ORIGINAL RESEARCH

# Evaluation of the Effect of Injectable Platelet-rich Fibrin on Palatal Wound Healing: A Two-arm Randomized Controlled Clinical Trial

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## Abstract

**Aim:** This study aimed to evaluate the effect of injectable platelet-rich fibrin (i-PRF) as a potential catalyst for the acceleration of palatal wound healing after subepithelial connective tissue graft (SCTG) harvesting.

**Materials and methods:** Referred patients to the Department of Periodontology with the complication of the gingival recession were examined. Thirty participants were chosen for root coverage surgeries with SCTGs, and randomly distributed into two groups; the study group ($n = 15$) with i-PRF was applied, and the control group ($n = 15$) without i-PRF. The wound healing index was evaluated on the 7th, 14th, and 30th days of the treatment. Palatal tissue thickness was measured before the treatment and at the 1st, 2nd, and 3rd months after the treatment.

**Results:** The study group improved significantly the early healing over the control group on days 7 and 14 ($p < 0.01$), whereas no difference in the first month ($p > 0.05$) between the groups. Moreover, the study group showed higher tissue thickness mean in the first and second month ($p < 0.01$), but in the third month, there were no significant differences ($p > 0.05$) between both groups.

**Conclusion:** The i-PRF has favorable effects on the healing process by enhancing wound healing and increasing the tissue thickness in the palate after SCTG harvesting.

**Clinical significance:** For clinicians, it is important to know that we can use biological materials to accelerate healing in general, such as i-PRF. In this study, we used it in the palate, which may accelerate the healing so that we can repeatedly use the same area of the patient's palate for more than one occasion faster.


**Keywords:** Injectable platelet-rich fibrin, Palatal tissue healing, Periodontal surgery, Subepithelial connective tissue graft, Tissue thickness.

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## Introduction

Gingival recession (GR) is defined by dispositioned of the gingival margin apically to the cementoenamel junction, which causes many problems like the possibility of tooth loss, hypersensitivity, and poor esthetics.[1] The prevalence and severity of GR increase with age where the percentage of people affected ranges from 30% to 100% depending on the population surveyed.[2] Several surgical techniques have been reported to treat GRs such as lateral pedicle flap, free gingival graft (FGG), coronally advanced flap, semilunar coronally repositioned flap, and subepithelial connective tissue graft (SCTG) with a combination of grafting techniques and flap designs.[3] The SCTG achieves the most predictable root coverage according to ample evidence.[4] The palate is usually used as a donor site when considering autogenous soft tissue grafting, especially between the mesial to the mid-point of the maxillary first molar and the distal of the canine to avoid damaging the greater palatine artery and nerve.[5] So another surgical site extends the complexity of the procedure and increases complications happening.[4] Soileau and Brannon reported that the desired thickness of the graft for root coverage should be about 1.5–2 mm for a typical result, and the reharvesting of tissue from the same donor site performed earlier than 9 weeks might result in poorer graft quality.[5] Platelet-rich fibrin (PRF) is a second-version platelet preparation that contains various growths factors that are believed to contribute to periodontal regeneration including the platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), transforming growth factor (TGF), epidermal growth factor (EGF), fibroblast growth factor, and bone morphogenetic protein.[6,7] Injectable platelet-rich fibrin (i-PRF) is a liquid form of PRF, it is gained by low-speed centrifugation (700 rpm for 3 min).[8] The low-speed concept may directly improve tissue regeneration by increasing fibroblast migration, proliferation, and collagen messenger ribonucleic acid (mRNA) levels.[9] The i-PRF has many advantages, as it increases the total growth factors release, collagen-1 synthesis, and osteoblasts migration.[10] The i-PRF has more regenerative cells with higher concentrations of growth factors than PRF.[11] A lot of studies confirm the ability of i-PRF on tissue regeneration and approve the feasibility of i-PRF application as a promising regenerative adjunct to dental procedures.[12] Among the favorable

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effects of i-PRF are reducing the bacterial count like Karde et al.'s research,[13] where they compared the antimicrobial property of i-PRF with other platelet concentrates, such as PRF and palate-rich plasma obtained from chronic generalized marginal gingivitis patients. They found that i-PRF has maximum antimicrobial efficacy compared with other platelet concentrates, therefore, demonstrating a superior regenerative potential increasing the amount of growth factors inside the wound, helping with wound healing, and accelerating orthodontic tooth movement like Karakasali and Erdur study,[14] where they assessed the efficiency of i-PRF injection in the retraction rate of the maxillary incisor. They concluded that the movements of incisors were significantly greater in the i-PRF group compared with the control group at all-time intervals as well as periodontal regeneration like Aydinyurt et al.'s study,[15] where they inspected the efficacy of i-PRF in rats with experimental periodontitis. They claimed positive effects of sub-gingival i-PRF injection on periodontitis by decreasing bone loss and regulating the inflammatory process, bone regeneration like Kyyak et al.'s study,[16] where they assessed the effect of an allogenic bone substitute material and a xenogeneic bone substitute material with and without i-PRF that was assessed on cell characteristics of human osteoblasts. They found that the human osteoblast proliferation, attachment, viability, and expression of differentiation and proliferation markers significantly increased in the i-PRF-added bone substitute material (BSM) compared to BSM without i-PRF, cartilage regeneration like Albilia et al.'s study.[17] They assessed the effect of i-PRF in patients suffering from temporomandibular joint dysfunction and pain. They found after 8 weeks, and at 3, 6, and 12-month follow-ups a significant decline in pain scores for responders to intra-articular injections of liquid PRF due to possible remodeling of damaged cartilage surfaces and pulp regeneration.[12] Sometimes, it is a mandatory procedure to repeat the use of the same area of the patient's palate on more than one occasion, such as for a patient who has multiple recessions.[5] So, we have to find a method to accelerate the healing in the palate. Therefore, depending on the properties of i-PRF that may accelerate tissue regeneration[18] as it is rich in platelets, leukocytes, and growth factors, and out of that PRF was used to stimulate palate healing after SCTG harvesting with promising results,[19] it was decided to conduct this study. Moreover, there are no randomized clinical trials (RCTs) on the effect of i-PRF on palatal wound healing. So, the current study aimed to evaluate the role of i-PRF on palate wound healing and tissue thickness after SCTG harvesting.

## Materials and Methods

### Study Design, Settings, and Ethical Approval

This parallel randomized double-blinded clinical trial has utilized a superiority design with a 1:1 allocation ratio to compare two techniques in the management of the donor site in the palate after SCTG harvesting with i-PRF and without it during the therapy of deep (≥3 mm) recessions on the anterior teeth. This study was conducted from November 2020 and September 2021 at the Department of Periodontology. The study protocol, questionnaires, and informed consent are in full accordance with the ethical guidelines of the Declaration of Helsinki. The research project was ethically approved by the Local Research Ethics Committee of the Faculty of Dentistry (UDDS-24082020/SRC-2794). The project was self-funded and it was registered at the ISRCTN registry under ID number: ISRCTN46963726. This RCT has been written according to the new CONSORT statement.

### Recruitment and Eligibility Criteria

Sixty patients aged between 19 and 40 years were referred to the Department of Periodontology during the study period because of the presence of GR in the upper and lower anterior teeth. The patients were investigated by the principal researcher. The principal investigator searched for healthy patients who have GR with class I or II according to Miller's classification (≥3 mm in depth) at the buccal gingiva of upper or lower anterior teeth. A preoperative clinical examination was done to assess the probing depth, GR classification, and palate tissue thickness (where 2.5 mm thickness or more was considered to be sufficient) to determine the included cases. Those who met this condition were 45 patients. Fifteen patients were excluded due to the presence of systemic diseases that compromised their general immune status. Moreover, smoker patients, those who were contraindications for surgery, or patients who have undergone connective tissue graft harvesting previously were also excluded. Therefore, 30 patients with one recession in need of SCTG in the upper and lower teeth were included in the current research. All included patients, who accepted to participate in this study, signed an informed consent sheet after explaining all the details about the trial and the therapeutic part of it.

### Sample Size Calculation

The sample size was calculated using G* Power 3.1 (Heinrich-Heine-Universität, Düsseldorf, Germany). The sample size was estimated depending on a minimum clinically significant difference of 1 mm in tissue thickness values. A total sample size of 28 patients (14 in each group) was found to be minimally sufficient for a level of significance of 0.05, and a power of 80%. The size was raised to 15 in each group to avoid any withdrawals.

### Randomization

Randomization was performed by allocating each patient to one of the two techniques: A—SCTG and i-PRF on the donor site and B—SCTG with nothing on the donor site. The sequence of allocation was performed using a computer random generator (allocation ratio of 1:1). The sequence of allocation was concealed in opaque sealed envelopes, which were identified by the initials of the patient's name. Each patient's envelope was opened immediately before surgery.

### Blinding

The presented study was double-blinded; as the current study was an interventional study, the treating clinician could not be blinded regarding the technique used during procedures. However, the involved patients were completely blinded. Moreover, the outcome assessor was also unaware of the patient's allocation during data analysis.

### Clinical Procedure

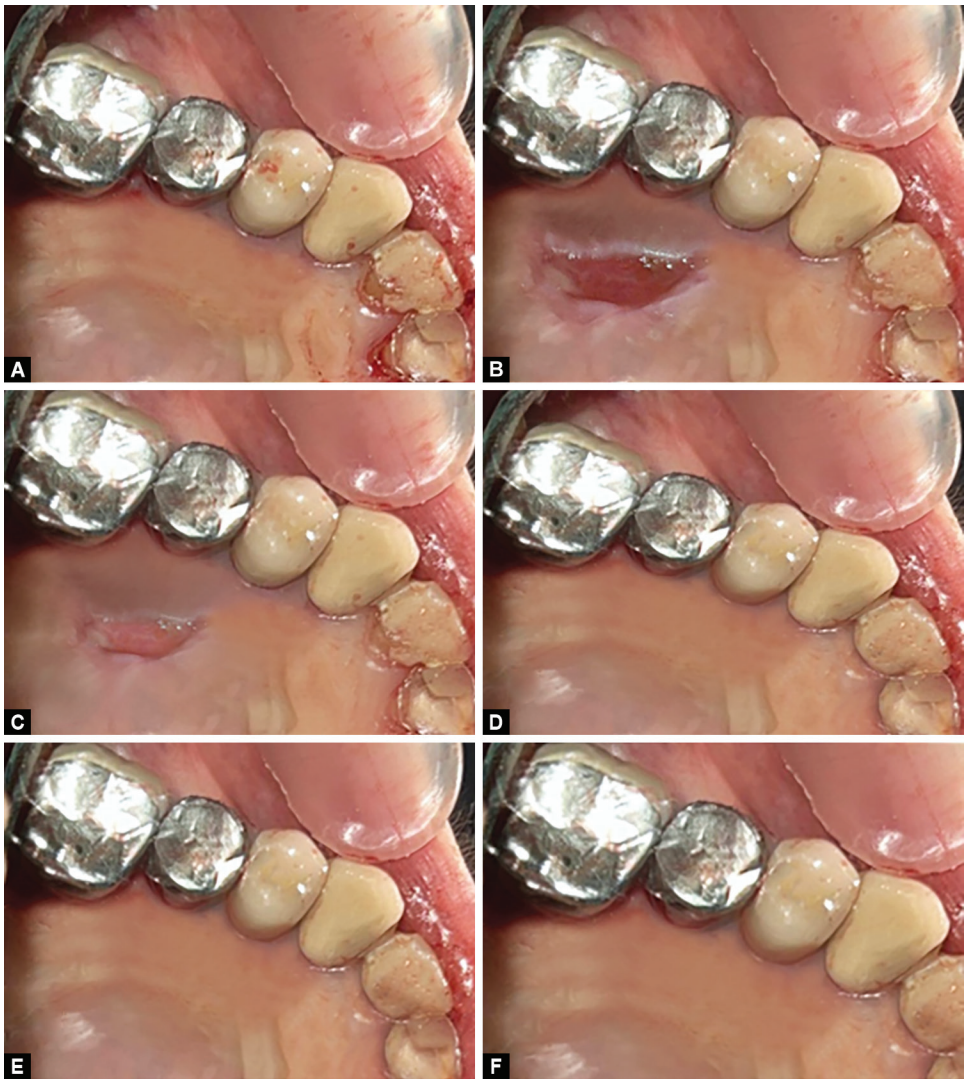
Medical and dental histories of all patients were taken.

### Presurgical Phase

A comprehensive clinical examination was performed and the patient was prepared for the surgical procedure by scaling and root debridement. All of them received detailed instructions for oral hygiene control.

### Surgical Phase

After recipient site preparation as described by Zucchelli.[20] A SCTG was harvested from palate mucosa after block anesthesia of the




**Figs 1A to F:** Healing in study group during following time periods. (A) Before surgery; (B) After 7 days; (C) After 14 days; (D) After 1 month; (E) After 2 months; (F) After 3 months

greater palatine nerve (Lidocaine HCL 2% + Epinephrine [1: 80,000] Huons Lidocaine HCL, Seoul, Korea) by Single Incision Technique.[4,21]

One horizontal incision initiating from the distal of the first molar to the lateral incisor, 2 mm from the gingival margin. The standardized graft size was 15 mm × 8 mm and 1.5–2 mm in thickness. By four internal incisions onto the bone level, the SCTG was demarcated and harvested with periosteal elevator from the bone surface including the periosteum. Interrupted suturing was performed for primary wound closure by nylon (4–0) (Jinhuan Medical Products Co. Ltd., Shanghai, China).

After suturing, in the study group, 30 mL of venous blood was drawn from each patient, and the samples were centrifuged at 700 rpm for 3 minutes in plastic tubes by centrifuge (Laboratory centrifuge EBA 200 series Andreas-Hettich-gmbh & Co.KG, 78532 Tuttlingen, Germany), then it was injected into the donor site and surrounding areas. Meanwhile, in the control group, no materials were added after suturing.

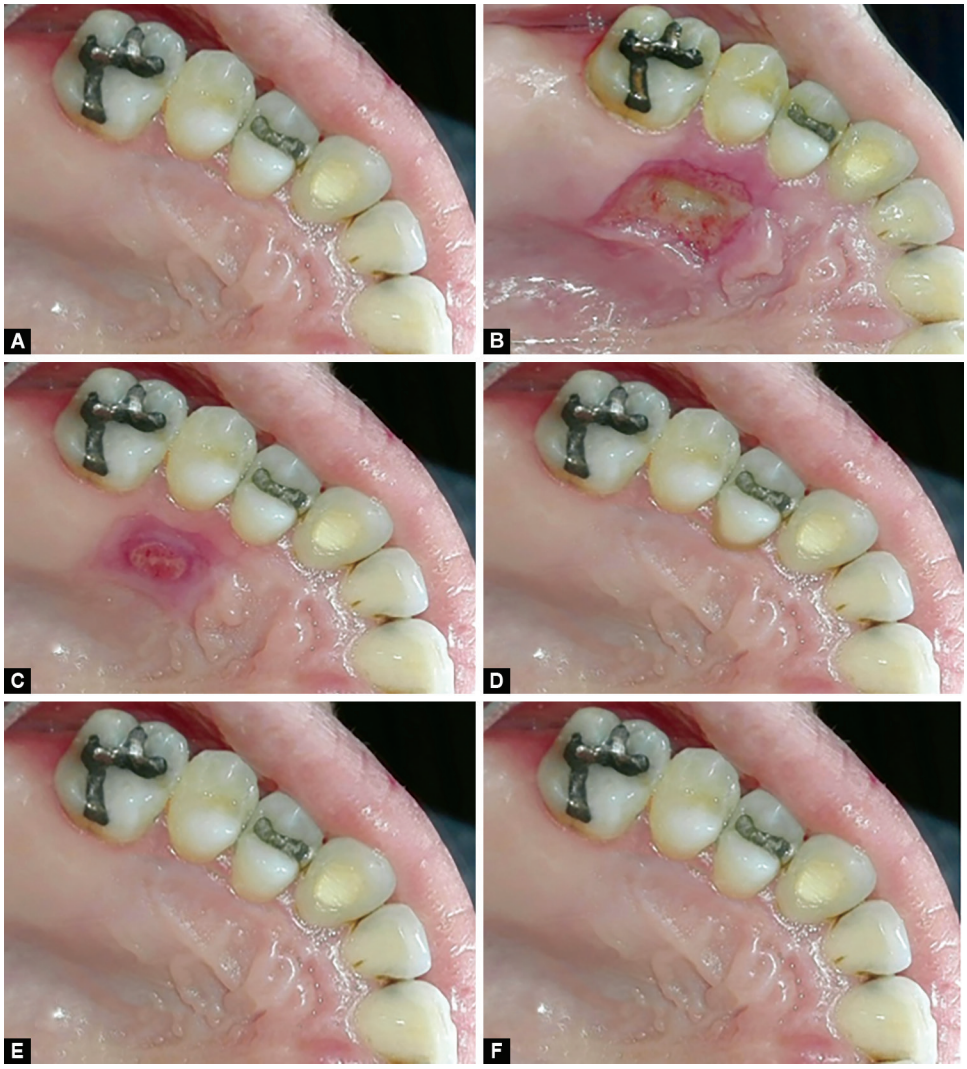
## Postsurgical Instructions and Follow-up

Amoxicillin and clavulanic acid (1000 mg, tab, two times a day for 5 days), paracetamol (500 mg, tab when needed), and 0.2% chlorhexidine rinses were prescribed. Patients were recommended to report any problems or complications after surgery and were recalled on the 7th, 14th, 30th, 60th, and 90th days of the procedure (Figs 1 and 2). The sutures were removed after 7 days.

## Outcomes Measures

Early wound healing index (EHI): It is an index that evaluates the quality of healing in donor site by evaluating the flap closure and fibrin formation in the interproximal area as a 5-level score index ranging from 1 to 5.[4,22] It was evaluated on the 7th, 14th, and 30th day.

Palatal tissue thickness: Before surgery, palatal tissue thickness was measured through three established points (4 and 7 mm from the gingival margin) from the operated region by a hard vacuum plate with three corresponding holes. It was done to regulate the points to be measured. The plate was placed, and with a probe, the points were made. Then, the plate was removed and values were taken by using a #25 spreader to reach the palatine bone plate and an electronic digital caliper with a precision of 0.01 mm (Ruifeng Machinery and Tools Co, Jiangxi, China). It was measured at baseline, first, second, and third month (Fig. 3).




**Figs 2A to F:** Healing in the control group during following time periods. (A) Before surgery; (B) After 7 days; (C) After 14 days; (D) After 1 month; (E) After 2 months; (F) After 3 months

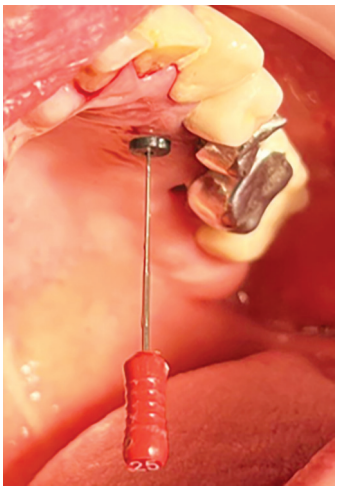


**Fig. 3:** A #25 endodontic spreader to record the measurements

The CONSORT diagram of the patients is reported in Flowchart 1.

### Statistical Analyses

Mean and standard deviation was used during the descriptive phase. Shapiro–Wilk test showed an abnormal quantitative measurement distribution. Hence, the Mann–Whitney *U*-test was used for the comparisons between groups. All analyses were conducted using SPSS for Windows software (SPSS Version 22, IBM SPSS Inc., Chicago, IL, USA).

## Results

Thirty patients (12 males and 18 females) were included in the study. The mean age of the patients was 35.6 ± 5.7 years.

### Early Wound Healing Index

On the 7th and 14th day of the procedure, the EHI scores of the i-PRF group were significantly better than the control group ($p < 0.01$ in both periods). Meanwhile, there was no significant

**Flowchart 1:** Flowchart of the patient

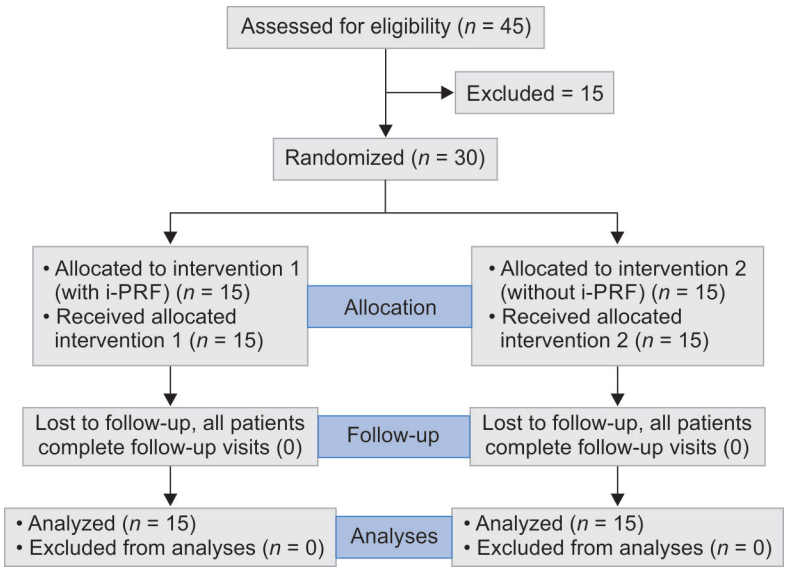



**Table 1:** Descriptive statistics of the EHI index values between both groups and the *p*-values of significance testing

| | | | *EHI score* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Follow-up period* | *Group* | *Number* | *1* | *2* | *3* | *4* | *5* | *Mean ± SD* | *U-value* | *^p-value* |
| 7th day | Without i-PRF | 15 | 0 | 2 | 8 | 3 | 2 | 3.33 ± 0.89 | 5.00 | <0.01* |
| | With i-PRF | 15 | 10 | 5 | 0 | 0 | 0 | 1.33 ± 0.48 | | |
| 14th day | Without i-PRF | 15 | 4 | 6 | 5 | 0 | 0 | 2.06 ± 0.79 | 35.00 | <0.01* |
| | With i-PRF | 15 | 14 | 1 | 0 | 0 | 0 | 1.06 ± 0.25 | | |
| 1st month | Without i-PRF | 15 | 12 | 3 | 0 | 0 | 0 | 1.20 ± 0.41 | 90.00 | 0.073 |
| | With i-PRF | 15 | 15 | 0 | 0 | 0 | 0 | 1.00 ± 0.00 | | |

^Mann–Whitney *U*-test; *Significant difference

**Table 2:** Descriptive statistics of the palatal tissue thickness values between both groups and the *p*-values of significance testing.

| *Follow-up period* | *Group* | *Number* | *Range* | *Mean ± SD* | *U-value* | *^p-value* |
|---|---|---|---|---|---|---|
| Baseline | Without i-PRF | 15 | 2.60–3.90 | 3.24 ± 0.34 | 84.00 | 0.234 |
| | With i-PRF | 15 | 2.60–4.00 | 3.12 ± 0.36 | | |
| 1st month | Without i-PRF | 15 | 1.40–2.10 | 1.80 ± 0.21 | 0.00 | <0.01* |
| | With i-PRF | 15 | 2.30–3.10 | 2.68 ± 0.23 | | |
| 2nd month | Without i-PRF | 15 | 2.00–3.00 | 2.50 ± 0.35 | 6.50 | <0.01* |
| | With i-PRF | 15 | 2.90–4.00 | 3.08 ± 0.25 | | |
| 3rd month | Without i-PRF | 15 | 2.90–3.90 | 3.16 ± 0.28 | 103.00 | 0.689 |
| | With i-PRF | 15 | 3.00–4.00 | 3.09 ± 0.25 | | |

^Mann–Whitney *U*-test; *Significant difference

difference in EHI scores between both groups in the first month ($p = 0.073$) (Table 1).

### Thickness of Palatal Tissue Scores

The thickness of tissue was detected in three points. With these three values, the mean of tissue thickness of the study area was calculated. This procedure was accomplished during the pre-surgery phase and the first, second, and third month after the operation. No statistically significant difference was found between both groups at the preoperative phase ($p = 0.234$) signifying that the palatal tissue thickness was similar in both groups. In the first and second month, the tissue thickness measurements of the i-PRF group were significantly higher than the control group ($p < 0.01$ in both periods). Meanwhile, there was no statistically significant difference between both groups in the third month ($p = 0.69$, Table 2).

## Discussion

Recently, the use of platelet concentrates has increased in periodontal regeneration.[23] A lot of research has proved that PRF has enhanced healing in many ways, such as cell proliferation,

angiogenesis, and matrix remodeling by growth factors such as IGF-1, PDGF, vascular endothelial growth factor (VEGF), and EGF and that are released from α-granules of platelets.[19,23] Moreover, PRF provides regulated release of growth factors from platelets for at least 7 and up to 28 days after its application, which corresponds to the required period of tissue remodeling.[19] Platelets play a vital role in wound healing,[24] where PRF contains seven times more platelets than blood, so it releases high quantities of proinflammatory cytokines.[25] A high concentration of growth factors in PRF boosts the manufacturing of fibroblasts and myofibroblasts and induces angiogenesis resulting accelerate early wound healing.[19] The i-PRF is based on a similar concept as PRF. It contains a higher number of regenerative cells and a higher concentration of growth factors because of the slower and shorter centrifugation spin.[26] Miron et al. reported that i-PRF had higher levels significantly of long-term secretion of growth factors [platelet-derived growth factor AA (PDGF-AA), platelet-derived growth factor AB (PDGF-AB), EGF, and IGF] after 10 days in comparison with platelet-rich plasma (PRP).[27] Moreover, i-PRF induced significantly the highest migration of fibroblasts in comparison with PRP.[21] In addition, it had the highest collagen 1 expression at both 3 and 7 days, mRNA levels of TGF-β at 7 days, and PDGF at 3 days in comparison with PRP.[27] These growth factors have significant effects on wound healing because they induce substrate adhesion and migration for neutrophils, macrophages, fibroblasts, and endothelial cells.[28] In addition, the i-PRF has the highest concentration of leukocytes/platelets in comparison to other preparations of PRF according to Miron et al.[29] So, because of the features of i-PRF, it was decided to use it to experience healing in the palate. According to the search of the PubMed and Google Scholar databases, this is the first research that studies the effects of i-PRF on wound healing and tissue thickness in the donor site of connective tissue at the palate. In the present study, the EHI results of the i-PRF group were significantly better than the control group on the 7th and 14th day, which means that the i-PRF provided better wound healing. The reason might be that TGF-β, which is found in the i-PRF, has good effects in various phases of palatal epithelium and connective tissue development and formation.[27,30] In addition, the i-PRF improved cell migration, proliferation, and spreading of fibroblasts.[31] The current findings are similar to Alpan et al. who evaluated the application of PRF to the palatal tissue after CTG harvesting and found that PRF provided better wound healing clinically on the 14th day.[19] The time between both separate surgeries in the same palatal donor site, when the patient needs many grafting procedures due to limitations caused by vascular anatomy or bad tissue, is an important factor in deciding when it can be repeated to harvest another SCTG again.[5] Therefore, it was necessary to search for catalysts to regenerate the thickness of the donner site in an ideal way to repeat the harvesting process when necessary. The current study revealed that palatal tissue thickness measurements of the i-PRF group were significantly higher than those of the control group at first and second month. Kiziltoprak et al. evaluated the effect of autologous fibrin glue (AFG) and i-PRF on palatal healing. They noted that there was no significant difference between i-PRF, AFG, and control groups concerning changes in tissue thickness.[23] The study of Samani et al. agreed with the previous one, where they did not report any significant difference between PRF and control groups in terms of changes in tissue thickness.[32] The difference between the current study and the two previous studies can be explained by the different grafts taken from the palate, FGG vs SCTG, where the mechanism of healing is different between them.[33] On the other hand, the findings of the presented study agreed with Ustaoğlu et al., whom they reported that tissue thickness in the titanium-PRF group was better than the control group.[34] According to this study, clinically we can repeat harvesting from the palate with i-PRF faster than the palate without it. Histologically, the minimum time required for re-intervention on the palate to obtain an adequate quality of the SCTG is 9 weeks.[5] However, no histological analysis was conducted in this study as a main limitation. Moreover, better results could have been obtained with the split-mouth study design. Another limitation is related to the small sample size because of strict conditions of inclusion criteria, so more reliable results could be achieved by considering these limitations. Clinicians should know the benefits that can be obtained from the use of PRF concentrates, especially injectable PRF, as it can be used to accelerate the healing in the palate. In addition, it improves tissue re-thickness sooner.

## Conclusion

From the mentioned limitations of this study, it can be revealed that the i-PRF accelerates healing and returning of the donor site in the palate to its original thickness. However, more researches with histological investigations and longer follow-up periods are required to assess the healing and regenerative features of the i-PRF on palatal healing after SCTG harvesting.

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