



Gingival phenotype changes after different periodontal plastic surgical techniques: a single-masked randomized controlled clinical trial

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Abstract

Objectives Aim of this clinical study was to evaluate the effects on gingival thickness of three surgical techniques for root coverage: the coronally advanced flap (CAF) alone, with a sub-epithelial connective tissue graft (SCTG) or with leukocyte- and platelet-rich fibrin (L-PRF) membranes.

Methods Sixty patients with RT1 single maxillary gingival recession were treated with CAF+L-PRF (20 patients), CAF+SCTG (20 patients) or CAF alone (20 patients). At baseline and 6-month after treatment, gingival thickness (GT), keratinized tissue width (KT), gingival recession (GR), clinical attachment level (CAL), probing depth (PD), PROMs, and the aesthetic outcome were recorded.

Results CAF+SCTG and CAF+L-PRF groups showed a significantly greater mean GT increase than CAF alone (0.31 ± 0.10 mm) with no significant differences between CAF+SCTG (0.99 ± 0.02 mm) and CAF+L-PRF (0.92 ± 0.52 mm) groups ($p=0.55$). CAF+SCTG was associated with a significantly greater KT gain (3.85 ± 1.04 mm), while in CAF+L-PRF (2.03 ± 0.53 mm) and CAF (1.50 ± 0.69 mm) groups, KT was not significantly increased. Both GR and CAL showed a significant within groups' improvement, without among-groups differences. No significant among-groups difference for the aesthetic outcome but greater discomfort and pain-killer consumption in CAF+SCTG group was detected.

Conclusion All investigated surgical techniques produced significant GR reduction and CAL gain. GT was similarly augmented by CAF+L-PRF and CAF+SCTG techniques; however, the CAF+SCTG technique produced a more predictable KT and GT increase.

Clinical relevance The results of our study suggest that the CAF+SCTG technique represents the most predictable method for the clinician to improve the gingival phenotype, an important factor for long term gingival margin stability.

Keywords Clinical trial · Gingival recession · Grafts · Gingival thickness · Plastic periodontal surgery

Introduction

The 2017 Consensus Report of the World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions recommends the use of “periodontal phenotype” to indicate the combination of gingival phenotype and buccal bone morphotype [1]. Gingival phenotype refers to the three-dimensional gingival volume covering the buccal bone plate; it may be affected by environmental factors and modified by clinical interventions [1]. Gingival phenotype is determined by gingival thickness (GT) and keratinized tissue width (KT); these anatomic features allow us to classify the “phenotypes” in three categories: thin scalloped, thick flat, and thick scalloped [2]. The influence of soft tissue thickness on both the

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etiology of gingival recessions (GRs) and the clinical outcomes after root coverage procedures has been highlighted by several authors [3, 4]. It is commonly accepted that the thin phenotype is more prone to develop GRs [5]; similarly, worsening GRs are often associated with a thin phenotype [6]. Therefore, when covering exposed roots, it is desirable to obtain a thick gingival tissue to reduce the risk of recurrence [7], especially when the initial flap thickness is less than 0.8mm [8]. A review conducted by Hwang and Wang concluded that complete root coverage (CRC) was directly related to flap thickness [4]. Currently, the coronally advanced flap (CAF) alone or with the addition of a sub-epithelial connective tissue graft (SCTG), are the two main approaches for treating GRs [9]. CAF+SCTG demonstrated to be a predictable treatment of GRs, particularly in a long-term scenario [10], but the resulting palatal wound unavoidably increases patient's morbidity [11].

On the other hand, CAF technique, while avoiding a second surgical site, reduces the surgical time and shows a better post-operative course, but is less effective in preventing GRs recurrence [12].

Leukocyte- and platelet-rich fibrin (L-PRF) may represent a valuable alternative to autogenous soft tissue grafts. L-PRF belongs to a group of second-generation blood autologous products, obtained from peripheral blood centrifugation without any anticlotting agent, to obtain a dense three-dimensional clot architecture concentrating platelets, fibrin, leukocytes, cytokines, and growth factor [13]. It promotes stabilization and revascularization of the flaps [14], contributes to soft tissue wound healing, and reduces post-operative discomfort [15]. L-PRF is used in various fields of regenerative medicine as well as in periodontal plastic surgery [16, 17].

The main aim of surgical procedures for the treatment of gingival recessions is to achieve a predictable root coverage [1]. This outcome has been extensively investigated by previous literature, while only few studies assessed the role of GT in obtaining and maintaining the complete root coverage over time [18].

Little is known about the occurrence of significant changes of the gingival thickness following CAF+L-PRF treatment [19–21]; GT increase seems to occur when a L-PRF membrane is used in association with CAF [20], while KT augmentation is still controversial [19, 22].

The purpose of this study was to investigate in what extent gingival thickness changes may affect the root coverage after three surgical techniques, CAF, CAF+SCTG, and CAF+L-PRF.

Materials and methods

Experimental design

This is a prospective, randomized, and controlled single-blind clinical trial comparing the effects of three

surgical treatment modalities for RT1 [23] single gingival recessions on gingival thickness: 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. The study protocol was registered at ClinicalTrials.gov as NCT03712852 after the approval obtained by the G. D'Annunzio University ethic committee and the first participant was enrolled in November 2018. Figure 1 shows the CONSORT [24] flow diagram.

Patients' inclusion criteria

(1) To be systemically healthy; (2) no medications affecting the periodontal status in the previous 6 months; (3) no pregnancy/lactating; (4) never-smoker/former-smoker ≥ 10 years; (5) full-mouth plaque score (FMPS) [25] and full-mouth bleeding score (FMBS) [26] $< 20\%$ at surgery, (6) ≥ 20 teeth without dental mobility, (7) no periapical lesions at experimental sites, (8) one maxillary RT1 buccal gingival recession. Each patient with a single maxillary recession was screened among patients diagnosed with gingival recession after having visited at the Unit of Periodontology of the G. D'Annunzio University of Chieti-Pescara, Italy.

Patient's concern for GR progression, aesthetics, and dentinal hypersensitivity was 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 sample size was determined using a multi-arm clinical trial model. An expected standard deviation of 0.3 mm in GT was set according to a previous study [27]. A sample size of 18 patients per group was calculated using three pairwise test (familywise adjusted by the Bonferroni methods) to detect at the 6-month follow-up a minimum difference of 0.3 mm in GT among the three groups with an α set at 0.05 and a power of 0.80. The number of patients was increased of 10% (20 patients per each arm) considering possible dropouts.

Randomization and blinding protocol

Random allocation of defects to groups was obtained by a computer-generated table (R Core Team 2020), opaque envelopes were used to conceal group allocation and opened during surgery after defect's debridement. Matching between

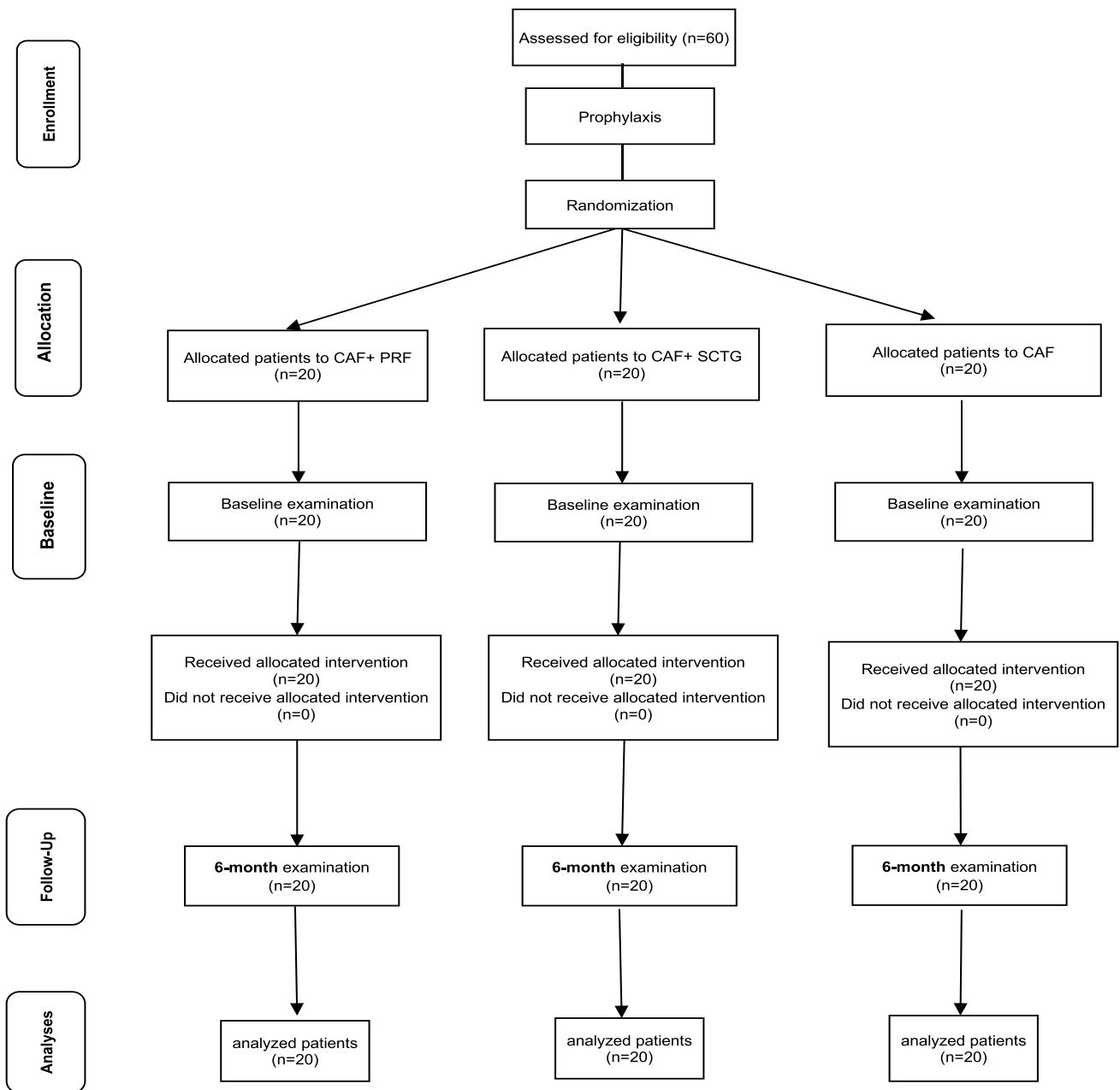


Fig. 1 Consort diagram showing the study layout

group and treatment was known only to the study coordinator, not involved in the trial, responsible even for keeping and breaking the blinding.

Two blinding levels were planned. The first related to one examiner and data collector (LR), trained with a previous intra-examiner calibration exercise (20 patients measured for all periodontal parameters twice, 24 h apart to assure a Fleiss-Cohen's $Kappa_w \geq 0.6$).

At the second level was the surgeon (PS), blinded until envelopes opening. The statistician analyzed letter-labeled

data groups which were re-matched with the treatment labels by the coordinator after blinding breaking.

Pre-surgical treatment

Participants were instructed with adequate oral hygiene methods. It was suggested the use of an electric toothbrush with pressure control (Oral-B Pro 6000 Cross Action; Procter & Gamble Italia SPA Gattatico (RE)) and extra-soft head (Oral B Sensitive EBS17; Procter & Gamble Italia SPA Gattatico (RE)). 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. All patients received an upper arch impression to obtain a cast model. Subsequently, a single measurement point was measured at the mid-buccal site of each study tooth on the study model. Then, an occlusal customized resin acrylic stent was prepared for each participant and stored for all the study duration [28].

Clinical measurement

All clinical parameters were measured by the same investigator (LR) at baseline and 6 months later. After the customized stent was applied, GT was measured by probing with a #15 endodontic reamer [29] (Mani, Reamer length 25 mm, size 15, Utsonomiya shi, Japan) 1 mm apical to the sulcus depth at the reference point (mid-buccal) marked on the stent. 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 fixed 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 digital caliper (Mitutoyo, Model CD-6" B, Andover, UK) accurate to the nearest 0.01 mm. Other measurements were accurately recorded to the nearest millimeter with a periodontal probe (XP 23/UNC15, Hu-Friedy MFG-Co, Inc., Chicago, IL, USA). 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. Complete root coverage (CRC) was evaluated for each experimental site. The percentage of obtained root coverage was also calculated for each GR.

Patient-reported and aesthetic outcomes

Participants were required to record the number of painkillers assumed during the first post-operative week. At the 2-week follow-up the patients evaluated on a VAS (1–10) the level of discomfort suffered after the surgery. The final gingival aesthetic was evaluated using the root coverage esthetic score (RES) at the 6-month follow-up, according to Cairo et al. [30].

Surgical technique

All surgical procedures (Fig. 2) were performed by the same experienced clinician (PS). Root debridement was accurately performed using sharp curettes. According to De Sanctis

and Zucchelli [31], 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. A trapezoidal-shaped flap was elevated using a split-full-split approach [31] 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 diepithelialized to supply a connective tissue surface for flap's surgical papillae suturing. 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 positioned on the root defect, stabilized by sutures with 4–0 braided resorbable polylactic sutures (Ethicon, Johnson & Johnson, Pomezia, Italy), and covered by the flap that was coronally positioned. This coronal position was maintained through 4–0 silk sutures (Ethicon, Johnson & Johnson, Pomezia, Italy) about 1–2 mm over the CEJ in a tension-free position.

L-PRF preparation

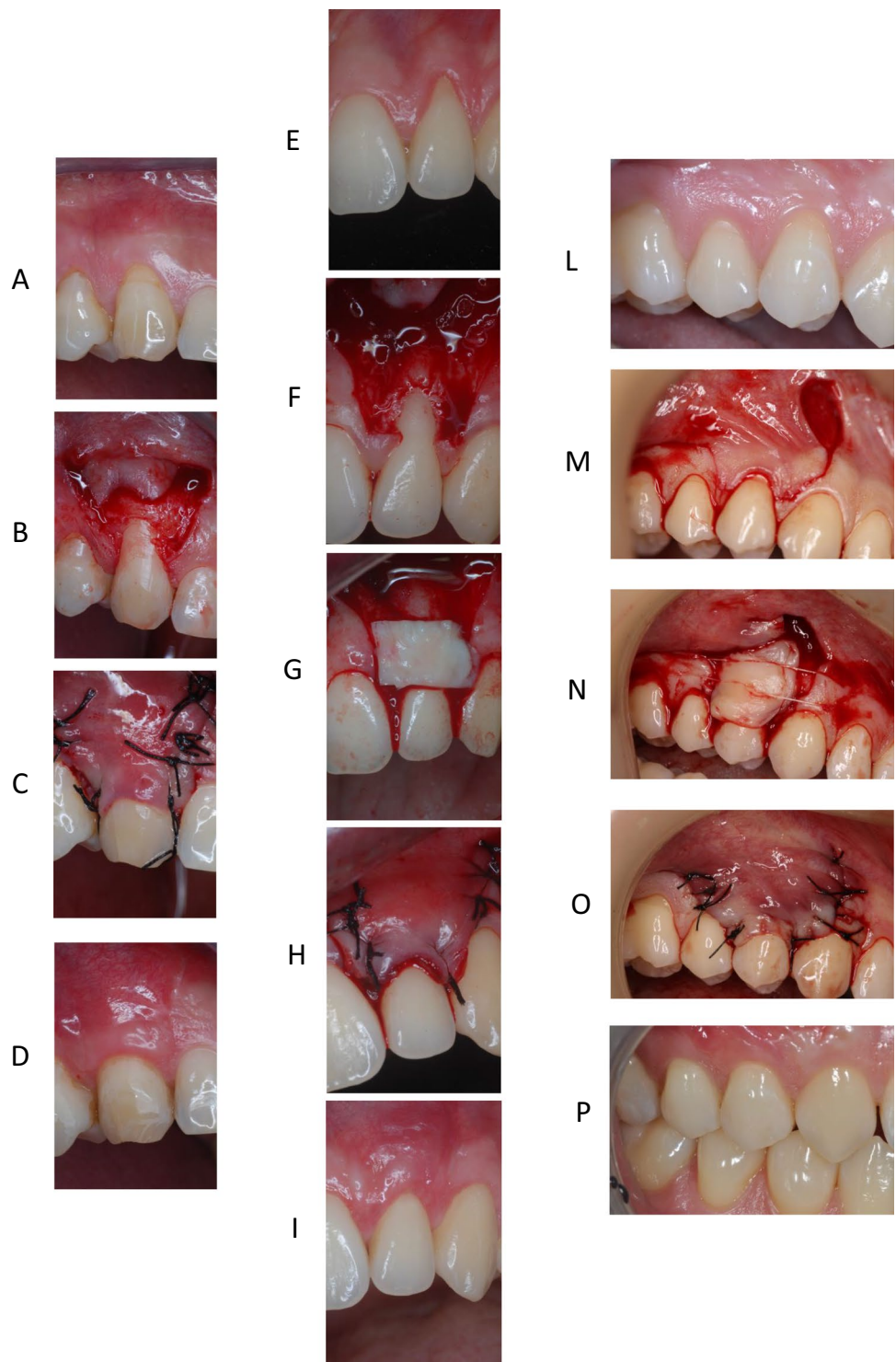
The Dohan-Choukroun et al. [32] protocol was applied to produce L-PRF immediately before surgery. From each patient of the L-PRF groups, 30 ml of blood was collected in three 10-ml sterile tubes without anticoagulant, and it was quickly centrifuged (IntraSpin™, Intra-Lock System Europa SpA, Salerno, Italy) at 2700 revolutions/minute for 12 min (RCF-clot = 408 g; RCF-max = 653 g).

The fibrin clot (L-PRF) was collected and squeezed in the L-PRF box to obtain 2 membranes: each membrane was turned in on itself, and two membranes placed one over the other to obtain a 1.5-mm-thick L-PRF graft measured by a standard caliper (Mitutoyo, Model CD-6" B, Andover, UK) [33].

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. [11]. Briefly, two horizontal and two vertical incisions delimited 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 an about 1.5-mm-thick graft measured by a standard caliper (Mitutoyo, Model CD-6" B, Andover, UK).

Fig. 2 **A** CAF treatment. Clinical aspect of the control site at baseline. **B** The trapezoidal-shaped flap is elevated. **C** The flap is advanced and sutured coronally. **D** Clinical aspect of the site 6 months after surgery. **E** CAF+SCTG treatment. Clinical aspect of the control site at baseline. **F** The flap elevation with the split-full-split approach. **G** SCTG is placed over the exposed root surface. **H** The graft is covered by the flap that was coronally positioned and sutured. **I** Clinical aspect of the control site 6 months after surgery. **L** CAF+L-PRF treatment. The test site before treatment. **M** The flap elevation with the split-full-split approach. **N** L-PRF membranes is positioned and fixed on the recession site. **O** Coronally advanced flap is sutured over the L-PRF membranes. **P** Clinical aspect of the gingival recession after 6 months



Postoperative care

Analgesic consumption was recommended in case of pain after the surgical procedure (400 mg of oral ibuprofen, Nurofen Express 400 mg, Reckitt Benckiser Group, Slough, Berkshire, UK); all participants received 2 g/day amoxicillin + clavulanic acid (Augmentin, SmithKline Beecham,

Milan, Italy) for 6 days for post-operative infection control, and 0.12% chlorhexidine rinses (Dentosan 0.12 Trattamento Mese, Johnson & Johnson, Pomezia, Italy), twice daily for 3 weeks. Sutures were removed after 14 days, after that patient were instructed to cautious brushing by a soft toothbrush and interdental brushing; meantime, the patients used a 1% chlorhexidine gel (Corsodyl Dental gel,

GlaxoSmithKline Consumer Healthcare S.p.A. — Baranzate, Italy) twice daily. Weekly supragingival professional hygiene and motivational reinforcement were administered to the patients for 6 weeks.

Statistical analysis

Descriptive statistics of GT, KT, CAL, REC, and PPD were planned, expressed as observed means \pm SDs and 95% confidence intervals. Multiple univariate analyses for each variable were performed. All outcomes were first analyzed with linear models assuming residuals normality (ANOVA for GT, ANCOVAs for all the others but CAL, which was analyzed with a moderated regression followed by Johnson-Neyman technique) with nonparametric case-resampling bootstrap (Figure-1-Supplementary Material). GT was analyzed by pairwise Cliff's delta tests, not assuming equal-shaped distributions, and by a heteroscedastic ANOVA with Games-Howell post hoc tests. A sensitivities analysis with several types of robust methods (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% Bonferroni-Holm simultaneous confidence intervals.

All the outcomes were analyzed by post hoc distribution-free Nemenyi's tests. Multiplicity adjustments used Bonferroni's or Tukey's method. Statistical software R 3.5.1 (R Core Team (2020). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.) was used.

Results

Sixty patients (twenty-seven women), between 18 and 47 years (mean 32.4 ± 5.0 years), were enrolled in this study. All patients completed the trial fitting with specifications, and no postoperative complications were detected. Experimental groups were balanced by age and gender ($p > 0.05$).

Out of 60 maxillary recessions examined, 17 (6CAF + L-PRF; 6CAF + SCTG; 5CAF) incisors, 23 canines (8CAF + L-PRF; 7CAF + SCTG; 8CAF), and 20 premolars (6CAF + L-PRF; 7CAF + SCTG; 7CAF) were included. The results obtained in this study are summarized in Tables 1 and 2, and in Figs. 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. CRC was 74% in CAF + L-PRF group, 83% in CAF + SCTG group, and 73% in CAF group, while the mean root coverage was 80,15% with CAF + L-PRF, 86% with CAF + SCTG, and 75% with CAF technique. 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 baseline CAL in the CAL gain regression for the CAF + PRF and CAF-SCTG group comparison. No significant difference was detected for RES among the three groups at 6 months but the CAF + SCTG group showed both a greater patient-reported discomfort VAS values (CAF + SCTG vs. CAF $p = 0.011$, CAF + SCTG vs. CAF + L-PRF $p = 0.038$) and painkillers consumption (CAF + SCTG-CAF $p < 0.001$, CAF + SCTG-CAF + L-PRF $p < 0.001$) than the other 2 groups.

Discussion

This study evaluated the results of 3 different surgical techniques CAF, CAF + SCTG, and CAF + L-PRF in changing GT at recession defects. To our knowledge, this was the first study to perform a multilevel analysis of three groups CAF + L-PRF, CAF + SCTG, and CAF, having GT as a primary outcome; although the main outcome of these surgical procedures is to offer a root coverage, GT was chosen as primary outcome considering the growing interest in gingival biotype as a promising potential factor of root coverage. The transgingival probing is the method chosen to measure GT. Although other new procedure have been proposed (ultrasound, CT), it is the first [29] and still the gold standard method according to the literature [34]. Our data reported a significant increase in GT when using L-PRF membranes; however, the CAF + L-PRF group showed a remarkable different distribution in GT increase as compared with the CAF + SCTG.

Table 1 Clinical parameter scoring in mm, mean \pm standard deviation and 95% confidence intervals ($n=20$ in each group)

Parameter	Group	Baseline mean \pm SD	Baseline 95% CI	6 Months mean \pm SD	6 Months 95% CI	Baseline–6 months mean \pm SD	Baseline–6 Months 95% CI
GT	CAF+L-PRF	1.05 \pm 0.32	0.90 to 1.20	1.97 \pm 0.55	1.72 to 1.23	0.92 \pm 0.52	0.68 to 1.17
	CAF+SCTG	0.96 \pm 0.26	0.83 to 1.08	1.95 \pm 0.28	1.82 to 2.08	0.99 \pm 0.02	0.98 to 1.00
	CAF	1.07 \pm 0.44	0.87 to 1.28	1.39 \pm 0.46	1.18 to 1.60	0.31 \pm 0.10	0.27 to 0.36
KT	CAF+L-PRF	1.95 \pm 0.51	1.71 to 2.19	2.03 \pm 0.53	1.78 to 2.27	0.08 \pm 0.25	-0.04 to -0.19
	CAF+SCTG	1.85 \pm 0.67	1.54 to 2.16	3.85 \pm 1.04	3.36 to 4.34	2.00 \pm 0.97	1.54 to 2.46
	CAF	1.65 \pm 0.59	1.38 to 1.92	1.50 \pm 0.69	1.18 to 1.82	-0.15 \pm 0.67	-0.46 to 0.16
CAL	CAF+L-PRF	5.18 \pm 0.69	4.85 to 5.50	1.80 \pm 0.41	1.61 to 1.99	3.38 \pm 0.89	2.96 to 3.79
	CAF+SCTG	5.35 \pm 0.81	4.97 to 5.73	1.75 \pm 0.64	1.45 to 2.05	3.60 \pm 0.60	3.32 to 3.88
	CAF	4.95 \pm 0.69	4.63 to 5.27	1.55 \pm 0.51	1.31 to 1.79	3.40 \pm 0.89	2.99 to 3.81
GR	CAF+L-PRF	4.08 \pm 0.47	3.86 to 4.29	0.80 \pm 0.41	0.61 to 0.99	3.28 \pm 0.59	3.00 to 3.55
	CAF+SCTG	4.35 \pm 0.43	3.91 to 4.79	0.65 \pm 0.49	0.42 to 0.88	3.70 \pm 0.73	3.36 to 4.04
	CAF	4.00 \pm 0.65	3.70 to 4.30	0.95 \pm 0.76	0.59 to 1.31	3.05 \pm 1.10	2.54 to 3.56
PPD	CAF+L-PRF	1.20 \pm 0.34	1.04 to 1.36	1.00 \pm 0.00	1.00 to 1.00	0.20 \pm 0.34	0.04 to 0.36
	CAF+SCTG	1.00 \pm 0.56	0.74 to 1.26	1.10 \pm 0.31	0.96 to 1.24	-0.10 \pm 0.64	-0.40 to 0.20
	CAF	0.95 \pm 0.22	0.85 to 1.25	0.51 \pm 0.51	0.26 to 0.74	0.45 \pm 0.51	0.21 to 0.69

CAF+L-PRF Coronally advanced flap+leukocyte-platelet rich fibrin, CAF+SCTG coronally advanced flap+subepithelial connective tissue graft, CAF coronally advanced flap, GT gingival thickness, KT keratinized tissue, CAL clinical attachment level, GR gingival recession, PD probing depth

The GT increase distribution in the CAF+L-PRF group looked hardly Gaussian; it was more dispersed, right-skewed, and apparently bimodal. At least 7 cases (35%, with best performances) should be excluded to make it normal; hence, the CAF+L-PRF group estimate would result dramatically lower (Fig. 4). The records from these 7 patients were promptly checked out, but no gross errors appeared. Consequently, the confirmatory analysis required both distribution-free hypothesis tests and robust resampling methods to get confidence intervals in a usual metric.

Since the diagnostics suggested a possible misspecification of the parametric model, two further attempts to improve the fit were made. We must emphasize that due to the exiguous sample size these exploratory post hoc analyses aimed to understand the idiosyncratic features of this sample rather than to provide a generalizable model (Fig. 4).

The best-responding L-PRF cases were all showing GT baseline scores below a threshold, so baseline GT was included to split the cases into 2 subgroups. Although a cut-off of 0.8 mm was reported in the literature [8], the value selected for this specific sample was 1.2 mm.

The 2-way Welch ANOVA with treatment and subgroup factors showed a not-significant interaction treatments-by-subgroup ($p=0.13$). As for subgroups, only those within the L-PRF treatment differed significantly ($p=0.002$): patients treated with L-PRF having initial GT < 1.2 mm obtained a greater increase than patients with GT > 1.2 mm. This result observed in the CAF+L-PRF group, in line with previous authors [8], was not detected in the SCTG group. However,

a second was required since this attempt fixed the residuals' asymmetry, but not the variances heterogeneity.

The second attempt was to divide into two the L-PRF group only, according to the outcome performance similarity, using the K-means algorithm. The resulting four-group "one-way ANOVA" test showed all significant group differences, with SCTG group in between the L-PRF-best and -worst groups. The residuals were finally showing a good behavior.

It might be supposed that L-PRF membranes offered a variable GT increase, with some L-PRF grafts that offered a better performance than others. This result could be ascribed to different biological characteristics of the L-PRF membranes in each patient. In fact, although the L-PRF protocol was strictly standardized, and identical among patients (same centrifuge, tubes, and centrifugation time), it was reported that mechanical vibrations might impact the final L-PRF architecture and composition [35]. Therefore, this range of vibrations could produce a slight variation in L-PRF clots, with different biological and clinical effects [35].

Our data agree with those reported by several authors, confirming a significant GT gain when comparing CAF+L-PRF with CAF treatments [19–21]. Aroca et al. [20] hypothesized that this result may be related to the space-making effect of L-PRF membrane and to the growth factors anabolic activity influencing the gingival and periodontal ligament fibroblasts. However, we reported a significant GT increase in the CAF group from the baseline [36], contrary to what reported in aforementioned studies [19–21].

Table 2 Comparison of treatment pairwise differences between groups. Estimated means and 95% simultaneous confidence intervals and hypothesis tests

	Omnibus statistical test	At covariate score (mm)	Pairwise comparison	Estimate + 95% BCa nonparametric bootstrap simultaneous CI (Holm)			
				CAF+SCTG-CAF+L-PRF	CAF-CAF+SCTG	CAF-CAF+L-PRF	
GT	Nonparametric BOOT-STRAP ANOVA $p=0.00009$		Holm adjusted BCa	0.07 (-0.27 to 0.19) NS ($p=0.53$)	-0.68 (-0.73 to -0.63) $p<0.0001$	-0.61 (-0.91 to -0.39) $p<0.0001$	
			Games-Howell	NS ($p=0.73$)	$p<0.0001$	$p<0.0001$	
			Cliff's Delta	NS ($p=0.18$)	$p=0.0001$	$p=0.0001$	
			Nemenyi test	NS ($p=0.73$)	$p<0.0001$	$p<0.0001$	
KT	Nonparametric BOOT-STRAP ANCOVA $p=0.0005$	1.9	Holm adjusted BCa	1.89 (1.48 to 2.51) $p=0.000$	2.21 (1.70 to 2.90) $p=0.000$	-0.32 (-0.62 to 0.02) NS ($p=0.053$)	
			Nemenyi test	$p=0.000001$	$p=0.0000001$	NS ($p=0.74$)	
CAL	OLS interactive model $p=0.0000$	4	Johnson-Neyman	0.94 (0.23 to 1.64) $p=0.0019$	-0.57 (-1.36 to 0.21) NS	0.37 (-0.42 to 1.16) NS	
			4.75		0.43 (0.00 to 0.86) $p=0.016$	0.10 (-0.58 to 0.37) NS	0.30 (-0.16 to 0.76) NS
			6.31		-0.63 (-1.25 to -0.00) $p=0.016$	0.72 (-0.03 to 1.48) NS	0.17 (-0.67 to 1.39) NS
			7		-1.10 (-1.84 to -0.35) $p=0.005$	1.19 (-0.00 to 2.36) NS	0.09 (-1.20 to 2.36) NS
			Nemenyi test	NS ($p=0.66$)	NS ($p=0.72$)	NS ($p=0.99$)	
GR	Nonparametric BOOT-STRAP ANCOVA NS ($p=0.637$)	4.21	Holm adjusted BCa	0.19 (-0.15 to 0.47) NS ($p=0.37$)	-0.35 (-0.82 to 0.12) NS ($p=0.25$)	-0.16 (-0.55 to 0.20) NS ($p=0.41$)	
			Nemenyi test	NS ($p=0.27$)	NS ($p=0.087$)	NS ($p=0.83$)	
PPD	Nonparametric BOOSTRAP ANCOVA $p=0.014$	1.1	Holm adjusted BCa	-0.11 (-0.34 to -0.01) NS ($p=0.13$)	0.60 (0.33 to 0.92) $p=0.00008$	0.49 (0.24 to 0.75) $p=0.0003$	
			Nemenyi test	NS ($p=0.36$)	$p=0.012$	NS ($p=0.304$)	

See Table 1 legend

Similar findings were detected in previous studies [37, 38], offering the hypothesis that this increase may be related to the split-full-split flap elevation. To our knowledge, a few studies compared GT increase between SCTG and L-PRF [22, 39] treatments, and their results are in good agreement with ours, reporting no statistical difference in GT between groups 6 months after surgery. A new finding from our study compared to the previous ones, is that we reported a relevant variability for GT increase in the CAF+L-PRF group, with some sites increasing more than 2 mm and others, instead, only 0.4 mm (Fig. 4). This result may be explained by the different vascularization process that occurs after CAF+SCTG in comparison with CAF+L-PRF procedures. In fact, histological analysis in gingival sites treated by L-PRF and SCTG, respectively, reported that, although L-PRF stimulate an earlier vessel formation, SCTG-treated sites show a greater number of blood vessels than L-PRF treated ones [40]. Furthermore, Hwang et al. [4] reported that flap survival depends on blood supply, and this vascular system is essential to minimize the tissue shrinkage while promoting wound healing. Hence, the pre-existence of a vascular network in SCTG could easily reactivate circulation in

the graft [41], reducing tissue shrinkage and explaining the more predictable GT increase in the CAF+SCTG-treated sites.

Previous studies showed different effect size on GT increase after CAF+L-PRF technique, these values ranging from 0.3 to 0.5 mm [19–22, 42]. However, these results could be ascribed to different methods used to measure GT. This parameter was recorded at the middle of keratinized tissue (Kuka et al. [21]), at 3 mm (Aroca et al. [20] and Thamaraiselvan et al. [19]) and at 2 mm (Eren et al. [22]) from gingival margin. Furthermore, these results derives from studies carried-out with different CAF techniques (i.e. treatment of single/multiple recessions): in fact, multiple gingival defects treated in a single surgical session represent a further clinical challenge compared with the treatment of isolated GR [20]. Similarly, the different GT increase [19–22, 42] may be related to the thickness of the L-PRF membrane to cover the exposed root: some authors did not measure the L-PRF graft [19, 22, 42], while Aroca, et al. [20] used a thinner layer (0.5-mm) compared to the present study (1.5-mm).

Graphs 1-5: Pairwise Nemenyi's tests for all the outcomes, 6 CAL Gain: Johnson-Neyman significant region of the Baseline by Treatment interaction

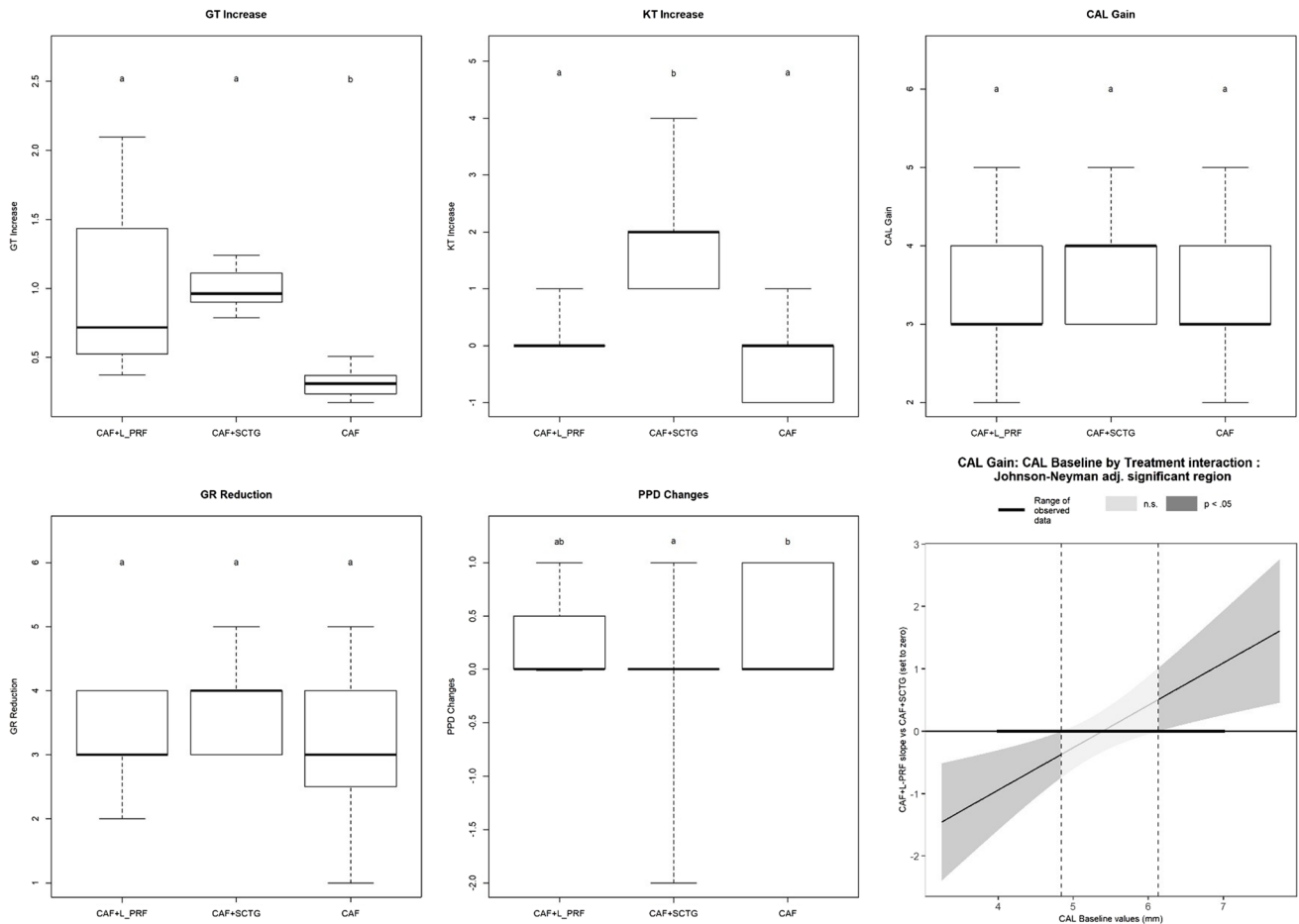


Fig. 3 Pairwise Nemenyi's test group comparisons for all clinical parameters and the Johnson-Neyman technique probing the interaction between treatment and CAL baseline in the CAL gain regression

for the CAF+L-PRF and CAF-SCTG group comparison. (Johnson-Neyman significant region is outside the CAL baseline range 4.76 to 6.31 $p < 0.017$)

In our study, KT did not increase significantly in both CAF group and CAF+L-PRF group at 6 month in line with previous result [20]. A significant KT increase in CAF+SCTG group compared with CAF and CAF+L-PRF groups was detected according to the literature [6]; this result could be related to the potential of the palatal graft to activate epithelial keratinization [43].

The present study shows no statistically significant differences for GR reduction among the 3 groups. Therefore, all the 3 techniques similarly improved root coverage after 6 months. These observations are congruent with those from literature [21, 22, 39, 44], the only exception being Aroca et al. [20] who concluded that L-PRF addition produces less root coverage. However, this study [20] shows some limitations: as observed by Del Corso et al [45], Aroca et al. [20] used a single L-PRF membrane (0.5-mm-thick) to cover multiple recessions, while it is suggested that at least two layers of L-PRF membranes should be applied in periodontal plastic surgery procedures [45]. Furthermore, Aroca et al.

[20] stored the L-PRF membranes at low temperature until surgery, affecting the potential activity of this living biomaterial [46]. In addition, clinical measurements were recorded by two non-masked investigators [20], probably affecting the conclusive results.

In the present study a significant CAL gain in the 3 groups was obtained without differences among groups, confirming previous results [20, 42].

We reported significant differences in PD changes among the 3 groups; this result was mostly due to the high statistical power of the study: in fact, all values ranged between 0.1 and 0.5 mm with confidence interval < 1 mm, thus resulting not-clinically relevant. The role of the graft in offering a better aesthetic outcome is still controversial. Some authors [47] reported a significant higher RES when the exposed root is grafted with SCTG or L-PRF, while other authors [48, 49] reported that CAF alone showed an overall higher aesthetic outcome compared with CAF+SCTG. This study reported no differences in terms of overall aesthetic score between

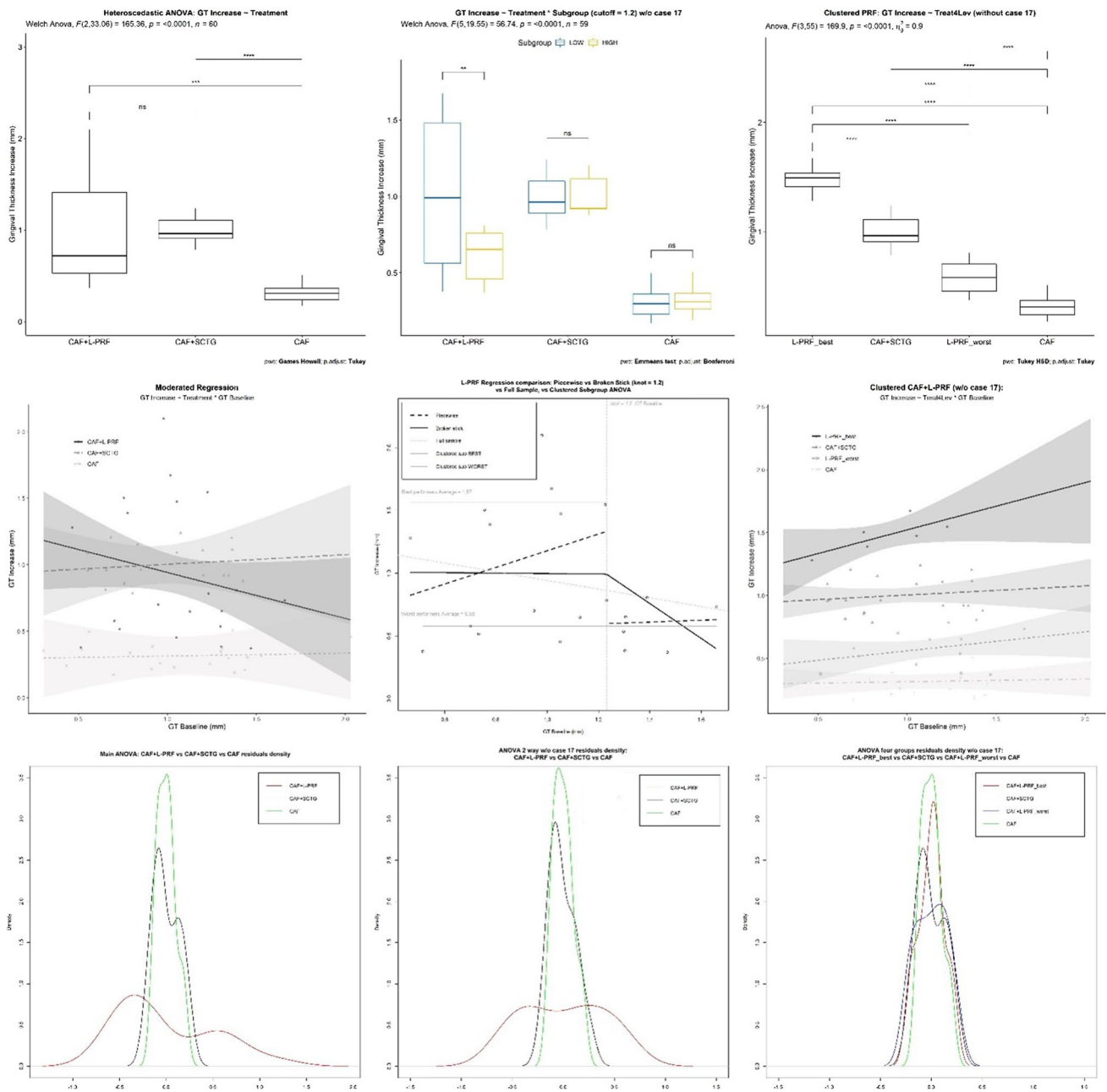


Fig. 4 A comparison between graphs about the confirmatory analysis (first column) and to two post-hoc analyses (second and third columns) to obtain the best possible fit. The exploratory analyses, described in the text, are a 2 way-ANOVA per sub-groups identified by a baseline threshold value of 1.2 mm in the second column, and an ANOVA for four groups (the L-PRF group was divided into high- and

low-responders). The first row shows the boxplots, the second row reports the per-treatment regression lines of the GT increase on GT baseline values (with several attempts of modeling the two subgroups in column two), and the third row reports the residuals density plots, showing the treatments variance not explained by each model

the three groups at 6 months. This result may be related to the largely sensitivity of overall RES for the amount root coverage achieved [48], the baseline GT [8] as parameter for adding or not a graft, and the relative short-term follow-up (6 months). Furthermore, both CAF and CAF+ L-PRF groups needed less analgesic consumption and discomfort compared with CAF+ SCTG treatment that required a

second surgical wound on the palate and longer operative times [11].

A growing body of literature investigated the role of GT in surgical procedures for root coverage, reporting that thick gingival tissues show greater resistance to inflammatory damages [50], prevent GR relapse [6], and promote CRC [4]. These conclusions about thick soft tissues may be ascribed

to the greater volume of collagen matrix [4], the higher tissue vascularization [4], and the multiple layers of keratinized epithelium [4]; these features favor a better wound healing and provide a mechanical barrier from traumatic injuries [4]. These findings suggest that thick gingival tissues should be considered as a major factor to improve long-term tissue stability [51]. In fact, it was demonstrated that CAF is similarly effective to CAF + SCTG in the treatment of GRs [52] in the short term; however, in the long-term follow-up,

GR recurred in 39% of the CAF-alone-treated sites [53]. On the other hand, the additional use of SCTG under the flap seems to be effective in improving gingival thickness, producing a predictable result in the long term [51, 54].

Although it is reported that 6-month follow-up period is enough to evaluate the gingival margin stability after CAF procedure [55] and all previous studies that compare the CAF alone or CAF + SCTG with the L-PRF membrane showed a 6-month follow-up, this aspect can be considered the main limitation of this study since it may be too-short to fully assess the changes of some clinical parameters as KT [36, 56]. This could explain the absence of a significant KT increase after CAF procedure in this study, contrary to what has been reported in studies with longer follow-up periods [31, 44]. The use of the silk sutures may be considered a limitation of our procedures. However, according to Tatakis et al. 2016 [57], there is no significant difference in the number of sites obtaining CRC based on the type of suture material used; furthermore, to minimize the potential inflammation risk of silk sutures, linked to the greater accumulation of bacterial plaque, the patients rinsed with a chlorhexidine mouthwash for a period of time that exceeded the time the sutures were in place.

Conclusion

Within its limitations, this study shows that, while CAF + L-PRF, CAF + SCTG, and CAF are similarly effective in reducing GR, both CAF + SCTG, and CAF + L-PRF produce positive increases of gingival thickness. CAF + L-PRF technique avoids palatal wound, implying a reduced operative time and less discomfort but offers less predictable results in terms of GT increase. CAF + SCTG technique produces better results for KT increase and most predictable results for GT gain, confirming this technique as the current gold standard for root coverage.

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Author contribution PS, PdN, MP and BF are co-first Authors having designed, written, revised and edited the work; LS and GP collaborated in designing, writing and editing the study; MS and IR cooperated in the study design, article editing and revising; LR collaborated in interpretation of data, article editing, and data collection with IR and BF. PdN did the statistical analysis.

Data availability The authors confirm that the data supporting the findings of this study are available within the article, its supplementary materials, and from the corresponding author [author MP] on request.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval The study protocol was registered at ClinicalTrials.gov as NCT03712852 after the approval obtained by the G. D’Annunzio University ethic committee. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained according to the protocol from all participants included in the study.

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