ACTA ODONTOLOGICA SCANDINAVICA, 2016
VOL. 74, NO. 7, 558–564
http://dx.doi.org/10.1080/00016357.2016.1219045

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ORIGINAL ARTICLE

The role of titanium-prepared platelet-rich fibrin in palatal mucosal wound healing and histoconduction

Gülbahar Ustaoğlu[a], Esra Ercan[a] and Mustafa Tunali[b]

[a]Department of Periodontology, Karadeniz Technical University, Trabzon, Turkey; [b]Department of Periodontology, Haydarpasa Training Hospital, Gulhane Military Medical Academy, İstanbul, Turkey

ABSTRACT

Objective: We aimed to determine the clinical effects of titanium-prepared, platelet-rich fibrin (T-PRF) on human palatal mucosal wound healing (PMWH) and to identify its effect on time-dependent changes in palatal soft-tissue thickness (PSTT) in terms of histoconduction, which is a new concept.
Materials and methods: Free gingival graft (FGG) donor sites were treated with T-PRF and compared with an untreated control group. The results of colour match and H_2O_2-bubbling tests for complete wound epithelization (CWE) were recorded on days 3, 7, 14 and 21. Pain level, number of analgesics used and bleeding status were recorded for the first 7 days. PSTT was measured at baseline and after 1 and 6 months.
Results: Colour match scores of the test group were significantly higher than those of the control group at 7 and 14 days. CWE was observed at a higher frequency in the test group than in the control group on day 14. Post-operative bleeding prevalence was lower in the test group than in the control group for the first 2 days. A time-dependent decrease in PSTT was observed at 1 and 6 months in the control group compared with baseline (baseline, 4.23 ± 0.62 mm; 1 month, 4.01 ± 0.68 mm; and 6 months, 3.93 ± 0.69 mm). However, no significant difference was found in the test group (baseline, 4.29 ± 0.64 mm; 1 month, 4.61 ± 0.51 mm; and 6 months, 4.51 ± 0.58 mm).
Conclusion: The T-PRF membrane exhibited positive effects on PMWH. T-PRF, which is a promising autogenous matrix for histoconduction, may also be preferred as an autogenous alternative to connective tissue grafts in the treatment of gingival recessions and peri-implant mucosal recessions.

ARTICLE HISTORY
Received 22 March 2016
Revised 26 May 2016
Accepted 26 July 2016
Published online 17 August 2016

KEYWORDS
Gingiva; histoconduction; platelet-rich fibrin; tissue transplantation; wound epithelization

Introduction

Adequate attached gingiva is essential for maintaining periodontal health and optimal oral hygiene.[1] A free gingival graft (FGG) is an autogenous soft-tissue graft that is widely used and accepted as a predictable method for treating mucogingival problems, such as inadequate attached gingiva and gingival recession defects.[2] The FGG technique was developed by Sullivan and Atkins. In this technique, a soft-tissue graft is taken from the palate, which forms an open wound that heals by secondary intention.[3,4] However, some complications may occur at the donor site during the post-operative period, including excessive bleeding, retarded wound healing, post-operative pain and sensation loss.[5,6] Several studies have attempted to determine the best way to reduce post-operative complications. These studies have tested hemostatic agents, ozonated oil, herbal products, antibacterial agents, bioactive materials, low-level laser therapy and platelet concentrates.[6–12]

Palatal donor site healing can be a suitable model for oral mucosal wound healing. Fibroblast proliferation, collagen synthesis and deposition, revascularization and wound contraction occur during wound healing.[13] Inflammation and cellular migration, proliferation and differentiation are crucial for successful healing.[14,15]

Platelet-rich fibrin (PRF) is an immune and platelet concentrate obtained by a simple procedure that does not involve extra biochemical blood handling in a glass tube. PRF is a three-dimensional fibrin network that promotes accelerated wound healing, immunity and effective neovascularization [16–18], and it supports the three crucial steps of wound healing: 'angiogenesis', 'immunity' and 'epithelial proliferation'.[19] In addition, PRF, an autogenous fibrin matrix formed only of concentrated blood, provides successful clinical outcomes for the treatment of soft-tissue defects and comparable keratinized tissue gain and tissue thickness when combined with connective tissue grafts.[20–22] However, using glass-evacuated collection tubes with silica activators might be problematic for blood.[23] A protocol for producing titanium-prepared, platelet-rich fibrin (T-PRF) was recently developed by Tunali et al. [24] that avoids any negative effects caused by dry glass or glass-coated plastic tubes. Titanium-activated platelet aggregation is similar to glass, but the fibrin carpet formed in titanium tubes exhibits a firmer network structure and a longer *in vivo* resolution time than do fibrin carpets that form on glass.[24,25] These properties allow T-PRF to play an important role in increasing soft-tissue thickness. The histoconductive efficacy of autogenous soft tissue grafts (i.e. T-PRF, connective tissue grafts) is similar

CONTACT Esra Ercan esraercan82@gmail.com Karadeniz Technical University, Faculty of Dentistry-Periodontology Department, 61080 Trabzon, Turkey

to the osteoconductive efficacy of hard-tissue grafts. The term 'histoconduction' might explain this feature of soft tissues, which has not been described previously in the literature.

The aims of this study were to evaluate the effects of T-PRF treatment on palatal wound healing and patient complaints, such as bleeding and pain sensation, and to observe the changes in palatal soft-tissue thickness (PSTT) after an FGG procedure.

Materials and methods

Subjects and study design

Approval was obtained from the Karadeniz Technical University Faculty of Medicine Ethics Council (number: 24237859-545; date: 08.09.2014) at the beginning of this single-centre, randomized, prospective controlled study. This study evaluated healing time, particularly complete wound epithelization (CWE) and post-operative morbidity after harvesting an FGG from the palate. The wounds were treated with sterile wet gauze pressure (control group) or T-PRF (test group).

Forty systemically healthy, non-smoking patients requiring an FGG were selected.

The inclusion criteria were as follows:

1. Systemic factors (no systemic disease, no coagulation disorders, no medications affecting periodontal status and no pregnancy or lactation)
2. Non-smoking
3. Good oral hygiene
4. No periodontal surgery at the experimental site
5. No nausea

The trial was conducted between November 2014 and January 2016. Each participant signed an informed consent form after receiving detailed verbal and written information about the technique.

To minimize differences related to the surgical technique, all surgical procedures were performed by a single experienced clinician (G.U.).

Pre-surgical treatments

All selected patients underwent professional periodontal prophylaxis and were given appropriate oral hygiene instructions. Patients who had a full-mouth plaque score of 20% and a full-mouth bleeding score of 20% were included in the study. Another clinician (E.E.) used a coin toss to randomly assign patients to the control or test group.

Surgical technique

Under local anaesthesia, the FGG was harvested as follows. The donor site was extended from the distal line angle of the canine to the mesial line angle of the maxillary first molar using the conventional scalpel technique.[4] An FGG of approximately 1.5 mm with a split thickness and a rectangular shape (7 × 12 mm) was obtained and then sutured at the prepared recipient bed using a size 5-0 resorbable suture (Pegelak, Doğsan, Turkey).

T-PRF

Blood samples were collected from the patients. The participants took no aspirin or other medications that could interfere with coagulation for 2 weeks prior to the procedure. The 20 mL blood samples were drawn from the antecubital vein of the subject's right or left arm in one attempt.

The blood was quickly and gently transferred to titanium tubes. The tubes were immediately centrifuged at 2800 rpm for 12 min. After centrifugation, T-PRF clots were removed from the tubes using sterile tweezers, separated from the red blood cell base using scissors and placed on sterile woven gauze. Each clot was deprived of its serum on the sterile-woven gauze slowly over 20 min and then pressed between two pieces of gauze. The T-PRF membrane was then prepared to be compatible with the FGG donor site (Figure 1).

Post-operative care

A non-eugenol pack was placed over the secured FGG. An acrylic stent was prepared to protect the palatal donor site and was placed after surgery; the patient was instructed to wear the stent for 7 days. Patients were prescribed 500 mg paracetamol (if needed) and 0.2% chlorhexidine oral rinse twice daily for 2 weeks. Patients were advised to report any adverse outcomes and were recalled on days 3, 7, 14, 21, 30, 90 and 180.

Evaluated parameters

All evaluations were performed by one author (E.E) who was blinded to the type of treatment. Patients were monitored

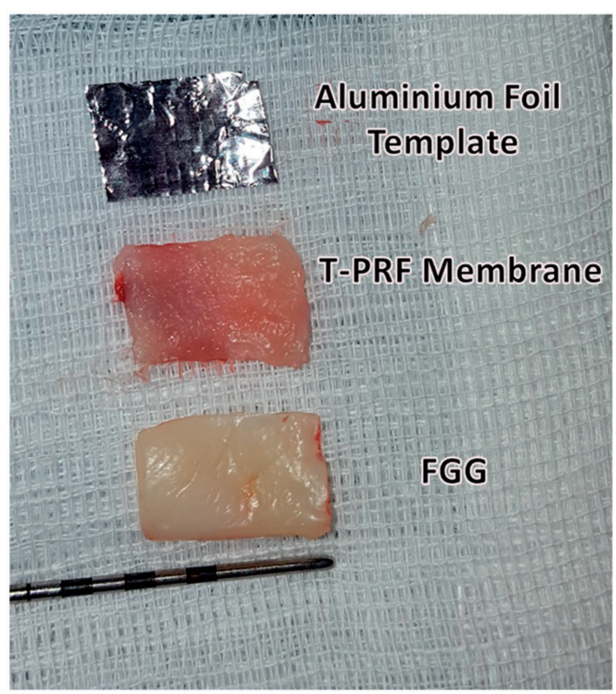

Figure 1. T-PRF membrane, FGG and aluminium foil template.

on days 3, 7, 14 and 21 after surgery (Figure 2). The following parameters were recorded: the H_2O_2-bubbling test to screen for complete epithelization, colour match, pain sensation measured using the visual analogue scale (VAS) score, bleeding (yes/no), number of analgesics taken (S) and PSTT.

Intra-surgical measurements: soft-tissue thickness

The PSTT in the harvested area was measured after the administration of local anaesthesia.[26] The measurement was made approximately 5 mm apical to the gingival margin of the first premolar using a #15 endodontic reamer. The penetration depth was measured using a digital caliper. The measurements were obtained before harvesting the FGG and at 1 and 6 months after the operation.

The graft thickness was measured in the same way at the middle points of all four edges of the graft. The mean of the obtained values was calculated and considered to be the graft thickness. The dimensions of the graft were standardized using a sterile aluminium foil template of 7×12 mm.

Colour match

On days 3, 7, 14 and 21, the colour of the palatal mucosa was assessed by comparing it to the colours of the adjacent and opposite sides using the objective VAS score $(0-100)$, as judged by a clinician (E.E.) who was blinded to the treatment groups. A score of 0 indicated no colour match, and a score of 100 indicated a perfect colour match with the adjacent tissues.

Wound epithelization

CWE was evaluated clinically using the H_2O_2-bubbling test. This test is based on the principle that if the epithelium is discontinuous, it diffuses into the connective tissue, and the enzyme catalase acts on H_2O_2 to release water and oxygen, which is clinically shown by the production of bubbles on the wound. The area to be evaluated was dried, and 3% H_2O_2 was sprinkled on the wound using a syringe. The appearance of bubbles suggested that the surgical site was not completely epithelialized.

Subjective assessment

For 7 days after the FGG procedure, patients were asked to assess pain and burning sensations using the VAS. The VAS score for pain ranged from 0 (no pain) to 100 (severe pain). In addition, patients were asked to report post-operative bleeding for 7 days.

Number of analgesics taken

Patients were informed that the number of analgesics they took for post-operative pain relief was recorded for 7 days.

The sutures were removed from the palate after one week and from the recipient site after 2 weeks.

Power analysis

The primary outcome of this study was the H_2O_2-bubbling test result measured on day 14. A power analysis was performed using http://www.openepi.com/Menu/OE_Menu.htm. The power of this study was calculated as 91.17%.

Statistical analyses

Data homogeneity was tested using the Shapiro–Wilk test. Non-parametric tests were used. SPSS v.17.0 (SPSS Inc., Chicago, IL) was used to analyse the data (License no: 2Z7ZHN12FA70P1JXWYNCMWY5).

The Mann–Whitney U-test was used to analyse intergroup differences. The Friedman and Wilcoxon tests were used for intragroup analyses. Time-dependent changes were analysed using the Wilcoxon test at a significance level of $p < 0.05$.

Results

The experimental groups were balanced by age and gender ($p > 0.05$). Two patients in the control group and four patients in the T-PRF group were dropped from the study due to irregular follow-up. A flow chart of this study is shown in Figure 3.

The T-PRF group showed a better colour match based on the VAS scores than did the control group on days 7 and 14. However, this discrepancy disappeared on day 21 (Table 1).

The H_2O_2-bubbling test results showed incomplete epithelization on days 3 and 7 in both groups. However, the T-PRF group showed a significantly higher CWE than did the control group on day 14 (68.7% for the test group; 16.7% for the control group) ($p < 0.001$). CWE was observed in all patients in both groups on day 21 (Figure 4).

Post-operative pain, as measured using the VAS, did not differ between the two groups during the first week ($p > 0.05$). The number of analgesics taken also did not differ between the two groups for all follow-up periods ($p > 0.05$).

The difference in the prevalence of bleeding between the T-PRF and control groups was statistically significant on days 1 and 2 ($p \leq 0.001$). On the first day, all control group patients exhibited bleeding compared to only 87.5% of the T-PRF group patients. On the second day, the difference between the T-PRF and control groups remained statistically significant (12.5% and 66.7%, respectively) ($p < 0.001$).

The PSTT decreased (4.23 ± 0.62 mm to 4.01 ± 0.68 mm) in the control group and increased (4.29 ± 0.64 mm to 4.61 ± 0.51 mm) in the T-PRF group after 4 weeks of healing. These differences were not statistically significant. The PSTT decreased significantly in the control group at the end of month 6 compared to baseline (4.23 ± 0.62 mm to 3.93 ± 0.69 mm) ($p < 0.01$) but did not change significantly in the T-PRF group (4.29 ± 0.64 mm to 4.51 ± 0.58 mm) ($p > 0.05$) according to the Wilcoxon test results (Table 2). The PSTT in the T-PRF group was significantly thicker than that in the control group after 6 months of healing (4.51 ± 0.58 mm in the T-PRF group and 3.93 ± 0.69 mm in the control group) ($p < 0.05$).

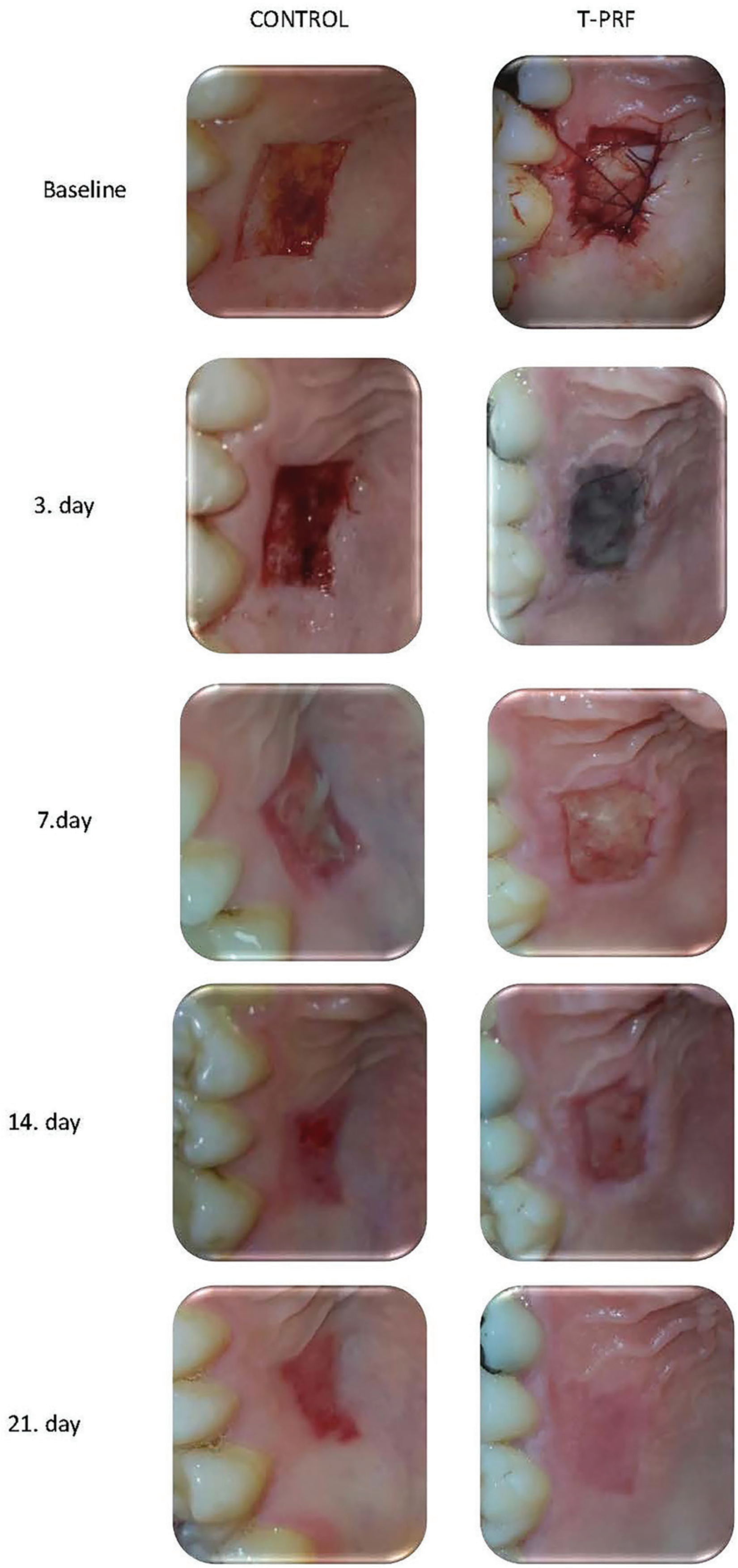

Figure 2. Clinical photographs of donor sites.

Discussion

In this study, we used T-PRF to enhance palatal wound healing. T-PRF significantly enhanced the frequency of CWE compared to the control group on day 14. These results were consistent with the results of a case study showing that the PRF membrane could be used as a 'palatal bandage'. Those authors reported better healing of palatal soft tissues than at non-PRF sites based on H_2O_2-bubbling test results. The non-PRF donor sites healed completely at 4 weeks. However, the PRF sites healed at 18 days.[27] In a similar study, Femminella et al. studied the healing of a palatal FGG donor site using PRF or a gelatin sponge. CWE at the palatal site was observed after 3 weeks in all PRF-group patients and in 25% of patients in the control group. Only 35% of the patients in the PRF group were observed to have CWE at day 14. However, in our study, CWE was detected in 68.7% patients in the T-PRF group on day 14 and in 100% of patients on day 21. This may be explained by the superior features of T-PRF, such as the long resorption time compared with conventional PRF and the well-organized fibrin matrix structure. In addition, in our study, the secondary wound-healing area, which was covered by the T-PRF membrane, showed similarity with the primary wound-healing pattern. Therefore, the T-PRF membrane may change the healing pattern by covering the non-epithelized donor site.

In this randomized, controlled clinical study, we harvested an FGG in a standardized manner using an aluminium template in a standardized size (7 × 12 mm). The harvested graft tissue thickness was also measured (1.5 mm). This method provided a standardized wound areas for the study groups. The CWE was evaluated with an objective H_2O_2-bubbling test. The CWE, which is an important phase of secondary wound healing, indicated the effects of the tested method on epithelization. One limitation of our study is that we did not perform a histological analysis.

When we assessed bleeding status, we observed statistically significant differences in reported bleeding between the T-PRF and control groups during the first two days. A lower incidence of bleeding was reported by patients in the T-PRF group compared with the control group. This rapid haemostasis feature can be explained by the more stable fibrin structure of PRF compared with blood clots.[28] Bleeding after graft harvesting from a palatal donor site is a frequent problem that leads to patient discomfort. The treatment of

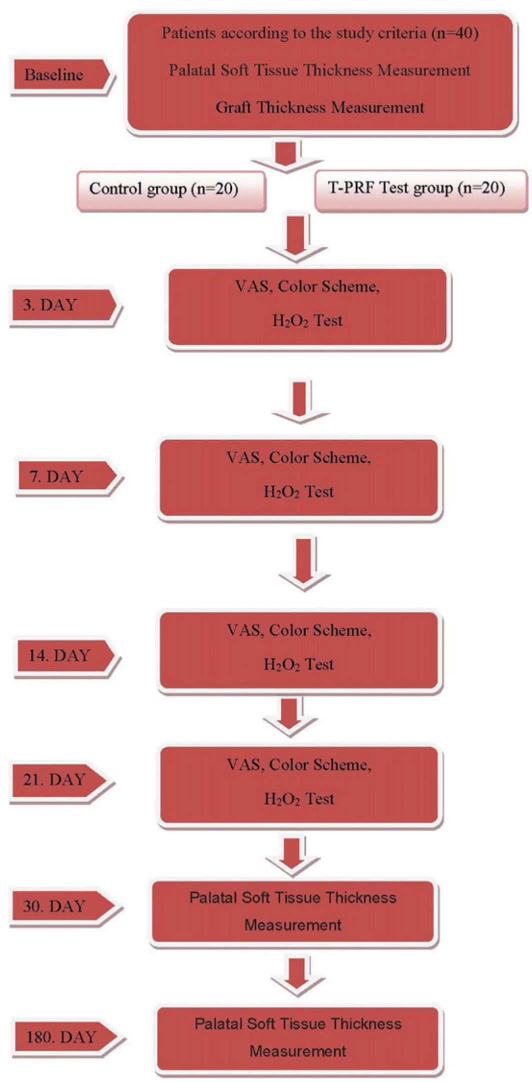

Figure 3. Flow chart of the study.

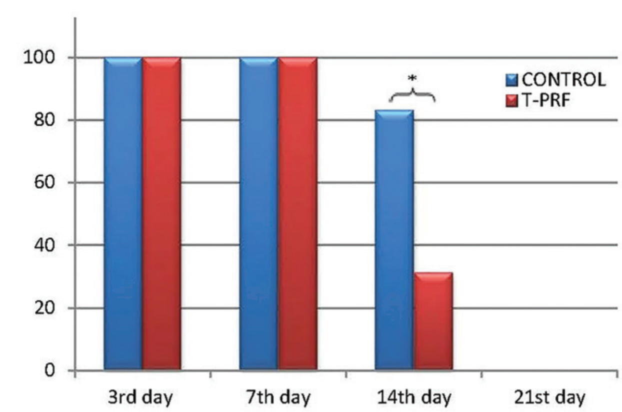

Figure 4. H_2O_2-bubbling test results.

Table 1. Colour match scores.

Group	Colour 3 median; min–max	Colour 7 median; min–max	Colour 14 median; min–max	Colour 21 median; min–max
CONTROL ($n = 18$)	0; 0–0	30; 15–45	65; 45–90	90; 80–95
T-PRF ($n = 16$)	0; 0–0	50; 40–60	85; 60–95	90; 85–100
p	NS	$p<0.001$	$p<0.001$	NS

Table 2. The time-dependent alterations in palatal soft-tissue thickness.

Group	Pre-op palatal tissue thickness Mean ± SD	First month palatal tissue thickness Mean ± SD	Sixth month palatal tissue thickness Mean ± SD	p
CONTROL ($n = 18$)	4.23 ± 0.62^a	4.01 ± 0.68	$3.93 \pm 0.69^{a,b}$	$^a p<0.01$
T-PRF ($n = 16$)	4.29 ± 0.64	4.61 ± 0.51	4.51 ± 0.58^b	NS
p	NS	NS	$^b p<0.05$	

T-PRF may help to enhance a patient's post-operative comfort by decreasing bleeding.

It was recently reported that PRF has positive effects on wound healing.[29] PRF acts through several mechanisms, including the modulation of angiogenesis by growth factors that are found in the alpha granules of platelets. In addition, clot formation regulates neutrophil activity and causes faster epithelial cell migration to the wound area. These positive effects occur in response to growth factors and the stable fibrin scaffold. Therefore, fibrin has been referred to as a 'healing biomaterial' in the literature.[29,30] The function of platelets has been investigated in recent years. An important finding is that platelets have a role in orchestrating the immune response by cross talking to neutrophils and other cells.[31] Platelet–neutrophil interactions induce the neutrophils to release various antimicrobial molecules, such as lysozyme, myeloperoxidase and antimicrobial peptides called cathelicidins and α-defensins. Neutrophils are also important in the regulation of wound healing and re-epithelization.[32,33]

We also evaluated post-operative morbidity by recording VAS scores for pain and the number of analgesics taken during 7 post-operative days. There were no statistically significant differences between the two groups. However, studies have shown that PRF acts as a healing biomaterial by enhancing epithelization and decreasing inflammation.[28,34] Pain is a subjective sensation, and VAS scores vary from person to person. Therefore, further split-mouth studies are needed to evaluate the effect of PRF on post-operative morbidity at FGG donor sites.

We evaluated the effects of T-PRF on soft-tissue thickness at the palatal donor site. In the control group, tissue thickness decreased significantly after 6 months compared with baseline ($p < 0.01$). However, in the test group, tissue thickness increased at 1 and 6 months compared with baseline. It has been recommended to place a T-PRF membrane with a similar thickness to the FGG on the wound area to avoid any bias. Based on the results of this study, it can be concluded that a T-PRF membrane helps to restore soft-tissue thickness at a donor site through its histoconduction feature. This feature can be attributed to the stable structure of T-PRF and its long resorption time, as observed on post-operative day 14 (Figure 2). This healing biomaterial might represent a 'histoconductive' membrane and serve as a scaffold for epithelial and connective tissue cells during wound healing.

In a recent study, palatal mucosa augmentation was performed using two different commercial products, including collagen.[35] The aim was to thicken the thin masticatory mucosa for connective tissue graft harvesting. The authors observed a thicker palatal mucosa at the end of 8 weeks with mature fibrous connective tissue. Similarly, in our study, we observed thicker palatal mucosa in the T-PRF group at the end of months 1 and 6 compared to baseline. T-PRF has significant advantages for clinicians during the augmentation of palatal mucosa because it can be obtained in the desired amount, and it is completely autogenous.

Adequate soft-tissue thickness is important to obtain qualified soft-tissue grafts with sufficient lamina propria. In our study, we observed that palatal tissue thickness decreased at the end of month 6 in the control group. Therefore, it can be assumed that the lamina propria thickness would also decrease during palatal donor site healing, and high-quality grafts with adequate lamina propria thickness cannot be obtained from donor sites because of the slimming of soft tissue at the palatal donor site. If a T-PRF membrane is placed at the donor site, it may serve as a three-dimensional scaffold for connective tissue and epithelial cells and may help to maintain the thickness of the donor site lamina propria. In addition, the T-PRF membrane protects the wound area from bacteria and external stimulants during the healing phase so that the healing pattern at the donor site looks like primary wound healing rather than secondary healing.

Conclusion

We conclude that T-PRF can be used to accelerate wound healing at FGG donor sites by simulating a primary wound-healing pattern. In addition, unlike the control group, the T-PRF-treated donor sites maintained baseline tissue thickness at the end of 6 months. Therefore, it can be concluded that a T-PRF membrane is a histoconductive material and is preferred for use at palatal donor sites after FGG procedures in terms of PSTT maintenance. However, further clinical controlled studies with long follow-up periods are warranted to investigate the effects of this technique.

Disclosure statement

Mustafa Tunali hold intellectual property releated to T-PRF. The other authors declera no competing interest.

The authors thank to Dr. Tamer Tüzüner for his supports during statistical analysis.

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