession height (2.79 ± 0.79 mm for the FGG group and 2.56 ± 0.73 mm for the FGG+CA group) [53]. In another study, Laney et al. demonstrated that the mean distance of GRH decreased from 2.136 mm to 1.301 [54]. Ersahan et al. performed FGG operations to treat recessions and reported that the recession depth was reduced from 4.42 ± 0.64 mm at baseline to 1.07 ± 0.26 mm at 6 months after surgery [55]. In the present study, a significant reduction was observed in recession height. Although recession decreased in both groups, better coverage was seen in the FGG+I-PRF group. In the FGG group, the gingival recession height before the operation was 4.1 mm and it was 0.6 mm after the operation. In the FGG+I-PRF group, 3 months after the operation, the gingival recession height, which was initially 4.7 mm, was reduced to a mean value of 0.65 mm.

Conclusions

We conclude that application of I-PRF to the root surface may have a positive effect on closure of the root surface in the context of free gingival graft operations. Further studies with larger samples and other mucogingival surgery methods, such as connective tissue grafts, should be done to support the valuable insights of our study.

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