Clinical Comparison of Platelet-Rich Fibrin and a Gelatin Sponge in the Management of Palatal Wounds After Epithelialized Free Gingival Graft Harvest: A Randomized Clinical Trial

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Background: Platelet-rich fibrin (PRF) promotes tissue regeneration by releasing various growth factors. The palatal donor site of the epithelialized connective tissue (CT) graft significantly influences the patient's morbidity. The aim of this study is to compare the effects of PRF and gelatin sponge on the healing of palatal donor sites and the patient's morbidity.

Methods: Forty patients with at least one site of Miller Class I or II gingival recession were treated by a coronally advanced flap with CT graft resulting from the de-epithelialization of a free gingival graft. In the test group (20 patients), a PRF membrane was placed over the palatal wounds; conversely, the 20 control group patients were treated with an absorbable gelatin sponge. Patients were monitored at 1, 2, 3, and 4 weeks after surgery for the complete re-epithelialization of the palatal wound (CWE), the alteration of sensitivity around the wound area, postoperative discomfort, and changes in feeding habits (CFH). Furthermore, the consumption of analgesics during the postoperative week 1 was assessed.

Results: The test group showed a significantly faster CWE ($P<0.001$); 35% of the test patients showed CWE at the end of week 2 (controls, 10%), whereas at the end of week 3, all palatal wounds in the test patients epithelialized completely (controls, 25%). Similarly, test patients reported significantly less discomfort and CFH ($P \leq 0.02$) and took a significantly lower dose of analgesics ($P = 0.02$).

Conclusion: The PRF-enriched palatal bandage significantly accelerates palatal wound healing and reduces the patient's morbidity. *J Periodontol 2016;87:103-113.*

KEY WORDS

Autografts; biocompatible materials; clinical trial; pain; palate; wound healing.

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Many surgical techniques have been proposed for the correction of gingival recession.[1] Although the amount of root coverage yielded by regenerative techniques[2,3] and pedicle grafts[4] is similar to that of free graft procedures, these techniques produce less of an increase in gingival thickness.[5] Conversely, bilaminar techniques (BTs) consisting of the association of a connective tissue graft (CTG) with a pedicle graft are presently considered the most predictable treatment choices to achieve exposed root coverage[6] while providing the greatest increase in gingival thickness.[2]

Gingival thickness is very important for long-term stability; in fact, gingival thickness plays an important role in preventing the recurrence of recession.[7] The main disadvantage of BTs is the need for a palatal wound, which often produces pain and discomfort.[4]

Many tissue-harvesting procedures have been described previously.[8,9] The epithelialized free gingival graft (EFGG)[9] is easy to perform and enables the harvest of large quantities of high-quality connective tissue (CT). Conversely, it produces a site of secondary-intention wound healing with discomfort and pain.[10,11]

To overcome this problem, different procedures with primary-intention healing

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have been proposed.[8] However, they are more difficult to perform and require the presence of thick palatal tissues to obtain a sufficiently thick graft and to avoid necrosis of the epithelial–connective layer left at the donor site. Furthermore, this graft usually contains a considerable amount of fatty and glandular tissues with less CT; it may be inadequate for root coverage.[12] Conversely, the more superficial tissue from the EFGG is stable and dense tissue, suitable for use in root coverage.

To reduce the postoperative problems in the EFGG donor site, Rossmann and Rees[13] suggest the use of a hemostatic dressing. Platelet-rich fibrin (PRF) is a platelet concentrate obtained by a simple and inexpensive procedure that does not require biochemical blood handling; its three-dimensional fibrin network promotes effective neovascularization, accelerated wound closing, and fast cicatricial tissue remodeling.[14] Therefore, PRF is used in many fields of regenerative medicine,[15] including orthopedics,[16] oral and maxillofacial surgery,[17] and sports medicine;[16] it has also been used with interesting results for the treatment of skin wound ulcers.[18]

Recently, Aravindaksha et al.[19] presented results from four patients whose palatal donor sites were covered by PRF membranes as palatal bandages, which showed very fast healing.

To the best of the authors' knowledge, to date, no randomized trials have evaluated the usefulness of PRF in the management of soft tissue donor sites by testing whether it could accelerate tissue healing and reduce the patient's morbidity compared with a conventional hemostatic material. This is the aim of the present study.

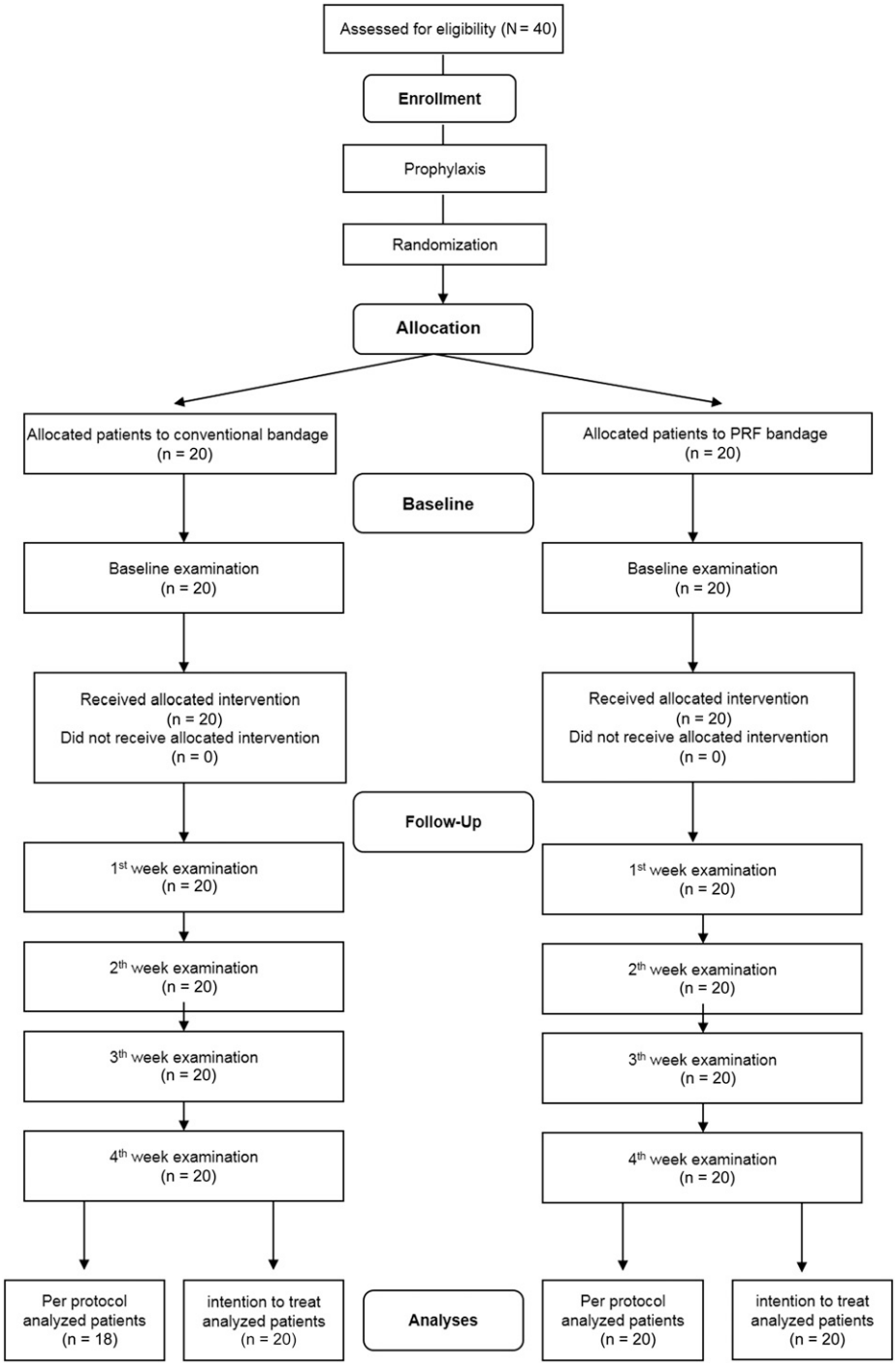

Figure 1.
CONSORT (Consolidated Standards of Reporting Trials)[20] diagram showing the study layout.

MATERIALS AND METHODS

Experimental Design

This was a prospective, controlled randomized clinical trial (RCT) with a parallel design, performed to evaluate the healing time and the patients' morbidity produced by the harvest of an EFGG from the palate. The wounds were treated with PRF (test group) and with an absorbable gelatin sponge (control group) (Fig. 1).[20]

Study Population

Forty patients (15 males and 25 females, aged 18 to 47 years

[mean age, 32.4 ± 5.0 years]) who sought treatment at the Unit of Periodontology of G. D'Annunzio University for at least one site of Miller Class I or II recession[21] (≥3 mm in depth) were selected for this study.

The inclusion criteria for this research were as follows: 1) systemic factors (no systemic diseases; no coagulation disorders; no medications affecting periodontal status in the previous 6 months; no pregnancy or lactation); 2) behavioral factors (no smoking habit); and 3) dental and periodontal factors (a full-mouth plaque score [FMPS][22] and a full-mouth bleeding score [FMBS][23] lower than 20% at the time of surgery; no periodontal surgery on the experimental sites; no inadequate endodontic treatment or tooth mobility at the site of surgery).

The participants volunteered for the study after they received verbal and written information and signed a consent form. The protocol was approved by the Ethical Committee of G. D'Annunzio University for human participants. The study protocol was in accordance with the Declaration of Helsinki of 1975, revised in Tokyo in 2004. This study was performed from May 2013 to January 2014.

This study is registered at ClinicalTrials.gov as NCT02438046.

Sample Size and Randomization

The primary outcome was to assess the time needed to obtain complete re-epithelialization of the palatal wound (CWE). Secondary outcomes were to evaluate the following: 1) the alteration of sensitivity (AS) around the palatal wound; 2) the postoperative discomfort (D); 3) changes in patients' feeding habits (CFH); 4) the consumption of analgesics; and 5) the existence of delayed bleeding from the palatal wound (DWB) during postoperative week 1.

The sample size was calculated to provide a power $1\text{-}\beta = 90\%$ to detect the difference in the proportion of patients who exhibited epithelialization after 3 weeks among patients undergoing FGG and single-incision procedures, as reported in the study by Del Pizzo et al.,[11] with $\alpha = 0.05$. At a minimum, 17.3 patients per treatment arm would have been required. Twenty patients per group were recruited to avoid a loss of statistical power as a consequence of patient drop-out. The balance of experimental groups by age and sex was tested by Student *t* test for unpaired samples and χ^2 analysis, respectively.

Each patient was allocated randomly to one of the experimental groups. Assignment was performed by a custom-made computer-generated table. To conceal allocation, opaque envelopes containing the treatment of the specific patients were assigned to the specific patient and were opened during surgery, immediately before fabricating the palatal bandage.

Surgeon Training

To minimize differences related to the surgical technique, all procedures were performed by one experienced clinician (MP).

To obtain grafts of similar size from all patients, producing wounds of similar characteristics and dimensions, the surgeon underwent preclinical training on animal tissues with the objective of withdrawing a 15 × 8–mm graft of an even 2-mm thickness, as measured in its central part by means of a caliper. The training was continued until the size of the graft (height, width, and thickness) differed by no more than 5% in five consecutive samples.

Presurgical Treatment

All selected patients underwent a session of prophylaxis with instruction in proper oral hygiene measures and professional tooth cleaning. The use of an electric toothbrush with an extrasoft head‡ with controlled pressure§ was recommended. The patients were instructed concerning the optimal use of the electric toothbrush, dental floss, and/or interdental brush.

Intrasurgical Measurement

After local anesthesia, the thickness of the palatal soft tissues in the harvesting area was measured according to Paolantonio.[2] The measurement was made at the midpalatal location, ≈5 mm apical to the gingival margin of the first premolar, by means of a no. 15 endodontic reamer. The reamer was inserted perpendicular to the mucosal surface through the soft tissue with light pressure until a hard surface was felt. The silicone disk stop was then placed in tight contact with the soft tissue surface and fixed with a drop of cyanoacrylate; after careful removal of the reamer, the penetration depth was measured with a caliper accurate to the nearest 0.1 mm. The thickness of the grafts was measured in both the test and control groups using a caliper positioned at the central part of the graft.

The mesial–distal dimensions and the apical-coronal dimensions of the grafts were measured with a manual probe‖ and rounded up to the nearest millimeter. Graft measurements were performed by a different examiner (BF).

Surgical Technique

The 40 patients had their gingival recession treated by a coronally advanced flap (CAF) + CTG surgical technique.

After local anesthesia, the EFGG was harvested as follows: the donor site extended from the distal line angle of the canine to the mesial line angle of the maxillary first molar. The most coronal horizontal incision, 15 mm long, was made ≈2 mm apical from

‡ Oral B Sensitive EBS17, Procter & Gamble, Gattatico, Italy.
§ Oral-B Pro 6000 CrossAction; Procter & Gamble.
‖ XP 23/UNC 15, Hu-Friedy, Chicago, IL.

the gingival margin; a second horizontal incision of the same length was drawn 8 mm away from the first, in a more apical position. Two vertical incisions were made to join the ends of the horizontal incisions and to delimitate the graft area. A rectangular-shaped partial-thickness incision was drawn to obtain a graft ≈2 mm in thickness. The EFGG was then measured; afterward it was trimmed, and the fatty tissue was eliminated. Then, the graft was de-epithelialized with a 15c blade and adapted to the tooth to be grafted.

In the test group (n = 20 patients), the palatal wound was protected by a quadruple layer of PRF, obtained by folding two PRF membranes on themselves; conversely, the control group patients (n = 20) had their wound medicated by an absorbable gelatin sponge.¶ Both bandages were maintained in situ by compressive sling 3-0 silk sutures.#

To prevent immediate and/or delayed bleeding from the donor site, in both experimental groups, two vertical mattress sutures were made mesial and distal to the tissue harvesting site. The ligatures were made with a 2-0 silk suture and a semicircular needle.** The needle was inserted ≈0.5 mm coronal to the coronal horizontal incision and 2 mm distal or mesial to the vertical distal or mesial incision. The needle was left to slide on the bony surface of the alveolar process and was more apically resurfaced beyond the apical horizontal incision of the donor site. These sutures were made with the aim of choking the blood vessels in the submucosa, thereby reducing the bleeding tendency.

PRF Preparation

The PRF was prepared according to Choukroun et al.[24] Immediately before surgery, a 40-mL blood sample was taken by venipuncture of the antecubital vein without anticoagulant, and it was divided into four tubes of 10 mL each. The tubes were centrifuged immediately by a dedicated centrifuge†† at 3,000 rpm for 10 minutes. Such a preparation protocol produces a structured fibrin clot in the middle of the tube, between the erythrocytes at the bottom and acellular plasma at the top. After removal of acellular plasma, the PRF was separated from the erythrocytes using sterile scissors; fibrin membranes were obtained by squeezing the serum from the clot with a specific mechanical press.‡‡

Each membrane thus obtained was turned in on itself, and two membranes placed one over the other (quadruple PRF layer) represented the palatal bandage of the test group.

Postoperative Care

All patients were administered 2 g/d amoxicillin plus clavulanic acid for 6 days;§§ pain was controlled by oral ketoprofen if needed.‖‖ Patients were advised to rinse twice a day with 0.12% chlorhexidine digluconate solution¶¶ for 3 weeks after surgery. Sutures were removed 14 days after the surgery. Plaque control in the surgically treated area was maintained by chlorhexidine rinsing for an additional 1 week after suture removal. Patients were instructed again in mechanical tooth cleaning of the grafted area using an ultrasoft manual toothbrush for 1 month. Patients were recalled once a week for the first 4 weeks after the surgery, when they underwent gentle supragingival professional tooth cleaning and oral hygiene reinforcement. Then, the patients were enrolled into a 3-month maintenance program.

Table 1.

Palatal Thickness and Graft Dimensions in the Experimental Groups

	Test Group	Control Group	Difference
Palatal thickness	3.62 ± 1.22	3.90 ± 1.30	NS
Graft thickness	2.11 ± 0.81	1.89 ± 0.76	NS
Graft width	14.89 ± 2.22	15.02 ± 3.01	NS
Graft height	8.11 ± 01.55	7.93 ± 1.67	NS

NS = not significant.

Wound Healing and Patient Morbidity

Patients were monitored at 1, 2, 3, and 4 weeks after surgery. The following parameters were recorded: 1) CWE; 2) AS; 3) D; and 4) CFH. Furthermore, postoperative pain during the first week was evaluated by recording the patients' mean analgesic (ketoprofen) consumption (in milligrams).[25] In particular, patients were instructed to take ketoprofen if they felt significant pain. Patients who did not fulfill this condition were excluded from the per-protocol analysis.

The number of episodes of DWB during the first postoperative week was also recorded.

CWE was evaluated clinically by the peroxide test.[26] This test is based on the principle that if the epithelium is discontinuous, then H_2O_2 diffuses into the CT; the enzyme catalase acts on H_2O_2 to release water and oxygen. This is shown clinically 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 with a syringe, waiting for the appearance of bubbles, which suggested that the surgical site was not completely epithelialized. CWE was recorded as a dichotomous variable (yes/no). For each observation week, the number of new patients experiencing CWE was

¶ Surgifoam, Ethicon, Johnson & Johnson, Pomezia, Italy.
# Ethicon, Johnson & Johnson.
** PC-02, Process, Nice, France.
†† IntraSpin Centrifuge, Intra-Lock International, Boca Raton, FL.
‡‡ IntraSpin Xpression fabrication kit, Intra-Lock International.
§§ Augmentin, SmithKline Beecham, Milan, Italy.
‖‖ OKi 80, Dompé, L'Aquila, Italy.
¶¶ Dentosan 0.12 monthly treatment, Johnson & Johnson.

Table 2.

Clinical Parameter Scores for Each Week and Pooled Data, Respectively, Using Univariate Tests

Dependent variable	Week 1			Week 2			Week 3			Week 4			Group × Time Effect, 2 × 4 Contingency Tables, Log-Likelihood Ratio, Weeks 1 to 4		
	Test	Difference	Control	Test	Difference	Control	Test	Difference	Control	Test	Difference	Control	Test	Difference	Control
	2 × 2 Contingency Tables (Bonferroni Adjusted Fisher Exact Tests)														
Distribution free CWE in the week (%)															
ITT	0	NS	0	35	NS	10	65	$P = 0.003$	15	0	$P < 0.001$	70		$P < 0.001$	
PP	0	NS	0	35	NS	11	65	$P = 0.003$	11	0	$P < 0.001$	72		$P < 0.001$	
	Univariate Analyses (Mann–Whitney *U* test)														
AS (VAS) PM_e															
ITT	4	NS	4	2.25	NS	3	2	NS	2	1.50	NS	1.50	10	NS	10.5
PP	4	NS	4	2.25	NS	3	2	NS	2	1.50	NS	1.50	10	NS	10.5
D (VAS) PM_e															
ITT	2	$P < 0.001$	4.50	2	$P < 0.001$	4	1	$P < 0.001$	2.50	0	$P = 0.02$	1	5.5	$P < 0.001$	12
PP	2	$P < 0.001$	4.50	2	$P < 0.001$	3.50	1	$P < 0.001$	2	0	$P = 0.02$	1	5.5	$P < 0.001$	11.5
CFH (VAS) PM_e															
ITT	3.50	$P = 0.01$	4.50	1.75	$P < 0.001$	2.50	1	$P < 0.001$	2.50	0	NS	0.50	7	$P < 0.001$	9.50
PP	3.50	$P = 0.02$	4.50	1.75	$P < 0.001$	3	1	$P < 0.001$	2.50	0	NS	0.50	7	$P < 0.001$	9.50
Analgesic usage (mg), mean															
ITT	204	NS	296												
PP	204	$P = 0.04$	328												
	Group × Time Effect, Bonferroni-Adjusted Univariate Tests in RM-MANOVA												Group Effect, Bonferroni-Adjusted Univariate Tests in RM-MANOVA		
Parametric AS (VAS), mean ± SE															
ITT	4.15 ± 0.18	NS	4.3 ± 0.18	2.6 ± 0.2	NS	2.85 ± 0.2	1.85 ± 0.11	NS	1.95 ± 0.11	1.5 ± 0.11	NS	2.52 ± 0.1	NS	2.67 ± 0.1	
PP	4.15 ± 0.18	NS	4.33 ± 0.19	2.6 ± 0.2	NS	2.88 ± 0.22	1.85 ± 0.11	NS	1.94 ± 0.12	1.5 ± 0.11	NS	2.52 ± 0.1	NS	2.68 ± 0.11	
D (VAS), mean ± SE															
ITT	2.4 ± 0.2	$P < 0.001$	4.6 ± 0.2	1.75 ± 0.22	$P < 0.001$	3.75 ± 0.22	1.1 ± 0.18	$P < 0.001$	2.6 ± 0.18	0.15 ± 0.17	$P = 0.004$	1.35 ± 0.12	$P < 0.001$	2.96 ± 0.12	
PP	2.4 ± 0.2	$P < 0.001$	4.5 ± 0.21	1.75 ± 0.21	$P < 0.001$	3.55 ± 0.22	1.1 ± 0.17	$P < 0.001$	2.44 ± 0.18	0.15 ± 0.17	$P = 0.02$	1.35 ± 0.11	$P < 0.001$	2.86 ± 0.12	

Table 2. (continued)
Clinical Parameter Scores for Each Week and Pooled Data, Respectively, Using Univariate Tests

			Group × Time Effect, Bonferroni-Adjusted Univariate Tests in RM-MANOVA						Group Effect, Bonferroni-Adjusted Univariate Tests in RM-MANOVA		
CFH (VAS), mean ± SE											
ITT	3.6 ± 0.19	$P = 0.005$	4.4 ± 0.19	1.7 ± 0.14	$P < 0.001$	2.75 ± 0.14	1.2 ± 0.13	$P < 0.001$	2.35 ± 0.13	0.25 ± 0.1	NS	1.68 ± 0.08	$P < 0.001$	2.46 ± 0.08
PP	3.6 ± 0.19	$P = 0.02$	4.38 ± 0.2	1.7 ± 0.14	$P < 0.001$	2.77 ± 0.15	1.2 ± 0.13	$P < 0.001$	2.27 ± 0.14	0.25 ± 0.1	NS	1.68 ± 0.08	$P < 0.001$	2.45 ± 0.08
Analgesic usage (mg), mean ± SE														
ITT	204 ± 32	NS	296 ± 43.2											
PP	204 ± 32	$P = 0.02$	328 ± 40.8											

ITT = intention-to-treat analysis; PP = per-protocol analysis; PM_e: Hodges-Lehmann pseudo-median score; NS = not significant.

calculated; patients whose palatal wounds were previously completely healed were not considered again.

AS, D, and CFH were evaluated by showing the intensity of the given event on a 100-mm visual analog scale (VAS)[27] divided into 10 segments of 10 mm each and numbered from 0 to 10. AS was scored (0 to 10) by asking the patient to compare the sensitivity he/she had when the surrounding areas of the wound were touched by the tip of a periodontal probe and comparing this feeling to the feeling that he/she felt on the other side of the palate. D was assessed as the level of pain (0 to 10) experienced by the patients during the postoperative experimental weeks as a result of the palatal wound. CFH was described as the degree of change in the patient's eating habits (0 to 10) resulting from the presence of the palatal wound.

DWB was considered to represent the occurrence of prolonged hemorrhaging during the first post-surgical week. This parameter was recorded as the number of observed episodes.

Data Analyses

A double parallel analysis was performed: 1) an "intention-to-treat" analysis on data from all the patients; and 2) a "per-protocol" analysis after excluding patients who did not respect the experimental protocol, i.e., they did not consume analgesics although they experienced significant pain.

The main outcome analysis was made by comparing experimental groups on the frequency distribution of patients who experienced CWE in each of the 4 postoperative weeks. The Williams-adjusted log-likelihood ratio was performed, followed by *post hoc* tests using Bonferroni-corrected Fisher exact test (two-tailed).

The other parameters were analyzed using a distribution-free test (Mann–Whitney *U* test). Because in a previous study[12] the dependent variables were analyzed in univariate contexts, multiple univariate analyses were performed,[28] reporting the correlation matrix among dependent variables according to Huberty and Morris.[28]

Similarly, for the VAS score analyses, the distribution-free test was compared with a repeated-measures multivariate analysis of variance (RM-MANOVA),[29] estimating the time × treatment effect and the confidence intervals (CIs).

Finally, for the first week, differences in painkiller consumption were evaluated by the Mann–Whitney *U* test, followed by a correlation matrix for D, CFH, AS, and painkiller consumption, assessing the association level between the outcome variables. The D, AS, and CFH scores were analyzed using the 4 weeks of pooled data and then the data from each single week. Student *t* test was used to analyze the differences between the control and test groups in the thickness of the palatal mucosa and the graft size. Significance was set at $\alpha = 0.05$.

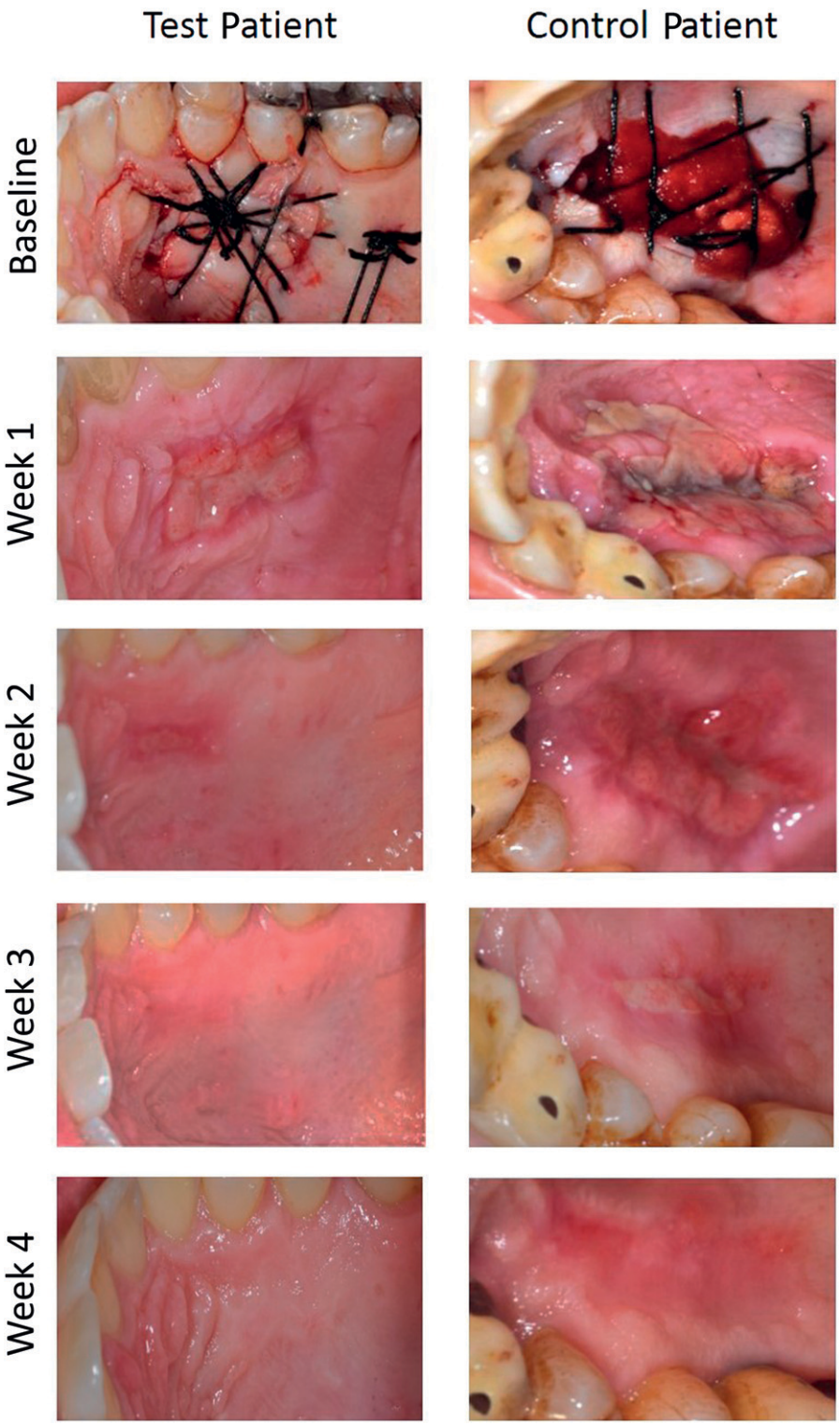

Figure 2.
Healing process at test and control donor sites.

RESULTS

The experimental groups were balanced by age and sex (P>0.05). No patients dropped out of the study, and no postoperative complications were reported by the patients.

The FMPS and FMBS remained <20% throughout the entire study without significant differences between the groups.

At the first week of examination, two patients in the control group reported that they did not take analgesics although they had felt significant pain. Therefore, two separate data analyses were performed: the first according to the intention-to-treat principle and the second according to the per-protocol analysis.

The thickness of the palatal tissues and the dimensional characteristics of the grafts from test and control patients did not show significant differences (Table 1).

Table 2 summarizes the results obtained in this study. The test group showed a significantly faster CWE (P <0.001); more than one third of test patients showed a CWE at the end of postoperative week 2, and at the end of week 3, the palatal wounds of all patients treated with PRF had completely epithelialized; in contrast, one patient from the control group did not show complete healing at the end of postoperative week 4 (Fig. 2).

A similar trend was shown by the assessments of D and CFH: in fact, as early as the postoperative week 1, patients in the test group reported a more favorable evolution of these parameters; these differences remained significant until the end of week 3.

A difference in the AS level between the experimental groups was never detected.

The per-protocol analysis showed that, in week 1, patients from the test group took a significantly lower dose of analgesics compared with the control group patients. This difference was not statistically significant

Table 3.
RM-MANOVA, Test-Control Univariate Differences Between the Means and 95% CIs for Each Week on Clinical Data

	RM-MANOVA: Group × Time Effect, Bonferroni-Adjusted Univariate Tests and CIs																			
	Week 1						Week 2						Week 3						Week 4					
				95% CI					95% CI					95% CI				95% CI				
Dependent Variable	Test – Control Difference Between the Means	SE_d	Bonferroni-Adjusted Significance P	Lower Limit	Upper Limit	Test – Control Difference Between the Means	SE_d	Bonferroni-Adjusted Significance P	Lower Limit	Upper Limit	Test – Control Difference Between the Means	SE_d	Bonferroni-Adjusted Significance P	Lower Limit	Upper Limit	Test – Control Difference Between the Means	SE_d	Bonferroni-Adjusted Significance P	Lower Limit	Upper Limit
AS (VAS)																				
ITT	−0.150	0.25	NS	−0.667	0.367	−0.250	0.29	NS	−0.850	0.350	−0.100	0.15	NS	−0.420	0.220	−0.100	0.16	NS	−0.425	0.225
PP	−0.183	0.26	NS	−0.727	0.361	−0.233	0.30	NS	−0.848	0.381	−0.094	0.16	NS	−0.433	0.244	−0.111	0.16	NS	−0.446	0.223
D (VAS)																				
ITT	−2.200	0.28	<0.001	−2.784	−1.616	−2.000	0.32	<0.001	−2.653	−1.347	−1.500	0.26	<0.001	−2.036	−0.964	−0.750	0.24	0.004	−1.241	−0.259
PP	−2.100	0.29	<0.001	−2.694	−1.506	−1.806	0.30	<0.001	−2.429	−1.182	−1.344	0.25	<0.001	−1.862	−0.826	−0.794	0.25	0.003	−1.304	−0.285
CFH (VAS)																				
ITT	−0.800	0.27	0.005	−1.346	−0.254	−1.050	0.20	<0.001	−1.465	−0.635	−1.150	0.19	<0.001	−1.535	−0.765	−0.100	0.14	NS	−0.399	0.199
PP	−0.789	0.28	0.008	−1.360	−0.217	−1.078	0.21	<0.001	−1.507	−0.648	−1.078	0.19	<0.001	−1.471	−0.685	−0.139	0.15	NS	−0.450	0.172
RM-MANOVA group × time effect: Multivariate test																				
ITT	P <0.001																			
PP	P <0.001																			

ITT = intention-to-treat analysis; PP = per-protocol analysis; SE_d = standard error of differences between means; NS = not significant.

when analyzing the data according to the intention-to-treat principle.

No episodes of DWB were reported by any test or control patient during week 1.

The similarity of the results from distribution-free and parametric tests allowed for the calculation of the CIs (Table 3).[28] The magnitude of the differences between the two experimental groups for the dependent parameters D and CFH suggests the existence of a significant difference from both the statistical and clinical points of view.

According to Huberty and Morris,[28] Table 4 shows the correlations among the dependent variables; a strong correlation among all variables was shown, with the only exception being AS.

DISCUSSION

Although CAF alone produces excellent root-coverage results in the short term,[30] it produces a considerable recurrence of recession in long-term follow-up.[31] However, the addition of a CTG to CAF, while improving the long-term results,[32] requires a palatal surgical site. The use of heterologous materials has been reported to yield fewer benefits than autogenous grafts.[33] The need for a palatal donor site makes periodontal plastic surgery an often painful procedure.[25,34]

Although many techniques with primary-intention healing have been described for CT harvesting,[8] it may sometimes be necessary to take a conventional EFGG, for example, when the palatal tissue is very thin. Moreover, EFGG is easier to obtain and requires less operative time. Furthermore, the high quality of the dense subepithelial tissue is very different from that of the deep palatal tissue, which is rich in adipose and glandular material, is less consistent and not suitable for root coverage, and produces a lesser increase in buccal soft tissue thickness.[35,36]

In a recent case report, Aravindaksha et al.[19] showed that the use of a PRF membrane as a palatal bandage is effective in accelerating soft tissue healing. The present results from this RCT on 40 patients confirm this observation by comparing the use of a PRF membrane with the use of a commonly used hemostatic agent.

When comparing the present data to those from other similar studies, it should be considered that great heterogeneity exists in the treatment of palatal wounds. In fact, Del Pizzo et al.[11] did not use any bandage, Wessel and Tatakis[25] protected the donor site with a stent,

Table 4.

Week 1 and Pooled Data Correlation Matrices From Clinical Data

Dependent Variable	Week 1 Correlation Matrix																Pooled Data Correlation Matrix									
	Analgesic Usage (mg)				AS (VAS)				D (VAS)				CFH (VAS)				AS (VAS)				D (VAS)				CFH (VAS)	
	Pearson		Spearman		Pearson		Spearman		Pearson		Spearman		Pearson		Spearman		Pearson		Spearman		Pearson		Spearman		Pearson	Spearmann
	R	*P*	ρ	*P*	*R*	*P*	ρ	*P*	*R*	*P*	ρ	*P*	*R*	*P*	ρ	*P*	*R*	*P*	ρ	*P*	*R*	*P*	ρ	*P*	*P*	*P*
Analgesic usage (mg)																										
ITT					0.231	NS	0.203	NS	0.191	NS	0.196	NS	0.275	NS	0.267	NS										
PP					0.223	NS	0.200	NS	0.336	0.04	0.360	0.03	0.340	0.04	0.360	0.03										
AS (VAS)																										
ITT	0.231	NS	0.203	NS					0.101	NS	0.073	NS	0.137	NS	0.085	NS					0.288	0.07	0.286	0.07	0.201	0.21	0.148	0.24
PP	0.223	NS	0.200	NS					0.130	NS	0.104	NS	0.148	NS	0.097	NS					0.333	0.04	0.318	0.051	0.206	0.22	0.185	0.27
D (VAS)																										
ITT	0.191	NS	0.196	NS	0.101	NS	0.073	NS					0.518	0.001	0.531	<0.001	0.288	0.07	0.286	0.07					0.785	<0.001	0.809	<0.001
PP	0.336	0.04	0.360	0.03	0.130	NS	0.104	NS					0.504	0.001	0.521	0.001	0.333	0.04	0.318	0.051					0.794	<0.001	0.792	<0.001
CFH (VAS)																										
ITT	0.275	NS	0.267	NS	0.137	NS	0.085	NS	0.518	0.001	0.531	<0.001					0.201	0.21	0.148	0.24	0.785	<0.001	0.809	<0.001		
PP	0.340	0.04	0.360	0.03	0.148	NS	0.097	NS	0.504	0.001	0.521	0.001					0.206	0.22	0.185	0.27	0.794	<0.001	0.792	<0.001		

ITT = intention-to-treat analysis; PP = per-protocol analysis; NS = not significant.

and Zucchelli et al.[12] treated the wound with equine-derived collagen. Conversely, Rossmann and Rees[13] evaluated three treatment modalities for the donor site: 1) oxidized cellulose; 2) an absorbable gelatin sponge; and 3) sterile gauze with external pressure.

In the present study, 35% of the test group showed CWE at the week-2 visit, and 100% showed CWE at week 3, with a significant difference relative to the control group ($P = 0.003$).

These data agree with results from Aravindaksha et al.,[19] who reported complete healing at week 3 in 100% of PRF-treated patients.

A similar outcome was reported by Del Pizzo et al.[11] in patients treated with the single-incision technique.[37] This method is currently considered to be less traumatic, leading to primary-intention healing.[11]

Although this study does not include patients treated with the single-incision technique,[37] a comparison of the present data to those from Del Pizzo et al.[11] suggests that the EFGG technique, along with a PRF bandage, may lead to similar postoperative morbidity and offer greater ease and speed in the procedure and a better graft quality.[35]

In this study, at week 4, complete palatal wound healing is observed in 95% of the control patients. This observation was different from that of Del Pizzo et al.,[11] who reported complete healing in 16% and 50% of patients at weeks 2 and 3, respectively, in EFGG donor sites without any bandage treatment. This can be explained by the difference in methods for evaluating healing. In fact, Del Pizzo et al.[11] assessed the complete wound epithelialization on the basis of a clinical evaluation; conversely, in the present study, the epithelial barrier was considered to be completely formed when no bubble formation occurred after H_2O_2 irrigation. The latter may be considered to be a more objective and reliable evaluation method.

It was hypothesized that the important biologic mediators within PRF are responsible for the shorter amount of time needed for CWE in the test group. The fibrin clot is enough in itself to account for the significant cicatricial capacity of the PRF: in fact, the physiologic three-dimensional fibrin network leads to more effective cell migration and proliferation.[14] PRF represents a combination of cytokines, structural glycoproteins, and glycanic chains that play a synergetic role in healing and stimulating angiogenesis, immunity, and epithelialization.[14] Angiogenesis is enhanced by soluble factors such as vascular endothelial growth factor, platelet-derived growth factor (PDGF), and fibroblast growth factor.[14] Fibroblast proliferation and epithelial cell migration are influenced positively by the presence of fibrin, fibronectin, PDGF, and transforming growth factors.[14]

Another interesting finding from the present study is the lower degree of D and the minor CFH observed in the test group. This observation cannot be compared

with others in the literature because the only other study of PRF palatal bandages[19] did not evaluate the patients' morbidity.

It could be hypothesized that the presence of multilayer PRF exerts mechanical protection, covering the injured sensitive structures exposed by surgery. This may explain why the PRF-treated patients showed significantly less D and CFH as early as week 1. Zucchelli et al.[12] suggested that the thickness of the remaining soft tissues covering the palatal bone plays a pivotal role in reducing the patients' postoperative discomfort and that ≥2 mm of soft tissue should be left to cover the bone. In the present study, the mean soft tissue thickness remaining on the palatal bone in the test group (1.51 mm) (Table 1) is inadequate to prevent significant discomfort. It could be speculated that the multilayered PRF placed on the wound may have produced the same protective effects as a thick residual layer of CT.

The accelerated healing process induced by PRF may explain the test patients' more favorable conditions during the following weeks. The reduced pain experienced by test patients was also demonstrated by their lower use of analgesics compared with control patients (P <0.05) (Table 2). When the lower mean discomfort VAS score from the test group of this study (2.4 ± 0.88) was compared to that from the study by Zucchelli et al.[12] (EFGG group; 3.2 ± 1.99) and bearing in mind that, in the above-mentioned study,[12] the collected gingival grafts were thinner than the present ones, it can speculated that, in adopting a PRF bandage, thicker EFGG can be collected while producing limited postoperative discomfort.

Although Zucchelli et al.[35] showed that thin grafts associated with CAFs obtain excellent root-coverage results with less patient morbidity and better esthetics, there are situations in which a thick graft may be preferable for the clinician (e.g., soft tissue ridge augmentation procedures; Miller FGG technique for root coverage).[38]

In the present study, no difference is observed between the experimental groups concerning the recovery of sensitivity at the donor site. Indeed, the presence of specific biologic agents able to influence the growth of nervous fibers in PRF was not reported. When investigating the nervous regeneration in a rat model, Lichtenfels et al.[39] reported that PRF was unable to significantly enhance peripheral nerve repair.

Finally, the lack of episodes of DWB in both experimental groups during week 1 may be explained by the palatal ligatures placed to constrict the blood vessels within the submucosa.

CONCLUSION

On the basis of the present results, it can be concluded that a PRF palatal bandage produces significant clinical advantages in accelerating palatal wound healing and reducing the patient's morbidity.

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REFERENCES

1. Chambrone L, Sukekava F, Araújo MG, Pustiglioni FE, Chambrone LA, Lima LA. Root-coverage procedures for the treatment of localized recession-type defects: A Cochrane systematic review. *J Periodontol* 2010;81:452-478.
2. Paolantonio M. Treatment of gingival recessions by combined periodontal regenerative technique, guided tissue regeneration, and subpedicle connective tissue graft. A comparative clinical study. *J Periodontol* 2002;73:53-62.
3. Pini Prato G, Clauser C, Cortellini P, Tinti C, Vincenzi G, Pagliaro U. Guided tissue regeneration versus mucogingival surgery in the treatment of human buccal recessions. A 4-year follow-up study. *J Periodontol* 1996;67:1216-1223.
4. Wennström JL. Mucogingival surgery. In: Lang NP, Karring T, eds. *Proceedings of the 1st European Workshop on Periodontology*. London: Quintessence Publishing; 1994:193-209.
5. Ahmedbeyli C, Ipçi ŞD, Cakar G, Kuru BE, Yılmaz S. Clinical evaluation of coronally advanced flap with or without acellular dermal matrix graft on complete defect coverage for the treatment of multiple gingival recessions with thin tissue biotype. *J Clin Periodontol* 2014;41:303-310.
6. Chambrone L, Pannuti CM, Tu YK, Chambrone LA. Evidence-based periodontal plastic surgery. II. An individual data meta-analysis for evaluating factors in achieving complete root coverage. *J Periodontol* 2012;83:477-490.
7. Müller HP, Eger T, Schorb A. Gingival dimensions after root coverage with free connective tissue grafts. *J Clin Periodontol* 1998;25:424-430.
8. Liu CL, Weisgold AS. Connective tissue graft: A classification for incision design from the palatal site and clinical case reports. *Int J Periodontics Restorative Dent* 2002;22:373-379.
9. Edel A. Clinical evaluation of free connective tissue grafts used to increase the width of keratinised gingiva. *J Clin Periodontol* 1974;1:185-196.
10. Farnoush A. Techniques for the protection and coverage of the donor sites in free soft tissue grafts. *J Periodontol* 1978;49:403-405.
11. Del Pizzo M, Modica F, Bethaz N, Priotto P, Romagnoli R. The connective tissue graft: A comparative clinical evaluation of wound healing at the palatal donor site. A preliminary study. *J Clin Periodontol* 2002;29:848-854.
12. Zucchelli G, Mele M, Stefanini M, et al. Patient morbidity and root coverage outcome after subepithelial connective tissue and de-epithelialized grafts: A comparative randomized-controlled clinical trial. *J Clin Periodontol* 2010;37:728-738.
13. Rossmann JA, Rees TD. A comparative evaluation of hemostatic agents in the management of soft tissue

graft donor site bleeding. *J Periodontol* 1999;70:1369-1375.
14. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101: e37-e44.
15. Bielecki T, Dohan Ehrenfest DM. Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF): Surgical adjuvants, preparations for in situ regenerative medicine and tools for tissue engineering. *Curr Pharm Biotechnol* 2012;13: 1121-1130.
16. Dohan Ehrenfest DM, Andia I, Zumstein MA, Zhang CQ, Pinto NR, Bielecki T. Classification of platelet concentrates (platelet-rich plasma-PRP, platelet-rich fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: Current consensus, clinical implications and perspectives. *Muscles Ligaments Tendons J* 2014;4:3-9.
17. Del Corso M, Vervelle A, Simonpieri A, et al. Current knowledge and perspectives for the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in oral and maxillofacial surgery part 1: Periodontal and dentoalveolar surgery. *Curr Pharm Biotechnol* 2012;13:1207-1230.
18. Cieslik-Bielecka A, Choukroun J, Odin G, Dohan Ehrenfest DM. L-PRP/L-PRF in esthetic plastic surgery, regenerative medicine of the skin and chronic wounds. *Curr Pharm Biotechnol* 2012;13:1266-1277.
19. Aravindaksha SP, Batra P, Sood V, Kumar A, Gupta G. Use of platelet-rich fibrin membrane as a palatal bandage. *Clin Adv Periodontics* 2014;4:246-250.
20. The CONSORT Group. CONSORT. Available at http://www.consort-statement.org/. Accessed May 2, 2013.
21. Miller PD Jr. A classification of marginal tissue recession. *Int J Periodontics Restorative Dent* 1985;5:8-13.
22. O'Leary TJ, Drake RB, Naylor JE. The plaque control record. *J Periodontol* 1972;43:38.
23. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. *Int Dent J* 1975;25:229-235.
24. Choukroun J, Adda F, Schoeffler C, Vervelle A. An opportunity in perio-implantology: The PRF (in French). *Implantodontie* 2000;42:55-62.
25. Wessel JR, Tatakis DN. Patient outcomes following subepithelial connective tissue graft and free gingival graft procedures. *J Periodontol* 2008;79:425-430.
26. Silva CO, Ribeiro Edel P, Sallum AW, Tatakis DN. Free gingival grafts: Graft shrinkage and donor-site healing in smokers and non-smokers. *J Periodontol* 2010;81: 692-701.
27. Aitken RC. Measurement of feelings using visual analogue scales. *Proc R Soc Med* 1969;62:989-993.
28. Huberty CJ, Morris JD. Multivariate analysis versus multiple univariate analysis. *Psychol Bull* 1989;105:302-308.
29. Koch GG. The use of non-parametric methods in the statistical analysis of a complex split plot experiment. *Biometrics* 1970;26:105-128.
30. Zucchelli G, Mele M, Mazzotti C, Marzadori M, Montebugnoli L, De Sanctis M. Coronally advanced flap with and without vertical releasing incisions for the treatment of multiple gingival recessions: A comparative controlled randomized clinical trial. *J Periodontol* 2009;80:1083-1094.
31. Pini Prato G, Rotundo R, Franceschi D, Cairo F, Cortellini P, Nieri M. Fourteen-year outcomes of coronally advanced flap for root coverage: Follow-up from a randomized trial. *J Clin Periodontol* 2011;38:715-720.
32. Pini-Prato GP, Cairo F, Nieri M, Franceschi D, Rotundo R, Cortellini P. Coronally advanced flap versus connective tissue graft in the treatment of multiple gingival recessions: A split-mouth study with a 5-year follow-up. *J Clin Periodontol* 2010;37:644-650.
33. Cairo F, Nieri M, Pagliaro U. Efficacy of periodontal plastic surgery procedures in the treatment of localized facial gingival recessions. A systematic review. *J Clin Periodontol* 2014;41(Suppl. 15):S44-S62.
34. Curtis JW Jr., McLain JB, Hutchinson RA. The incidence and severity of complications and pain following periodontal surgery. *J Periodontol* 1985;56:597-601.
35. Zucchelli G, Mounssif I, Mazzotti C, et al. Does the dimension of the graft influence patient morbidity and root coverage outcomes? A randomized controlled clinical trial. *J Clin Periodontol* 2014;41:708-716.
36. Harris RJ. Histologic evaluation of connective tissue grafts in humans. *Int J Periodontics Restorative Dent* 2003;23:575-583.
37. Hürzeler MB, Weng D. A single-incision technique to harvest subepithelial connective tissue grafts from the palate. *Int J Periodontics Restorative Dent* 1999;19: 279-287.
38. Miller PD Jr. Root coverage using a free soft tissue autograft following citric acid application. Part 1: Technique. *Int J Periodontics Restorative Dent* 1982;2:65-70.
39. Lichtenfels M, Colomé L, Sebben AD, Braga-Silva J. Effect of platelet rich plasma and platelet rich fibrin on sciatic nerve regeneration in a rat model. *Microsurgery* 2013;33:383-390.

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