GINGIVAL THICKNESS CHANGES AFTER USE OF PLATELET RICH FIBRIN MEMBRANE COMPARED TO SUB EPITHELIAL CONNECTIVE TISSUE GRAFT AND CORONALLY ADVANCED FLAP ALONE: A CONTROLLED AND RANDOMIZED CLINICAL TRIAL

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Study Protocol

This is a prospective, randomized and controlled clinical trial comparing the effects of three surgical treatment modalities for Miller's class I and II recessions on gingival phenotype: CAF with L-PRF (CAF+L-PRF group), CAF with SCTG (CAF+SCTG group) and CAF alone (CAF group). Clinical parameters were evaluated at baseline and 6 months after surgery. This study is registered at ClinicalTrials.gov as NCT03712852 from October 19th, 2018. First participants were enrolled in November 2018 and final data from last participants were collected in July 2019. Figure 1 shows the CONSORT flow diagram.

Patients' inclusion criteria

1)to be systemically healthy; 2)not having taken any medications related to periodontal status in the previous 6 months; 3)not being pregnant/lactating; 4)to be never-smoker/former-smoker≥10yrs; 5)to have a full-mouth plaque score (FMPS)[25] and full-mouth bleeding score (FMBS)[26] <20% at surgery, 6)to have ≥20 teeth without dental mobility, 7) not having periapical lesions at experimental sites, 8) to have at least one Miller's class I or II recession; each patient participated with a single recession. When more than one GR

was present and treated, the deepest one only was included in the analysis. Patient's concern for GR progression, aesthetics and dentinal hypersensitivity were the main indications for the surgical procedure. The patients signed a consent form approved by the ethical committee of the G. D'Annunzio University after having received comprehensive information. The study is in accordance with the declaration of Helsinki of 1975, as revised in 2013.

Sample Size

The primary outcome of the study was GT gain at 6 months. According to a previous study[20] a sample size of 16 patients per group was calculated to detect at the 6-months follow-up a minimum difference of 0.3 mm in GT between the groups, with an expected standard deviation of 0.3 mm, an α set at 0.05, and a power of 0.80. To compensate possible dropouts, 20 patients for group were recruited.

Randomization and Blinding protocol

The trial director was responsible for randomly assigning patients to treatment after enrollment and was not involved with the clinical interventions or the study measurements. A computer-generated table[1§] was used to make the random assignment, known only to the trial director. An opaque envelope concealed group allocation and was opened just before the intervention surgery. Matching between group and treatment was performed by a figure extraneous to the experimentation, responsible even for keeping and breaking the blinding, and known only to him.

A blood draw, needed for the CAF+L-PRF treatment, was done to all patients. Patients and examiners were masked to group membership; clinical examiners were blinded to each other. The study analyst was also blind to group membership. The analyst received the data by groups labeled as A and B and returned two 90%CIs for the differences (A minus B and vice versa). The blind was not broken until after study completion and the correct difference retained.

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Pre-Surgical Treatment

Participants were instructed with adequate oral hygiene methods. It was suggested the use of an electric toothbrush with pressure control[2‖] and extra-soft head[3¶]. Instructions on the optimal use of dental floss, and/or interdental brush were given. All patients underwent professional supragingival scaling, and they were strictly monitored about the maintenance of periodontal health.

Clinical Measurement

All clinical parameters were measured by the same investigator (PS) at baseline and at 6-month. GT was measured 1 mm apical to the sulcus depth, using a #15 endodontic reamer[4#] that was inserted at the mid-buccal site of each tooth. The reamer was inserted perpendicularly to the gingival surface until the hard tissue was reached. A silicon disk stop was moved to contact the soft tissue surface, and this position was mantained by a cyanoacrylate adhesive drop. Once the reamer was gently removed, the GT was calculated measuring the distances from the reamer's tip to the silicon disk with a caliper[5**] accurate to the nearest 0.1 mm. Other measurements were accurately recorded to the nearest millimeter with a periodontal probe[6††]. GR was measured from the cement-enamel junction (CEJ) to the mid-buccal point of the gingival margin. KT resulted from the distance of the mid-buccal site of the gingival margin to the mucogingival junction. PD and CAL were measured as the distance between the bottom of the pocket and the gingival margin and CEJ, respectively.

Surgical Technique

All surgical procedures (figure 2) were performed by a single expert clinician (MP).

‖ Oral-B Pro 6000 Cross Action; Procter & Gamble Italia SPA Gattatico (RE)
¶ Oral B Sensitive EBS17; Procter & Gamble Italia SPA Gattatico (RE)
# Mani, Reamer lenght 25 mm, size 15, Utsonomiya shi, Japan
** Mitutoyo , Model CD-6'' B , Andover, U.K.
†† XP 23/UNC15, Hu-Friedy MFG-Co, Inc., Chicago, IL, USA

The root surface was accurately treated using Gracey curettes. First, two horizontal incisions were performed mesial and distal to the GR at a distance from the tip of the anatomical papillae equal to the depth of the recession plus 1 mm. Secondly, two oblique incisions were made from the end of the two horizontal incisions to the alveolar mucosa. The achieved trapezoidal-shaped flap was elevated using a split-full-split approach[27] until the CEJ of the tooth could be passively covered by the marginal portion of the flap. The anatomic papillae coronal to the horizontal incisions were disepithelized to supply a connective tissue surface to which the flap's surgical papillae were sutured. In L-PRF+CAF and CAF+SCTG groups, the L-PRF grafts and the SCTGs were placed over the exposed root surface at the CEJ level. The grafts were stabilized by sutures and covered by the flap that was coronally positioned and sutured about 1-2 mm over the CEJ in a tension-free position.

L-PRF Preparation

The Choukroun et al. [28] protocol was applied to produce L-PRF immediately before surgery. From each patient of both groups, to avoid unblinding, 30 ml of blood was collected in three 10-ml sterile tubes without anticoagulant, and it was quickly centrifuged[7‡‡] at 3,000 revolutions/minute for 10 minutes.

The fibrin clot (L-PRF) was collected and squeezed in the L-PRF Box[8§§] to obtain 2 membranes: each membrane was turned in on itself, and two membranes placed one over the other (quadruple L-PRF layer) represented the L-PRF graft.

Connective Tissue Graft Preparation

The connective tissue graft was harvested from the palatal area on the opposite side of the gingival defect according to Zucchelli et al[12]. Briefly, two horizontal and two vertical

‡‡ IntraSpin™, Intra-Lock System Europa SpA, Salerno, Italy
§§ Xpression™ Fabrication Kit, Intra-Lock System Europa SpA, Salerno, Italy

incisions delimitated the donor area. The graft was separated from the underlying tissues by the scalpel's blade oriented parallel to the palatal surface to obtain an about 2 mm thick graft. Then, the graft was de-epithelialized by a 15c blade and the fatty tissue was eliminated until obtaining a graft with a thickness of about 1,5 mm. measured by a standard caliper[9]‖‖ .

Postoperative Care

All patients received 2 g/day amoxicillin+clavulanic acid[10]¶¶ for 6 days for post-operative infection prevention; 400 mg of oral ibuprofen[11]##, twice daily, controlled the pain; 0.12% chlorhexidine[12]*** rinses, twice daily for 3 weeks, were prescribed to the patients. Sutures were removed after 14 days. Only 2-4 weeks after sutures removal, respectively, cautious brushing by a soft toothbrush and interdental brushing were recommended; meantime, the patients used a 1% chlorhexidine gel[13]††† twice daily. Weekly supragingival professional hygiene and motivational reinforcement were administered to the patients for 6 weeks.

Statistical Analysis

Statistical software R 3.5.1 was used to analyze the clinical data. Descriptive statistics of GT, KT, CAL, REC, and PPD were planned, expressed as observed means ± SDs and

‖ Mitutoyo , Model CD-6'' B , Andover, U.K.
¶¶ Augmentin, SmithKline Beecham, Milan, Italy
Nurofen Express 400 mg, Reckitt Benckiser Group, Slough, Berkshire, UK
*** Dentosan 0.12 Trattamento Mese, Johnson & Johnson, Pomezia, Italy
††† Corsodyl Dental gel, GlaxoSmithKline Consumer Healthcare S.p.A. - Baranzate, Italy

95% confidence intervals. Multiple univariate analyses for each variable were performed. GT was analyzed by non-parametric Cliff's delta tests to assess group dominance and by a Heteroscedastic ANOVA with Games-Howell posthoc tests. A sensitivities analysis with different types of robust analyses (M-estimators and High Breakdown LTS Estimators, both with Huber’s, Hampel’s and Biweight’s loss functions) was conducted to get an effect-size estimate by means of Bootstrap Bias Corrected and accelerated (BCa) 95% Confidence Intervals. All the outcomes were analyzed by posthoc Nemenyi's tests; CAL with a Moderated Regression (Treatment by Baseline values) too, while their interaction probed by the Johnson-Neyman technique.

RESULTS

Study Population

Sixty patients (twenty-seven women), between 18 and 47 years (mean 32.4 ± 5.0 years), were enrolled in this study after having been visited at the Unit of Periodontology of the “G. D’Annunzio” University. All 60 patients completed the trial fitting with specifications, and no postoperative complications were detected. Experimental groups were balanced by age and gender ($p>0.05$).

Clinical Outcomes

A single recession from each patient was evaluated. Out of 60 recessions examined, 35 were classified as Miller Class I (10 incisors, 13 canines and 12 premolars), and 25 were classified as Class II (7 incisors, 10 canines and 8 premolars).

The results obtained in this study are summarized in Tables 1 and 2, and in Fig. 3 and 4.

GT significantly increased from baseline in all experimental groups.

CAF + SCTG and CAF + L-PRF groups showed a significantly greater GT increase (0.99 ± 0.02 and 0.92 ± 0.52 respectively) as compared to the CAF group (0.31 ± 0.10) while their direct comparison was inconclusive (NS).

KT showed a significant increase from baseline to the 6 months follow-up in the CAF+SCTG group, while the same result was not observed in CAF+L-PRF and CAF groups. In particular, KT has more than doubled in the CAF+SCTG group, and this increase was significantly greater when compared to CAF+L-PRF and CAF groups; KT increase was not significantly different between the latter two groups.

GR significantly decreased and CAL significantly improved in each group from baseline to the 6 months follow-up, without significant differences.

PD showed limited changes in the experimental period without significant differences among groups, the only exception being CAF+SCTG and CAF groups comparison ($p = 0.012$).

Figure 3 shows pairwise group comparisons for all clinical parameters and the Johnson-Neyman technique probing the interaction between treatment and CAL baseline in the

CAL gain regression for the CAF+PRF and CAF-SCTG group comparison.