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EFFECT OF PLATELET-RICH FIBRIN PALATAL BANDAGE ON PAIN SCORES AND WOUND HEALING AFTER FREE GINGIVAL GRAFT: A RANDOMIZED CONTROLLED CLINICAL TRIAL

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ABSTRACT

Background: This prospective randomized clinical trial was conducted to determine whether the application of a platelet-rich fibrin (PRF) palatal bandage after harvesting free gingival grafts (FGG) would improve healing of donor sites and decrease pain scores and patient discomfort.

Methods: Twenty-four patients received FGG to augment keratinized tissue dimensions. The application of a PRF bandage was decided randomly (n = 12 in each group). Patients reported their pain levels for the first 7 days of healing using the visual analog scale (VAS), 101-point numerical rating scale (NRS-101), and 4-point verbal rating scale (VRS-4). The patients' pre-operative anxiety levels were measured using three anxiety scales. The healing of the donor sites was evaluated and compared to preoperative records. Data were assessed and recorded before surgery and at 1, 2, 3, 4, and 8 weeks postoperatively.

Results: Dental anxiety, state-trait anxiety were evaluated at base line and correlated to the postoperative pain scores. There were no significant differences in anxiety levels between the two groups, yet they could potentially influence the outcome of any surgery and were included as covariates. The patients in the PRF group reported significantly lower pain scores and their pain levels returned to baseline levels earlier, compared to the control group. They were also assessed as having better wound healing over a 2-month follow-up period, based on color, contour, and texture indices.

Conclusion: PRF palatal bandages significantly reduced postoperative pain and discomfort, and facilitated wound healing after harvesting FGG.

INTRODUCTION

Although techniques such as subepithelial connective tissue graft are now widely used for root coverage procedures [1,2], free gingival graft (FGG) continues to be a common mucogingival procedure used to increase keratinized tissue dimensions [3]. However, the palatal donor sites heal with secondary intention and require a longer healing duration with

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more patient discomfort and pain. Therefore, a variety of periodontal dressings have been used to assist the healing of the donor site [4].

Platelet-rich fibrin (PRF) has been recommended for use as a palatal bandage to cover the donor sites for FGG [4]. PRF is a second generation platelet concentrate obtained from autologous blood with simplified processing without the need for biochemical blood handling [5,6]. It resembles a fibrin network, which leads to more efficient cell migration and proliferation, and thus cicatrization [7-9].

Many studies have reported that autologous PRF retained high levels of growth factors such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), and insulin-like growth factor-1 (IGF-1) for long durations [10]. The leukocytes present in the PRF fibrin network play a significant role in inflammatory response to infectious phenomena. In addition, this network facilitates the migration of endothelial cells, which is necessary for neoangiogenesis and vascularization [11]. Some authors have reported the presence of at least 60 different biologically active substances in platelet concentrates, all of which aid in repair mechanisms such as chemotaxis, proliferation, intracellular matrix deposition, immune modulation, antimicrobial activity, and remodeling [12].

PRF applications have been studied in various oral and maxillofacial procedures such as facial plastic surgery [13] ,sinus-lift procedure [14] and multiple gingival recessions treated with a coronally advanced flap [15] The autologous PRF was also used in implant surgery to improve bone healing [5] and it has been shown to act as a suitable scaffold for breeding human periosteal cells in vitro, which may be suitable for bone tissue engineering [16]. The ease and reliability of the PRF approach can provide a less expensive and more compatible mode of delivery of growth factors when compared to exogenous recombinant growth factors [4,11].

Prospective patient outcome studies regarding FGG postoperative experiences and healing at the donor site are generally lacking. This work aimed to evaluate the effect of using a PRF membrane as a palatal bandage on clinical healing parameters at the donor site after harvesting FGG, compared to surgical blood clot alone. An assessment of the patients' pain and discomfort scores (with or without the application of a PRF bandage) was carried out as well.

SUBJECTS AND METHOD

Study Population and Design

This human clinical trial followed a prospective, randomized design with an observation period of 8 weeks. Twenty-four adult patients were included (aged between 18 and 40 years old; mean age: 27.3 ± 2.6) and planned to receive FGG to treat a lack of keratinized tissue in the mandibular central incisor region after dental prophylaxis and oral hygiene instructions. Participants were recruited from among the patients seeking periodontal treatment in the Department of Periodontology of the Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia, from January 2014 to January 2016.

Exclusion criteria were smoking and/or any uncontrolled systemic disease that might contraindicate periodontal surgery, chronic use of any medication that may affect pain perception or the inflammatory cycle and wound healing, severe gag reflex preventing maxillary surgical procedures, and inability or unwillingness to provide informed consent. The study was approved by the King Abdulaziz University ethical committee, was carried out in accordance with the tenets of the Declaration of Helsinki [17, 18], and was registered at clinicaltrials.gov with the registration number NCT02797899.

Pre-surgical Assessment of Dental Anxiety and Patient Grouping

Because dental anxiety varies from one patient to another and may influence pain perception, each patient was asked to complete a state-trait anxiety inventory [19, 20] and a modified dental anxiety scale [21, 22] one hour before surgery to assess the correlation between pain scores and self-reported stress and anxiety.

Donor sites were assigned randomly to either receive PRF or not by a flip of a coin. This was performed by the dental assistant who informed the operator just before the surgical procedure. The operator then gave the patients a detailed description of the assigned procedure, and its advantages and disadvantages.

Surgical Procedures

Initial prophylaxis and periodontal therapy, consisting of full mouth scaling utilizing both hand and ultrasonic instruments was performed under local anesthesia.

All surgeries were performed under local anesthesia (2% lidocaine with 1:100,000 epinephrine). All free gingival grafts were done following the surgical technique of *Sullivan and Atkins* [23], with all grafts being placed on a periosteal bed. A custom foil template was placed over the palatal mucosa and used to outline the standardized dimensions (12 × 7 mm) of the graft for all patients. Graft thickness was standardized as well to be approximately 1 to 1.5 mm and confirmed with a caliper at 3 points (ends and center of the graft). The graft was positioned and firmly adapted to the recipient area and stabilized with suspensory periosteal sutures.

Sites receiving PRF were allocated to Group 1 (G1; n = 12). 10 ml of venous blood was drawn from the subject's antecubital vein to be collected in glass-coated plastic tubes free from anticoagulant agents. The blood-containing tubes were immediately centrifuged at 3000 rpm for 10 min [24]. The PRF bandages, in the form of a consistent membrane, were applied over the donor sites after harvesting the FGG and were secured in place with resorbable sutures. Patients in Group 2 (G2; n=12) underwent FGG without PRF coverage but sutures were made in the same manner. In both groups, non-eugenol periodontal pack (Coe-Pak ™) was applied as a protective bandage to the donor and recipient sites.

Pain Assessment

Patients were instructed to complete a pain diary every hour for the first 8 hours after surgery, and then three times a day on the second day, third day, fourth day, and seventh day. Three methods of measuring clinical postoperative pain intensity were used in this study. The visual analog scale (VAS), which consists of a 10-cm line anchored by 2 extremes: no pain and pain that could not be more severe. Patients were asked to make a mark on the line that represents their level of perceived pain. For the 101-point numerical rating scale (NRS-101), the patient was asked to rate his/her pain intensity on a numeric scale ranging from 0 to 100. The four-point verbal rating scale (VRS-4) was also used to measure the level of discomfort. In this measure, the patient has four options: no discomfort, some discomfort, considerable discomfort, or discomfort that could not be more severe [25, 26].

Post-Surgical Care

Postoperative instructions included discontinuing tooth brushing and flossing around the surgical sites for the first 3 postoperative weeks. They were also instructed to consume only soft food during the first week and to avoid any other mechanical trauma to the treated sites. Patients were placed on a 0.12% chlorhexidine gluconate mouth rinse for 2 weeks. Rescue medication (1000 mg acetaminophen) was given to each patient and they were instructed to take the drug only when necessary. Patients were

seen every week for the first month and at the end of 2 months for postoperative follow-ups. During the follow-up visits, clinical photographs and records of surgical sites were taken.

Analysis of Clinical Results

Five blinded senior postgraduate periodontal residents and one professional periodontist were asked to judge the clinical photographs taken from the post-surgical visits, which were masked regarding their group allocation. The examiners were provided with the pre-operative photographs and then asked to rate the site that exhibited better healing based on a proposed index by the authors. The wound healing criteria were based on the degree of color match, tissue texture, and contour of the surgical area compared to the adjacent tissue. Each examiner evaluated the clinical slides twice at two different times.

Descriptive statistical analysis was carried out to compare the clinical findings between the two groups as only seven cases in each group had a full set of follow-up clinical photographs. The scores were calculated as a weighted average of the following Likert scales:

Analysis of Color Changes

1 = exactly similar to the pre-operative photograph

2 = slightly red more than the pre-operative photograph

3 = very red and inflamed tissues

4 = extremely bad color mismatch

Analysis of Contour Changes

1 = exactly similar to the pre-operative photograph

2 = some tissue irregularities can be detected

3 = severe depression or extreme elevation of the palatine tissues detected

Analysis of Texture Changes

1 = exactly similar to the adjacent tissues

2 = slightly different than adjacent tissues

3 = totally different than adjacent tissues

Statistical Analysis

To evaluate the changes in pain scores over time, we performed a one-way repeated measures analysis of variance (ANOVA) for each pain scale (VAS, NRS-101, and VRS-4). We used two-way ANOVA to compare the pain scores at the different time-points between the two groups.

To assess the effects of pre-surgical anxiety on pain scores, we performed two mixed-design analyses of variance, one between factors and one within factors with three covariates: dental anxiety, state-trait anxiety. These analyses were conducted for the three measured variables: VAS, NRS-101 and VRS-4.All statistical analyses were performed using the SPSS software (Version 20; IBM Corp., NY, USA)

RESULTS

Base Line Data Analysis

The gender distribution was not statistically different in the two groups with seven males and five female patients in each group. The mean age and standard deviation (SD) of the PRF group was 27.8 ± 4.3, while for the control group it was 28.5 ± 3.7. Neither age nor gender had a significant effect on the anxiety or pain scores assessed in this study.

During the first week of postoperative follow up, three PRF subjects experienced the worst postoperative pain at the donor site started around midnight of day 1 and continued up to day 3 and they reported receiving the rescue medication (Acetaminophen 100 mg analgesic prescription).

Six of 12 control subjects started taking the pills during the same period but lasted up to day 7. 30.4% of the total studied subjects reported pain in the recipient site, and none reported pain elsewhere. Up to day 3, the PRF group reported having taken 8.1±2.3 pills, whereas the control group reported 23.1±4.2 pills taken.

For the three types of anxiety measured at baseline, the mean scores for dental anxiety, state-trait anxiety in the PRF group were 9.92 ±3.70, 29.00±7.59 and 27.83±7.58, respectively, and in the control group the scores were 9.67±2.77, 28.67±7.97, and 26.83±6.56, respectively. Although there were no significant differences in any of the scores between the two groups, we still considered them as potential confounders that could influence the outcome and therefore included them as covariates.

Using mixed-design ANOVA with three covariates: dental anxiety, state-trait anxiety, we found no significant differences between the VAS, NRS-101, and VRS-4 measures (Table 1).

TABLE (1) Results of repeated measure analysis of variance "mixed design" with three covariates: dental anxiety, state-trait anxiety

	VAS		NRS-101		VRS-4	
	F	P value	F	P value	F	P value
Dental Anxiety	1.180	0.291	0.830	0.374	2.638	0.121
State Anxiety	0.047	0.831	0.287	0.599	1.662	0.213
Trait Anxiety	0.170	0.685	0.060	0.809	0.374	0.548

VAS Analysis

The results of the one-way repeated measures ANOVA in the PRF group (Greenhouse-Geisser) were an F-value of 9.641 and a P-value of 0.00012, while in the control group, these values were F = 20.590 and P = 0.000. There was a significant change in the VAS scores with time within the subjects in each group (P = 0.000), and a significant difference between the subjects in the two groups (P=0.000). As shown in Figure 1, subjects in the PRF group had lower VAS scores at all time-points, compared to the controls, as well as a markedly lower peak pain level at 4 hours post-operatively (2.10 vs. 5.46 in the control group). Furthermore, patients in the PRF group reached normal scores earlier than the controls.

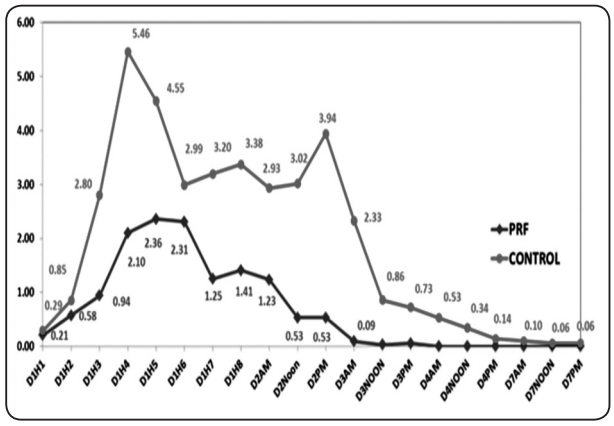

Fig. (1) Estimated marginal means of VAS with time in the two studied groups

NRS-101 Analysis

The one-way ANOVA in the PRF group resulted in an F-value of 8.606 and a P-value of 0.0001, while in the control group, the F-value was 23.233 and P-value was 0.0000. There was a significant change in the NRS-101 scores with time within the subjects in each group (P = 0.000), and a significant difference between the subjects in the two groups

(P = 0.000), with patients in the PRF group having lower scores at all time-points. In addition, the interaction of time and group was statistically significant (F = 6.851 and P = 0.0000). In the control group, the peak pain was recorded at 4 hours post-operatively (after the effect of the anesthetic solution was lost) and the score was 53.75, compared to 18.58 for the PRF group (Figure 2).

VRS-4 Analysis

One-way ANOVA of the VRS-4 scores within each group showed an F-value of 3.119 in the PRF group, and an F-value of 19.225 in the controls. There was a significant change in the VRS-4 scores with time within each group, and a significant difference between the two groups (P = 0.0000 for both). As shown in Figure 3, in both groups at noon on day 3, the VRS-4 scores reached 1 (no discomfort at all).

Analysis of Clinical Results

Representative clinical photographs are shown in the multi-panel Figure 4.

Analysis of Color Changes

A Likert scale with 4 points was used for assessing the color changes over time to help in evaluating the magnitude and direction of the difference between the two groups. Both groups had a score of 1 (normal color index) at day 0 (before surgery). Post-operatively, the scores in the control group were higher at every time-point, compared with the PRF group, indicating a poorer color match (Figure 5A).

Analysis of Contour Changes

Post-operatively, images from the control group were rated higher than those from the PRF group at every time-point (Figure 5B). Higher scores indicate a poorer contour match when compared to the pre-operative images.

Analysis of Texture Changes

Post-operatively, images from the control group were rated higher than those from the PRF group at every time-point (Figure 5C). Higher scores indicate a poorer texture match when compared to the adjacent tissues.

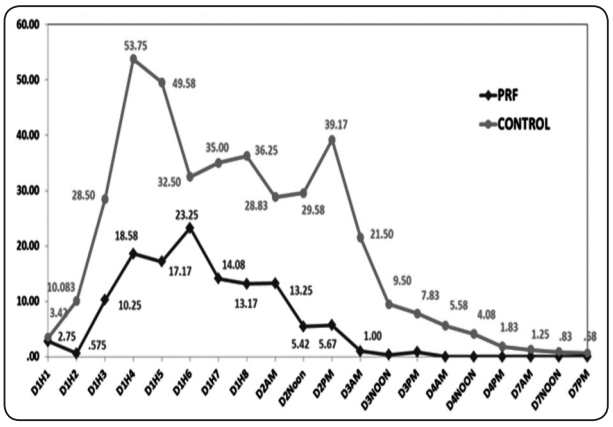

Fig. (2) Estimated marginal means of NRS-101 with time in the two studied groups

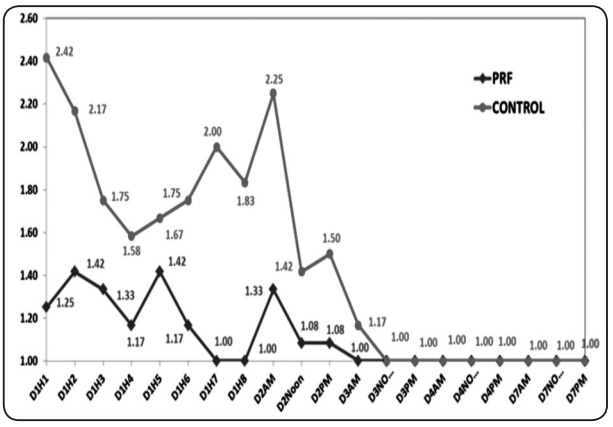

Fig. (3) Estimated marginal means of VRS-4 with time in the two studied groups

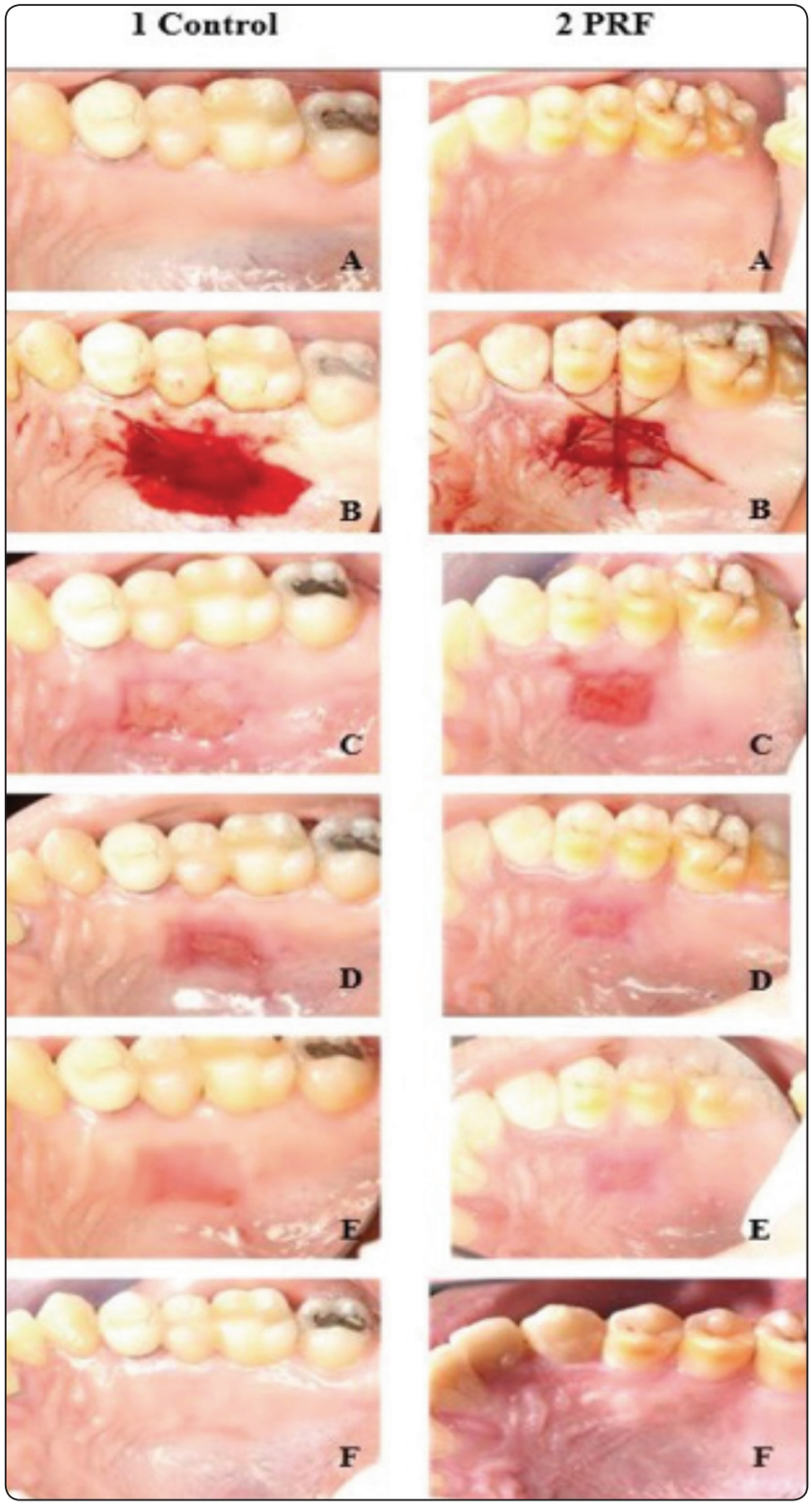

Fig. (4) Clinical photographs showing healing results of palatal donor sites (1=sample from control group and 2=sample from PRF treated group) (A)View at baseline, (B) Immediately after harvesting the graft, (C) 1 week, (D) 2 weeks, (E) 4 weeks, and (F) 2 months postoperatively.

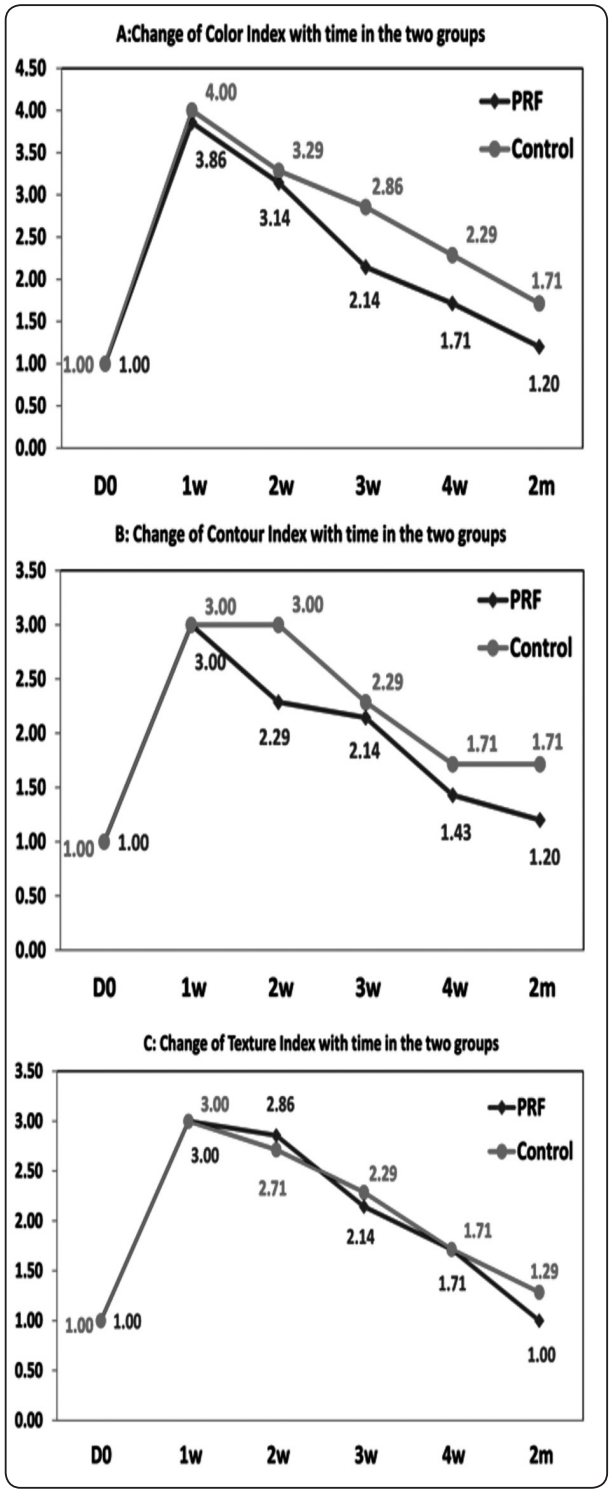

Fig. (5) Change in clinical results (A: Color, B: Contour, and C: Texture) with time in the two studied groups.

DISCUSSION

Autologous platelet concentrates constitute a safe means of delivering high concentrations of essential cytokines and growth factors to surgical wounds. Among the different types of platelet concentrates, PRF was first described by Choukroun et al. [5] They obtained the PRF by gentle centrifugation of peripheral blood and characterized it as being platelet-rich and fibrin-dense [27]. This study aimed to evaluate the effect of utilizing PRF as a palatal bandage on soft tissue healing of donor sites, as well as on pain scores and patient discomfort following FGG.

Studies have shown that PRF protects open wounds and accelerates natural healing mechanisms through its effects on angiogenesis, immunity, and epithelial proliferation. Its utilization seems to be of high interest mainly with non-healing wounds [6]. One of the most important advantages of PRF is wound protection from surrounding external irritants. Several studies have reported that the massive fibrin content of PRF protects growth factors and cytokines from proteolytic degradation, allowing them to maintain their activity for longer periods of time [28,29].

PRF is believed to contain platelets in a concentration seven times that of blood, and release high quantities of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α, as well as a main coagulation matricellular glycoprotein called thrombospondin-1 (TSP-1) during the first seven days of wound healing [12]. The clinical photographs in this study showed less bleeding immediately after application of the PRF bandage, better first-week soft tissue healing in terms of color match, contour, and texture, as well as less pain and discomfort. The PFR bandage not only provides mechanical occlusion, but also releases an arsenal of potent growth factors such as transforming growth factor beta-1 (TGFB-1), platelet derived growth factor AB (PDGF-AB), fibroblast-derived growth factors, and vascular

endothelial growth factor (VEGF), all of which can promote angiogenesis as well as healing and remodeling processes [30].

When PRF was compared to platelet rich plasma (PRP) in a previous study [31], the levels of TGFβ-1 and PDGF-AB in PRF were found to reach their highest levels on day 14, before gradually decreasing. In contrast, PRP showed a very short-term release pattern; the highest levels were reached on day 1 before rapidly declining . Another study reported that PRF has a slow sustained release of essential growth factors, lasting for up to 28 days, thus stimulating the wound healing process for a significant time [24]. These findings are in accordance with the better results seen in the PRF group in this study, even after a 4-week interval.

Soileau and Brannon [32] reported that at least 9 weeks are necessary for remodeling of the palatal wound after harvesting FGG to appear complete histologically. On the other hand, Yen et al. [33] found that administration of a platelet concentrate could accelerate the soft tissue healing and regeneration of palatal tissue thickness after a 6-week interval, as observed at both clinical and histological levels. In the current study, the PRF group had consistently better clinical results, during both the early healing events and at the end of the study interval (eight weeks postoperatively), when compared to the control group.

On the other hand, a recent study [11] on the profile of crevicular fluid growth factors released following the use of PRF in treating intrabony periodontal defects found that the PRF did not maintain extra-physiologic levels of growth factors. The authors stated that, based on the open nature and continuous bacterial colonization of periodontal defects, the ecology of periodontal pockets could be an unsuitable media that negatively affects the healing potential of platelet concentrate.

Donor site protection promotes faster healing and reepithelialization compared with unprotected sites or sites protected by periodontal dressing [4, 34]. Only resorbable suture material was used in the current study to secure the PRF in place and we highly emphasize that an improper PRF membrane size or improper suturing may lead to limited success of the procedure. The sutures were satisfactory for eliminating the need for surgical stents (as reported by many of our patients). Nevertheless, Jain et al. [12] used collagen membranes to act as a scaffold for the PRF. They reported that these two acting together led to complete epithelization without any signs of infection or inflammation within a 2-week interval.

The design of the present study allowed an evaluation of patient-centered outcomes, namely donor site pain and discomfort, which are the common problems encountered during FGG procedures. Additionally, highly anxious dental patients require greater attention during treatment, special behavioral management strategies, or additional pharmacological treatment. Dental anxiety, state-trait anxiety [19-22] were evaluated at base line and were correlated to the postoperative pain scores. Anxiety can act as a potential confounder that influence the outcome of any surgical procedure, yet there were no significant differences in the scores of these three scales between the two groups in the current study. Despite previous findings that higher anxiety levels are more prevalent among females as opposed to males and among younger individuals as compared to older ones [35], we did not observe these effects among the patients in the current study. The smaller sample size as well as the simple nature of the surgical procedure carried out in this study may explain this observation. In addition, patients with dental fear may have been underrepresented, because we sampled patients at general dental clinics rather than a clinic specializing in dentally fearful patients [19].

Regarding the pain scores, donor sites heal by secondary intention with longer healing duration, and previous studies have reported more discomfort

in the first 2 weeks postoperatively [1]. In the current study, the PRF group, during the first week of healing, showed significantly lower VAS and NRS-101 scores than the controls. Additionally, there were statistically significant changes in VRS-4 with time within each group and a significant difference between the two groups. During the first 2 hours postoperatively, pain scores were at their lowest levels because of the topical effect of the local anesthetic solution. Pain levels started to increase gradually, reaching a peak at 4 hours in the control group, but 1 hour later in the PRF group. Patients in the PRF group had mostly returned to normal pain levels by day 3 postoperatively, compared to 7 days for the same in the control group. Aravindaksha et al. [4] stated that the considerably shorter healing time required by PRF resulted in less postoperative discomfort to the patients. They reported uneventful comprehensive healing at all PRF sites by 18 days and that donor sites without PRF membrane healed completely after 4 weeks.

CONCLUSIONS

This controlled, randomized clinical trial indicated that application of a PRF palatal bandage after harvesting FGG is an effective procedure in terms of the early events of wound healing. PRF is an economical, autologous, and easy-to-procure biomaterial that can be used to decrease the pain and discomfort usually associated with FGG donor sites. Our 2-month data also showed that the application of PRF resulted in a statistically significant improvement in all clinical parameters tested (including color, contour, and texture of the keratinized palatal tissues after FGG procedure). Nevertheless, although the clinical evaluation showed excellent matches between the newly formed tissue and the surrounding tissue in the PRF group, further histologic analysis is recommended to evaluate the nature of this healing tissue.

Ethics approval and consent to participate

The study protocol was submitted and approved by the ethical committee of King Abdulaziz University (proposal number 054-13). All research procedures were explained to the patients, who provided signed consent to participate in the study. The study was retrospectively registered at ClinicalTrials.gov under Clinical Trial Registration Number NCT02797899.

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