



ORIGINAL ARTICLE

Multiple coronally advanced flap with a selective use of connective tissue graft: A 3-year prospective clinical and histological study

Giovanna Laura Di Domenico¹ | Maria Di Martino¹ | Gianluigi Arrigoni² | Sofia Aroca^{3,4} | Massimo de Sanctis¹

¹Department of Periodontology, Vita-Salute San Raffaele University, Milan, Italy

²Department of Pathology, IRCCS San Raffaele Scientific Institute, Milan, Italy

³26K Center for Clinical Research, Paris, France

⁴Department of Periodontology, Bern University, Bern, Switzerland

Correspondence

Giovanna Laura Di Domenico,
Department of Periodontology,
Vita-Salute San Raffaele University, 58 Via
Olgettina, Milan, Italy.
Email: giovannalauradd@gmail.com

Abstract

Background: The purpose of the present study was to prospectively evaluate the 3-year changes in the gingival dimensions following multiple coronally advanced flap (MCAF) with selective use of connective tissue graft (CTG). In addition, the secondary aim was to histologically identify the factors related to phenotype changes.

Methods: Twenty patients treated with MCAF and site-specific application of a CTG were available for the 3-year follow-up. Outcome measures included complete root coverage (CRC), recession reduction, keratinized tissue width (KTW), marginal tissue thickness changes, and primary flap position. Biopsies were harvested at one of the sites treated with the adjunct of CTG. All sections were stained with hematoxylin and eosin, Masson trichrome, Verhoeff-van Gieson, tenascin, and alcian blue stain for semiquantitative evaluation.

Results: At 3 years, CRC was detected in 86% of sites treated with MCAF alone and 81% of sites treated with MCAF + CTG. The 47% of sites treated with MCAF + CTG presented an apical shift of primary flap from its original position. Linear regression showed a significant association between KTW change and the initial KTW in MCAF-treated sites, while both initial KTW and position of primary flap were statistically significantly associated factors with KTW changes in the MCAF + CTG group. In all the biopsies examined, there is always a marked and clear separation between the connective tissue of the gingival flap and the palatal connective tissue of the graft.

Conclusions: The selective use of CTG is an effective treatment for multiple gingival recessions. Only a limited increase in KTW can be expected in a bilaminar technique if, during the healing phases, the connective tissue is maintained completely covered.

KEYWORDS

connective tissue, gingival recession, histology, surgical flaps



1 | INTRODUCTION

Gingival recessions can be successfully treated by several surgical approaches. Many studies attested that pedicle flaps, free soft tissue grafts, or combination of pedicle flaps with grafts, barrier membranes, or enamel matrix derivatives are all effective procedures to cover the exposed root surfaces.^{1–4}

The multiple coronally advanced flap (MCAF), proposed by Zucchelli and de Sanctis (2000),⁵ has become the most widely used root coverage procedure for multiple recession defects. It is the first-choice surgical technique in presence of an adequate keratinized tissue width (KTW) apical to the recession and it is very effective with advantages for the patients in terms of aesthetics and morbidity.^{2,6}

The use of a connective tissue graft (CTG) under MCAF has been introduced to increase the predictability of the procedure. The application of CTG is associated with the highest probability to obtain complete root coverage (CRC),^{1,2,4,7} as well as with an increase of gingival thickness (GT) and of the KTW over time.^{6,8,9}

The increased dimension in KTW was reported with different amounts by several authors. Cairo et al. (2016)⁶ in a randomized controlled clinical trial found that the addition of a CTG under MCAF was associated with a significant increase in both keratinized tissue (1.8 ± 0.6 mm) and GT (0.66 ± 0.17 mm) at 1 year, when compared with MCAF.

Since very often teeth adjacent to each other present different phenotype, Stefanini et al. (2018)⁸ in a case series proposed MCAF with site-specific application of CTG, reporting a significant increase in KTW in the MCAF + CTG treated-sites compared with baseline (3.14 ± 0.48 mm).

Various explanations have been proposed to justify the increase in KTW following bilaminar techniques, such as genetically predetermined position of mucogingival line,^{8,10–12} inductive potential of a dense connective graft on epithelial phenotype^{11,13,14} or repositioning of the primary flap in an apical direction during wound healing.^{15,16}

When discussing the effect of connective tissue to induce epithelial keratinization, several authors reported that the dense connective tissue of the graft could exert its influence over the epithelium, even when covered by the connective tissue of the covering flap.^{12–14} Nevertheless, it should be considered that the previous studies demonstrated that the stimulus which determine the histodifferentiation of the epithelium occurs when the connective tissue is left uncovered,^{17,18} while it is unclear how the differentiation of the mucosal epithelium into keratinized one, could occur when using a bilaminar technique, that is covering the connective tissue with the coronal advanced flap.

On the other hand, some authors have indicated that the postsurgical instability of the flap, which leads to the exposure of marginal part of the underlying connective tissue, could explain the epithelial differentiation and the increase in keratinization.^{15,16} Moreover, this localized increase in keratinization due to the shrinkage of the covering flap is reported by some authors as keloid formation¹⁹ or as an unaesthetic result.⁶

The main objective of the present investigation was to prospectively evaluate the 3-year results of a mini-invasive bilaminar technique, the MCAF plus selective use of CTG. In addition, the study evaluated the changes of the gingival phenotype and the increase of keratinized tissue. To identify the factors related to these changes, selective biopsies were taken and a semiquantitative analysis of the specimens was conducted.

2 | MATERIALS AND METHODS

2.1 | Study design and population

This study reported the 3-year follow-up of a prospective trial on the treatment of multiple gingival recessions. All subjects were treated with MCAF with selective use of CTG, where CTG was applied only to the sites showing a KTW <2 mm or when the phenotype was evaluated as thin (<1 mm), to reduce the invasiveness of the technique (Figure 1). Details of the study protocol were presented in a previous paper reporting 1-year results.²⁰

This study was approved by the Ethical Committee of San Raffaele Hospital (with number of protocol “KT” — EC Reg. N. 157/INT/2019) and performed in accordance with the Helsinki Declaration of Human Studies.

The study population was enrolled at the Department of Periodontology at San Raffaele Hospital (Milan, Italy) between January 2022 and March 2022. Details of inclusion and exclusion criteria have been reported in the previous paper.²⁰ All subjects were informed about all pertinent aspects of the study and gave written informed consent.

2.2 | Clinical evaluation

The following clinical parameters were collected at the treated sites:

Recession depth (REC) – measured at the midbuccal site from anatomical/restored cemento-enamel junction (CEJ) to the gingival margin.

KTW – measured as the distance from the gingival margin to the mucogingival junction at the middle buccal point. The measurements were repeated a

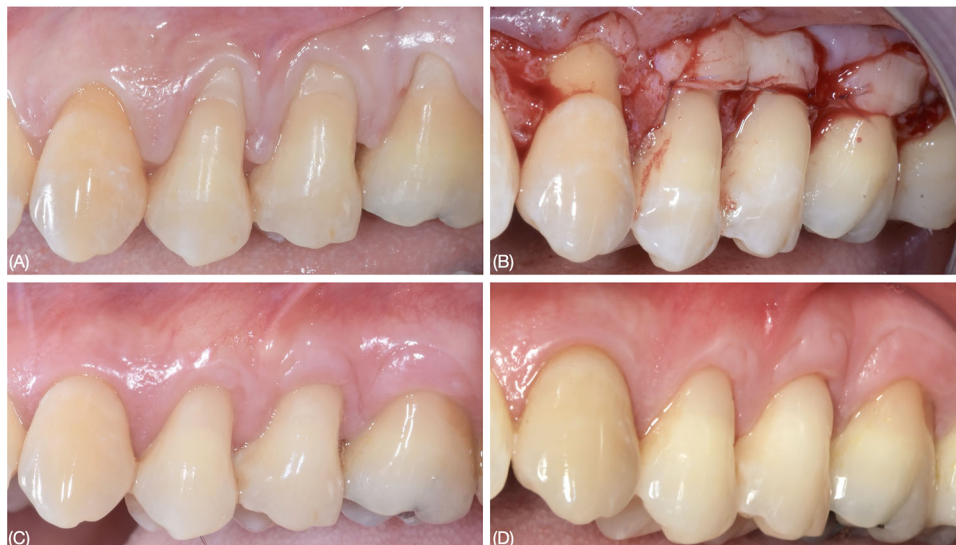


FIGURE 1 (A) Baseline situation showing multiple gingival recessions in the maxilla. (B) The connective tissue graft was positioned at the level of the first and second premolars and first molar. (C) Clinical outcome at 1 year. (D) Clinical outcome at 3 years.

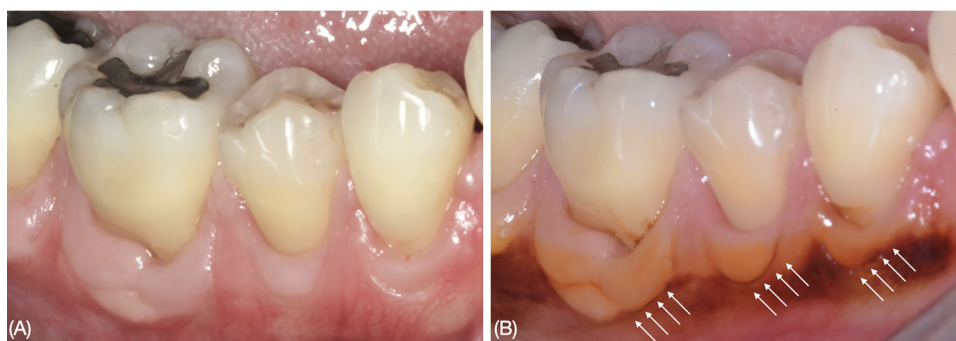


FIGURE 2 Identification of the primary position flap: the use of iodine solution (B) evidenced the apical shift of the margin of the flap (arrows).

second time with the use of iodine solution (Lugol solution 5%).²¹

Marginal tissue thickness (MTT) measured using a short needle for anesthesia with a silicon disk stop 2-mm apical to the gingival margin.

Primary flap position (PFP) — the position of the primary flap (the flap covering the CTG) following the healing phases. This was evaluated dichotomously as “coronal” when the original position was maintained and the connective tissue remained covered, and “apical” when an apical shift of the flap was observed, thus exposing part of the CTG. The use of iodine solution (Lugol solution 5%) evidenced the apical shift of the margin of the flap (Figure 2).

All clinical measurements were performed by two calibrated examiners (G.L.D.D. and M.D.M.) using a manual

probe.* To demonstrate an acceptable level of intra- and inter-examiner agreement, duplicate measurements for KTW and REC of five subjects presenting multiple gingival recessions, were repeated after 24 h following the first measurements. The intra-examiner intra-class correlation coefficient was 0.9775 (95% CI, 0.957–0.985) for G.L.D.D. and 0.96 (95% CI, 0.932–0.976) for M.D.M. The inter-examiner intra-class correlation coefficient was 0.97 (95% CI, 0.954–0.98).

2.3 | Biopsy and sample preparation

Informed consent was obtained by nine patients to execute a biopsy at one of the sites treated with the adjunct of CTG

* PCP-UNC 15 probe tip, Hu-Friedy, Chicago, IL.



for histologic evaluation. In addition, two biopsies of normal palatal and gingiva were obtained as references for the histological examination.

Gingival tissue samples were harvested using a 3-mm punch biopsy pen under local anesthesia. Incision was performed positioning the handheld disposable punch perpendicular to the tooth at 2-mm apical to the gingival margin at the edge between the exposed connective tissue and the residual covering flap, performing simultaneous rotational movements under gentle pressure (see Figure S1 in online *Journal of Periodontology*). The biopsies included only connective and epithelial tissues (they did not include periosteum) since they were harvested in a position coronal to the crest of bone.

Each specimen was positioned on blotting paper, and it was fixed in 10% neutral buffered formalin solution with the correct orientation, by an examiner (G.A.) of the Pathological Anatomy Laboratory (San Raffaele Hospital, Milan, Italy). Following dehydration, the specimens were embedded in paraffin, with proper positioning (at buccal-lingual direction) and oriented for sectioning as perpendicularly to the surface plane as possible. Five-micrometer-thick sections were collected serially and thaw-mounted on glass slides.

All sections were stained with hematoxylin and eosin (H&E) for morphological evaluation and Masson trichrome, Verhoeff-van Gieson, and alcian blue stain for evaluation of the collagen framework, elastic fibers, and extracellular matrix mucopolysaccharides, respectively. For immunohistochemical studies, the slides were stained with mouse monoclonal anti-tenascin,[†] performed in the automated immuno-stainer[‡] using standard procedures.

2.4 | Histologic, histochemical, and immunohistochemical analysis

From each sample, one histological section was randomly chosen and examined by a masked investigator (A.G.) using a light microscope[§] at $\times 50$ magnification. The images were captured at an original magnification of $\times 20$ using a dedicated software.^{||}

Semiquantitative analysis was performed to detect the presence of specific staining on each section to record information relative to the overall structure and composition of the soft tissues, including both the graft and the flap.^{21–25} The intensity of staining (intensity score) or the degree of staining (proportion score) were calculated.

[†] clone BC-24, Sigma-Aldrich, St Louis, MO.

[‡] ULTRA Benchmark, Ventana Medical Systems/Roche, Tucson, AZ.

[§] AxioVision System SE64 – Zeiss, Oberkochen, Germany.

^{||} Aperio ImageScope, Leica Biosystems Srl, Buccinasco, Milan, Italy.

2.5 | Statistical analysis

Statistical analysis was performed at tooth level using dedicated software.[¶] Mean values and standard deviations were calculated for continuous variables, such as REC, KTW, MTT, recession reduction, percentage of root coverage (%RC), KTW change (Δ KTW), MTT change (Δ MTT), and frequencies and percentage for ordinal ones, such as PFP and CRC.

Intergroup analysis (MCAF vs. MCAF + CTG and apical PFP vs. coronal PFP) of continuous variables were performed using paired *t*-test, while ordinal ones were compared using Chi-square test. Significance was set at 0.05.

A linear regression model was used to explore the possible relationship between the KTW changes at 3-year follow-up and some variables of interest (REC0, KTW0, MTT0, CTG thickness, CTG height, PFP).

3 | RESULTS

3.1 | Clinical evaluation

3.1.1 | Experimental population, patients, and defect characteristics at baseline

A total of 23 patients (11 women and 12 men; mean age: 46.2 ± 10.6 years) with multiple recession defects were included in the study, and 93 recession defects were treated. Of these, 59 recessions (63%, 14 patients) were located in the maxilla and 34 (37%, nine patients) in the mandible.

The surgical treatment involved MCAF + CTG for 54 sites and MCAF alone for 39 sites.

At MCAF sites, the mean REC at baseline was statistically significantly lower than MCAF + CTG group (MCAF: 1.97 ± 0.87 mm; MCAF + CTG: 2.91 ± 1.01 mm; $p < 0.001$), while mean KTW was statistically significantly higher (MCAF: 2.39 ± 1.02 mm; MCAF + CTG: 1.74 ± 0.89 mm; $p = 0.002$) (see Table S1 in online *Journal of Periodontology*).

3.1.2 | One-year outcomes

At 1-year follow-up, CRC was observed in 90% of all treated sites (84 of 93 gingival recessions), with no statistically significant differences between MCAF and MCAF + CTG sites (MCAF: 3.67 ± 1.52 mm; MCAF + CTG:

[¶] SPSS version 24.0, SPSS, Statistical Package for the Social Sciences, Chicago, IL.



TABLE 1 Changes in clinical parameters at 3-year follow-up.

Clinical parameter	MCAF (n = 36)	MCAF + CTG (n = 48)	<i>p</i> ^b	Apical PFP (n = 25)	Coronal PFP (n = 23)	<i>p</i> ^b
RecRed (mm)	1.69 ± 0.81	2.69 ± 1.08	0.000018 ^a	3 ± 0.98	2.35 ± 1.09	0.0037 ^a
%RC (%)	93 ± 21	93 ± 16	0.95	96 ± 10	89 ± 20	0.118
CRC	86	81	0.76 ^c	88	74	0.38 ^c
ΔKTW (mm)	0.72 ± 1.11	1.69 ± 1.59	0.0029 ^a	2.3 ± 1.34	1.02 ± 1.59	0.0049 ^a
ΔMTT (mm)	0.22 ± 0.89	1.47 ± 0.77	<0.001 ^a	1.62 ± 0.83	1.31 ± 0.67	0.182

Abbreviations: %RC, percentage of root coverage; CRC, complete root coverage; MCAF, multiple coronally advanced flap; MCAF + CTG, multiple coronally advanced flap plus connective tissue graft; PFP, primary flap position; RecRed, recession reduction; ΔKTW, keratinized tissue width changes from baseline to 3 years; ΔMTT, marginal tissue thickness changes from baseline to 3 years.

^aStatistically significant.

^bStudent *t*-test.

^cChi-square test.

3.85 ± 1.51 mm; *p* = 0.26), while a statistically KTW increase was observed in both groups when compared with baseline (*p* = 0.01).

3.1.3 | Three-year outcomes

Between 1- and 3-year follow-ups, a total of three dropouts were registered (one patient was untraceable, two patients moved to another town).

CRC was detected in 86% of sites treated with MCAF alone and 81% of sites treated with MCAF + CTG, without any significant difference (Table 1).

No statistically significant difference was observed in KTW at 3 years between groups (3.19 ± 1.21 mm at MCAF-treated sites and 3.33 ± 1.2 mm at MCAF + CTG-treated sites) (see Table S1 in online *Journal of Periodontology*), while a statistically significantly greater KTW increase was observed in the MCAF + CTG group, when compared with baseline (Table 1).

Linear regression showed a significant association between KTW change and the initial KTW, in both MCAF- and MCAF + CTG-treated sites. In addition, in the last group also the position of primary flap was a statistically significantly associated factor (Table 2).

The apical PFP was observed in 25 (52%) of the sites treated with MCAF + CTG.

The subgroup analysis showed a statistically significant difference between teeth presenting apical and coronal PFP in terms of mean KTW at 3 years (apical PFP: 3.82 ± 1.14 mm, coronal PFP: 2.8 ± 1.07 mm; *p* = 0.0027) (Table 3), and a statistically significantly greater KTW increase was observed in sites presenting an apical PFP (Table 1).

When comparing MCAF + CTG sites showing coronal PFP- with MCAF-treated sites, no statistically significant differences were observed both for mean KTW (coronal PFP: 2.8 ± 1.07 mm, MCAF: 3.19 ± 1.21 mm; *p* = 0.212)

and KTW change at 3 years (coronal PFP: 1.02 ± 1.59 mm, MCAF: 0.72 ± 1.11 mm; *p* = 407).

3.2 | Histologic evaluation

Eleven biopsies from nine different patients were examined. Nine of these biopsies were performed in MCAF + CTG-treated sites; the other two biopsies were performed in normal gingiva and in normal palatal mucosa.

In all the biopsies of treated sites examined, the differences in composition between the connective tissue of the flap and the connective tissue of the graft are conspicuous. As evidenced by the different stains used, there is always a marked and clear contrast between the gingival flap and the palatal graft.

3.2.1 | Masson trichrome (Figures 3A, F)

In the sections stained with Masson trichrome, as expected, a net differentiation between the two layer was evident: collagen fibers are denser in palatal graft than in gingival flap; only in two cases a significant difference in collagen fiber density between the two areas could not be observed (see Figure S2 and Table S2 in online *Journal of Periodontology*). This increase in collagen fiber is explained by the transformation of palatal connective stromal tissue into scar tissue due to remodeling processes after surgery.

3.2.2 | Verhoeff-van Gieson stain (Figures 3B, G)

Elastic fibers are present in both flap and graft connective tissue; in six out of nine biopsies they are abundant (++) in flap and mild (+) in graft tissue (see Figures S3, S4 and Table S3 in online *Journal of Periodontology*).

**TABLE 2** Linear regression with KTW changes (measured as difference between KTW at 3-year follow-up and KTW at baseline) of MCAF and MCAF + CTG-treated sites as outcome variable.

Clinical parameter	MCAF			MCAF + CTG		
	Estimate	Standard error	<i>p</i> ^b	Estimate	Standard error	<i>p</i> ^b
KTW0	-0.40	0.186	0.038 ^a	-1.09	0.19	<0.001 ^a
REC0	-0.14	0.228	0.546	0.26	0.18	0.152
MTT0	0.80	0.841	0.952	-0.97	1.38	0.487
CTG height	Na	na	na	0.15	0.30	0.619
CTG thickness	Na	na	na	0.51	0.99	0.607
PFP	Na	na	na	0.81	0.37	0.04 ^a

Abbreviations: CTG, connective tissue graft; KTW0, keratinized tissue width at baseline; MCAF, multiple coronally advanced flap; MCAF + CTG, multiple coronally advanced flap plus connective tissue graft; MTT0, marginal tissue thickness at baseline; PFP, primary flap position; REC0, recession depth at baseline.

^aStatistically significant.

^bStudent *t*-test.

TABLE 3 Clinical parameters (mean ± SD, mm) of MCAF + CTG-treated sites with apical and coronal primary flap position (PFP) at baseline and 3 years.

Clinical parameter	Apical PFP (<i>n</i> = 25)		Coronal PFP (<i>n</i> = 23)		<i>p</i> ^b	
	Baseline	3 years	Baseline	3 years	Baseline	3 years
REC (mm)	3.12 ± 0.97	0.12 ± 0.33	2.65 ± 1.07	0.3 ± 0.56	0.12	0.167
KTW (mm)	1.52 ± 0.82	3.82 ± 1.14	1.78 ± 0.96	2.8 ± 1.07	0.31	0.0027 ^a
MTT (mm)	0.38 ± 0.16	2 ± 0.8	0.46 ± 0.17	1.78 ± 0.66	0.08	0.321

Abbreviations: KTW, keratinized tissue width; MCAF, multiple coronally advanced flap; MCAF + CTG, multiple coronally advanced flap plus connective tissue graft; MTT, marginal tissue thickness; REC, recession depth.

^aStatistically significant.

^bStudent *t*-test.

3.2.3 | Alcian blue (Figures 3C, H, S5)

In seven out of nine histological sections from treated sites the expression of alcian blue was significantly higher in palatal graft than in gingival flap. In fact, in all gingival flaps the stain is mild, while in palatal graft can vary from mild (2/9) and moderate (2/9) to abundant (5/9) (see Figure S5 and Table S4 in online *Journal of Periodontology*). Moreover, it is interesting to notice that in both biopsies taken from normal palate and normal gingiva there was no expression of alcian blue (see Figure S6 in online *Journal of Periodontology*).

We can hypothesize that the insertion of the graft under the flap determines an inflammatory status, which is responsible of inflammatory modulators expression. These molecules, in fact, can modulate the production of all members of the metalloproteinases family (that is metalloproteinases and tissue inhibitors of metalloproteinases), which are involved in the stability of connective tissue. This mechanism is involved both in tissue development and tissue remodeling.²⁷ Moreover, in hyalinized and fibrous tissue the number of glycosaminoglycans and hyaluronic acid is increased²⁸ and so this could explain our observation of higher intensity of stain in palatal graft connective tissue compared with adjacent stromal tissue.

3.2.4 | Tenascin (Figures 3D, I)

Tenascin is an extracellular matrix glycoprotein of 1900 kD.^{29,30} In human skin and oral mucosa, tenascin is contained in the papillary connective tissue and near the basement membrane.³¹

In all specimens from treated sites, tenascin expression is increased in connective tissue of palatal graft, while its expression remains confined at the basement membrane and around vessels in gingival flaps (see Figure S7 and Table S5 in online *Journal of Periodontology*).

Tenascin expression is significantly induced and increased in areas where tissue remodeling is taking place, that is, areas with high rates of cell turnover and migration, such as in sites of neovascularization, wound healing, and tumorigenesis.³¹⁻³³

3.2.5 | Hematoxylin and eosin (Figure 3E, J)

Likewise, some differences in the epidermal layers between the flap and the epithelium formed after insertion of the palatal graft were revealed by hematoxylin and eosin stain. The epithelium covering the exposed graft,

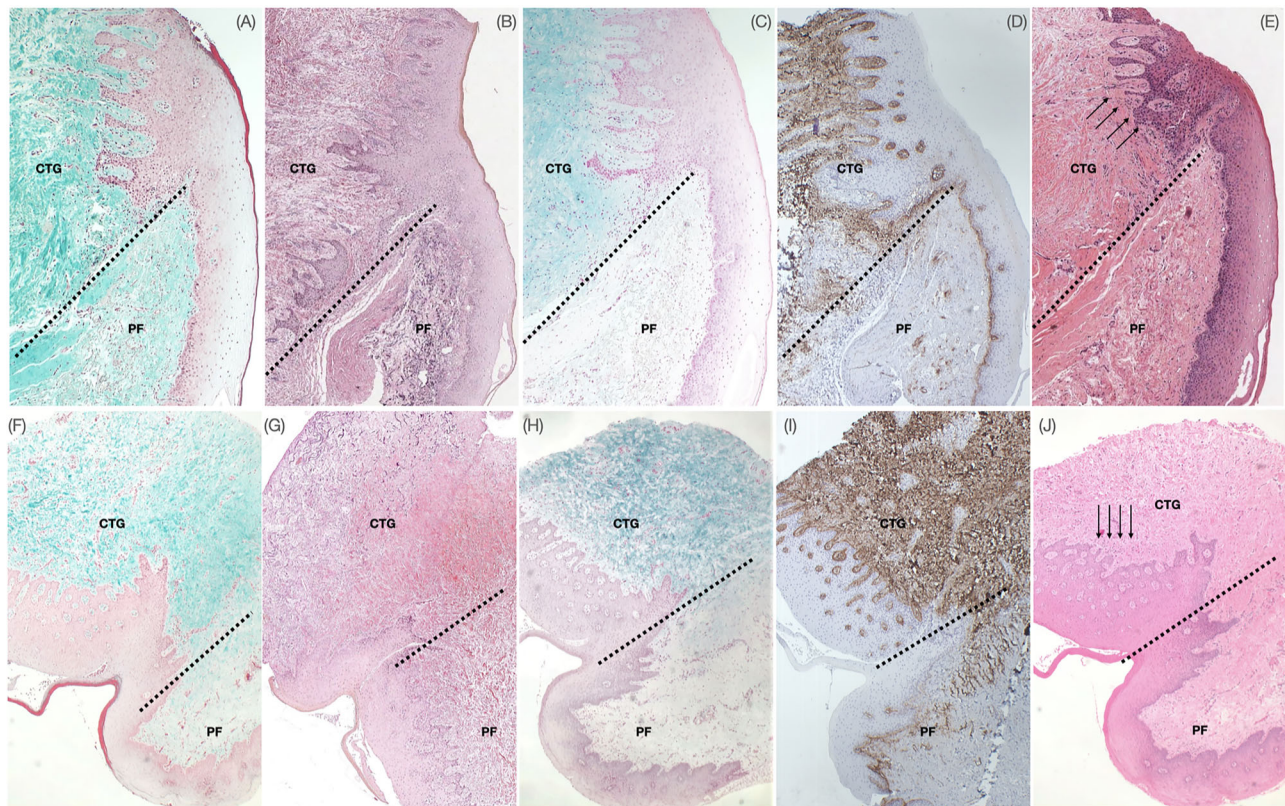


FIGURE 3 Histologic sections showing: (A, F) Masson trichrome stain: collagen fibers are denser in connective tissue graft (CTG) than in primary flap (PF); (B, G) Verhoeff-van Gieson stain: the number of elastic fibers is higher in PF than in CTG; (C, H) Alcian blue: the stain is abundant in CTG, while mild in PF; (D, I) Tenascin: note that its expression is increased in CTG; (E, J) Hematoxylin and eosin (H&E) stain: note as the epithelium covering the exposed CTG is hyperplastic, with elongation of rete ridges (arrows).

indeed, is hyperplastic, with elongation of rete ridges (see Figure S8 in online *Journal of Periodontology*).

All these modifications could be determined by the type of tissue in the lamina propria below. The density of the connective tissue, in fact, is different between the graft and the flap: palatal connective tissue has a greater density than gingival connective tissue.

Moreover, after surgery, the graft undergoes some modifications related to wound healing and inflammatory status that ends in a transformation of the connective tissue in scar tissue.

4 | DISCUSSION

The present study has investigated clinical and histological results at 3 years following an MCAF with site-specific application of CTG for the treatment of multiple RT1 recession type defects.

Sites treated with MCAF and MCAF + CTG were compared. Results obtained from this study showed that both approaches were effective in reducing REC, recording no statistically significant difference at the

final assessment at 3 years postoperatively. Furthermore, the results showed no statistically significant difference between groups at 1-year and 3-year assessments in terms of CRC.

It is important to underline that the sites where coronally advanced flap was used without the CTG, were selected on the basis of thick phenotype and the presence of at least 2 mm of KTW at baseline. The present data suggest that these successful outcomes achieved with both surgical approaches can be maintained in the long term, as per the previous literature.^{19,34}

In terms of postsurgical increase in KTW, the results showed that the variation was statistically significant higher in sites treated with MCAF + CTG (MCAF: 0.74 ± 0.21 mm, MCAF + CTG: 1.23 ± 0.2 mm, $p = 0.025$), in agreement with previous studies.^{7,8,19}

The higher efficacy in augmenting KTW is often described in the literature as the result of the induction of the dense CTG on the epithelium of the flap, thus inducing keratinization.^{12–14} This effect was described by Karring et al. (1971, 1975)^{17,18} in a fundamental experiment to evaluate epithelial-mesenchymal interaction. Nevertheless, one has to keep in mind that this effect was observed only when



the connective tissue, transplanted in an area of alveolar mucosa, was left uncovered.

In the present article, the PFP was evaluated, highlighting that in almost 50% of the sites it was possible to evidence an apical shift of the flap, leaving the most coronal part of the connective tissue exposed. These exposed areas were re-epithelialized during the healing phases: being the dense connective tissue exposed, the induction of keratinization could occur, by this augmenting the KTW. On the contrary, sites where the primary flap was maintained at the coronal position showed a minor amount of KTW increase. In fact, when comparing sites with CTG, where the position of the flap was maintained coronal, and sites without CTG (MCAF), there was no statistical difference in terms of KTW. It could be speculated that augmented KTW did not depend on the presence of dense connective tissue, but from the repositioning of mucogingival line, such as for the MCAF,^{8,12} or by the inclusion of the periosteum in the flap, being the technique split-full-split elevated as described by Zucchelli and de Sanctis (2000).⁵ Moreover, when comparing the apical PFP group with the coronal PFP group, differences in terms of both %RC and CRC did not reach statistical significance.

To explain the relative high percentage of sites where flap was showing an apical shift, it could be speculated that the areas where a CTG was used were composed of a thin or very thin gingiva phenotype thus more prone to recede^{1,7} and the interposition of the connective graft could also impair the vascular exchange between the covering flap and the underlying receiving bed.^{19,35}

At the histological examination, the areas of exposed graft showed an increase in collagen fibers, transforming the palatal connective stromal tissue into a scar tissue due to remodeling processes after surgery.

Clinically many authors have evidenced this change to occur when using a CTG, defining these areas as a keloid formation.¹⁹ The formation of a localized area of increased keratinization (keloid) is also responsible for less esthetic results, as described by Zucchelli et al. (2003, 2014)^{19,35} and Cairo et al. (2010)³⁶ previously.

The findings of the present study confirm that the quality of connective tissue determines the quality of the epithelium,^{17,18} nevertheless, in order for this phenomenon to occur, following a bilaminar technique, the CTG must be exposed.^{16,37,38} When the flap is covering the graft, this effect cannot be seen and the increase in KTW is similar to MCAF alone.³⁹

Furthermore, the histological observation allows us to speculate that the quality and density of the CTG maintain its characteristic, nevertheless it does not influence the quality and composition of the overlying connective tissue of the flap. In fact, with all the stain used, a net separation between the two layers (the CTG and the flap) is

always well evident. In other words, the connective tissue of the flap retains always its original characteristics in composition and density so, when covering the graft, it is acting as a “buffer zone”, impeding the dense connective tissue of the graft to induce any change to the overlying epithelium.^{19,35} Only in instances where the connective tissue is coronal to the margin of the flap, the epithelial modifications become evident.

Even when attempting to evaluate the tissue morphogenesis by the use of tenascin, the two areas evaluated maintained a net colorimetric separation. According to our observations, in palatal graft (that is scar tissue like) the expression of tenascin remains high for years after surgery. This is the first study that evidence gingival tenascin expression after surgery with a so long follow-up (3 years) and for this reason we could not compare our data with existing literature.

Due to the sample limitation of the present study more information is needed on the effective role and the need to use a connective graft under a coronal advanced flap. In fact, if it is confirmed that the effect of the graft is solely to act as a “phenotype modifier,” that is an instrument to increase MTT. Then, the use of selective, thin graft, with a less invasive surgical technique, together with an MCAF, could be the treatment of choice for multiple recession type defects.

5 | CONCLUSIONS

From the clinical standpoint, our data support that the selective use of connective tissue together with an MCAF is effective in reducing REC. No statistically significant difference between sites with and without the graft was assessed, both for root coverage and KTW at the final evaluation at 3 years postoperatively.

From a biological standpoint, it can be concluded that the CTG cannot exert any differentiation of the overlying epithelium in instances where the flap maintains its original position at CEJ. Only where a recession of the flap over the connective tissue is observed, there is an evident increase in the amount of KTW.

Also, it should be considered that a thick connective tissue positioned under an MCAF could impede a normal vascularization of the overlying flap, augmenting the risk of flap contraction, that will explain areas of increased keratinization.

AUTHOR CONTRIBUTIONS

Giovanna Laura Di Domenico was the coordinator of the study, including creation of the study design; collected the data; helped with their interpretation; and participated in writing the manuscript. Maria Di Martino created the



study design, recruited the patients, performed clinical evaluations, collected the data, and participated in writing the manuscript. Gianluigi Arrigoni performed the histological examination, helped with data interpretation, and participated in writing the manuscript. Massimo de Sanctis and Sofia Aroca created the study design, helped with data interpretation, and participated in writing and reviewing the manuscript. All authors reviewed and approved the submission of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare that are relevant to the content of this article.

ORCID

Giovanna Laura Di Domenico  <https://orcid.org/0000-0001-5956-2161>

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