

PROTOCOL 29/9/2017

Patient morbidity after palatal free gingival grafts with or without PRF membranes coverage: a comparative randomized clinical trial.

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Introduction

Gingival recessions could be described as the retraction of gingival margin apically at the enamel-cement junction which causes exposition of root surface.

Gingival recessions can be treated with several surgical techniques, the choice depends on factors such as dimensions of the recession, presence or lack of keratinized mucosa close to the recession, depth of vestibule, presence of frenulums and height of soft tissue between teeth (Wennstrom, 1994).

Scientific literature seems to show that the add of a connective tissue graft (CTG) to a coronally advanced flap (CAF) could improve the probabilities of success in the long period, especially in patient with thin biotype (Cortellini 2009).

With the purpose of achieving primary intention palatal wound healing some connective tissue-harvesting procedures have been described: Trap-door (Edel, 1974) and Single-incision technique (Hurzeler & Weng, 1999 ; Lorenzana & Allen, 2000), that are characterized by a primary split-thickness access flap elevation, the withdrawal of CTG and the complete closure of the palatal wound suturing the access flap.

Trap-door and Single-Incision techniques, involving the elevation of a primary flap, determine the withdrawal of a deeper connective tissue graft with more risk of including adipose and glandular tissue, reducing the efficacy of root coverage operation.

Furthermore, these techniques need an adequate thickness of the palatal fibromucosa to avoid necrosis or desquamation of the undermined superficial flap due to compromised vascularization (Harris, 2003; Del Pizzo, 2002; Jahnke, 1993) .

An alternative proposal is the withdrawal of an epithelium-connective graft which undergoes disepithelialization before its utilization (De-epithelialized gingival grafts, DGG) (Harris, 2003; Zucchelli, 2010), with subsequent secondary intention healing of the palatal donor site.

For DGG technique, instead, a lesser thickness of palatal fibromucosa is enough for obtaining a dense and stable connective graft, optimal for root coverage (Harris, 2003).

Few prospective comparative studies (Hurzeler & Weng, 1999; Griffin, 2006; Wessel&Tatakis, 2008) reported greater incidence of post-operative pain for DGG compared to CTG procedures, except for cases with necrosis or dehiscence of the primary flap.

However, limits of these studies are due to lack of data about dimensions of grafts harvested from the palate.

Zucchelli & co-authors 2010 demonstrated that post-operative morbidity after CTG and DGG techniques doesn't show statistically significant differences; factors that influence post-surgical consumption of pain killers are not the type of healing (primary or secondary) of the palatal wound, but the height and depth of the graft harvesting.

Furthermore, CTG techniques increase surgical time (Zucchelli, 2010) which is related to post-surgical pain (Cortellini, 2009; Griffin, 2006).

Since 1988 platelets concentrates have been proposed in several medical fields with the aim of improving wounds healing and reducing post-operative morbidity.

In dentistry only few authors investigated the use of platelets concentrates on palatal donor sites after connective tissue graft harvesting (Shayesteh, 2012; Yen, 2007) and none of them used L-PRF (Leucocyte and Platelet Rich Fibrin) (Choukroun, 2006; Giannini 2015).

The aim of this study is to evaluate complications and post-operative morbidity in patients that undergo epithelium-connective graft harvesting from palate with or without the use of autogenous PRF membranes for covering the donor site wound.

Material and methods

42 subjects were selected from those seeking care at the Department of oral surgery, Dental clinic G.Vogel, San Paolo hospital, Milan.

The study protocol, questionnaires and informed consent, in full accordance with the ethical principles of the Declaration of Helsinki of 1975, as rivisited in 2000, received the approval by the local ethic committee. All patients agreed to participate in the study and signed a written informed consent according to the above-mentioned principles.

All participants met the study inclusion criteria: age range 18-60 years,

- FMPS and FMBS < 20%,
- presence of two adjacent Miller's Class I and II recession defects on natural teeth (≥ 2 mm in depth);
- periodontally and systemically healthy;
- controindications for periodontal surgery
- taking medications or having deseases known to interfere with periodontal tissue health or healing and coagulation.
- Subject smoking more than 10 cigarettes a day
- Gingival recessions on molar teeth were excluded.

Study design

The study was a double-blinded, randomized-controlled clinical trial with a parallel design. The technique of covering the palatal wound with PRF membranes (Test Group) was compared with the use of hemostatic agents with oxidized and regenerated cellulosa (control group).

The study protocol involved a screening appointment to verify eligibility, surgical therapy, evaluation of patient morbidity 2 weeks after surgery, post-operative clinical evaluation 3,14,30 and 90 days after the surgery.

Sample size and randomization

To obtain a statistical power of 80% , $\alpha = 0,05$ and $\beta = 0,8$ were used, considering the previous randomized comparative study (Wessel&Tatakis, 2008) that had patient morbidity as the primary outcome.

As a minimum, 18 patients per treatment arm would have been required.

Patients were assigned to one of the two treatment groups using a computer-generated randomization table. Allocation concealment was achieved using two sealed coded opaque envelope containing the treatment of the specific subject.

The operator responsible of the blood collection (BC) was not part of the surgery team.

Before surgery the BC opened the first envelope determining the Group of the patient: blood collection, for preparing the platelets concentrate, took place only in Test Group.

Whereas the surgeons opened the second envelope only after the graft harvesting, before suturing.

Principal evaluated outcomes

The patient morbidity was evaluated with a questionnaire given to patients 1 week following surgery considering parameters such as post-operative pain, discomfort, bleeding, stress and inability to chew. The questionnaire included the evaluation of the intensity of these parameters on a visual analogical scale (VAS) of 100 mm (Hjermstad, 2011). Discomfort was defined as the level of soreness experienced by the patients during the first post-operative week due to the palatal wound and how it influenced the ability to work and the quality of the sleep.

Bleeding was considered to be the prolonged hemorrhaging during the post-surgical week reported by the patient. Stress was related to the level of apprehension and fear experienced by the patients of jeopardizing the palatal wound. Inability to chew was described as the level of variation of the patients' eating habits due to the presence of the palatal wound.

Patients were asked also to evaluate the pain related to the palatal wound and the pain related to the grafting site, determining how much they can discriminate them.

Post-operative pain was also indirectly evaluated on the basis of the mean consumption (in mg) of analgesics (Ibuprofen). A closed box of pills was given to the patients recommending them to bring it back at the control visits allowing to count the number of consumed pills.

A blind operator evaluated clinical healing of the donor site 3,14,30 and 90 days after surgery. Sutures removal took place after 2 weeks. At any control visit photos of the palatal sites were taken and any complication such as marginal tissue necrosis, hematoma, infection, sensibility disorders was recorded.

Secondary outcomes

- Surgical chair time was measured in both groups using a chronometer from the first incision to the last suture.
- Evaluation of the thickness of the epithelium-connective tissue graft that was withdrawn and estimate the thickness of the residual connective tissue in the donor site. A preliminary measurement was performed by using a needle with a silicon endodontic stop which pass through 5 holes of a standardized template which allowed to obtain the mean thickness of the palatal fibromucosa

The measurement was then repeated in the same 5 points on the withdrawn graft in order to obtain the mean thickness of the graft and indirectly the thickness of the residual tissue in the palatal donor site.

PRF Protocol

Venous blood was collected with a butterfly needle in two 10 mL tubes (Venosafe ref: VF- 109SP, Venosafe™, Teramo, Roma, Italia) without anticoagulants or other chemicals. The tubes were immediately centrifuged at 3000 rpm for 10 minutes (Hettich Zentrifugen EBA 20 - HETTICH ITALIA SRL - Via Prestinari, 4 - 20158 Milano, Italy).

At the end of centrifugation a fibrin clot (PRF) was obtained in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma at the top. The PRF clot was taken from the tube (Fig.3) and the red cells portion at the base of the clot was eliminated with sterile scissors (Fig.4) Then the PRF was enveloped in sterile gauzes and positioned between two glass plates: the compression drove out the serum from the clot obtaining PRF membranes (Fig.5) that were ready to be used in the surgical site (Fig.6).

Surgical techniques

After local anesthesia with Mepivacaine 2% + Adrenalin 1 : 100.000, bilaminar (CAF + CTG) technique was performed in both patient groups to accomplish root coverage.

Donor site and receiving site were contralateral, in this way the patient could more easily distinguish the pain related to the two surgical areas.

The connective harvesting was realized with the help of a single-use rectangular template with standardized dimensions 20 x 6 mm and 5 holes which permitted the measurement of the thickness of palatal fibromucosa in five repeatable points (Fig.1)

Standardized dimensions of the template were obtained estimating the average amount of CTG needed for the treatment of the types of gingival defects included in the study.

The template was kept adherent to the palatal mucosa in the premolar-molar area for guiding the incisions needed for the graft harvesting; the first incision was realized 1-1,5 mm apical to the gingival margin of the adjacent tooth.

The thickness of the graft was maintained uniform during the withdrawal. Once the graft was separated, the template permitted to repeat the measurements of thickness through its five holes (Fig.2). Then the disepithelization was performed using a 15C blade.

The exposed roots surfaces were then covered by the connective grafts which were sutured to the remaining soft tissue of interdental papillae.

The graft was covered by the coronally advanced flap which was sutured with sling sutured ancore around the palatal cingulum of teeth with gingival recessions.

The surgeons were then informed of which type of palatal wound covering to perform by opening the labelled envelope, which contained the patient's number with the assigned treatment.

In the Control Group hemostatic agents with oxidized and regenerated cellulosa (TABOTAMP; Ethicon Sarl. Rue de Puits Godet, 20. 2000 Neuchatel. Switzerland) were sutured to the palatal wound with mattress suture (silk 5/0).

In the Test Group the same suture technique was used for covering the donor site with PRF membranes (Fig.6).

Post-surgical protocol

All the patients were treated with amoxicillin + clavulanic acid 2g 1 hour before surgery and 1g 6 hours after, then the therapy continued for 5 days with 1g every 12 hours.

In case of penicillins allergy, patient received clarithromycin 500mg 1 hour before surgery and 250mg 6 hours after, then 1 dose every 12 hours for 5 days.

Patients received ibuprofen 600mg at the beginning of surgical procedure; subsequent doses were taken only if necessary to control pain and registered in the questionnaire.

No patient was allergic to ibuprofen.

Patients were instructed not to brush their teeth in treated area until suture removing but to rinse with chlorhexidine solution (0,12%) three times a day for 1 minute. Then the patient started to use a ultra-soft toothbrush and fit flos wire.

A diet based on soft and cold foods was suggested, taking care to chew on the opposite side of the mouth with respect to the donor site for the first week.

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