# Adjunctive benefit of a Xenogenic Collagen Matrix associated with Coronally Advanced Flap for the treatment of multiple gingival recessions. A superiority, assessor-blind, randomized clinical trial.

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**Short running title:** Collagen matrix for gingival recessions

**Summary:** After 12 months, CAF+CMX achieved similar root coverage to CAF alone, but resulted in a significant increase in gingival thickness.

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The study was financially supported in part by a grant from Geistlich Pharma AG, Switzerland. This research was conducted by the investigators who independently performed all phases of the study including protocol development, experimental procedures, data analysis, result interpretation and reporting.

## ABSTRACT

**Aim:** To evaluate the superiority of coronally advanced flaps (CAF) when used in combination with a xenogeneic collagen matrix (CMX) for root coverage of multiple adjacent gingival recessions.

**Material & Methods:** Participants with at least 2 upper adjacent teeth exhibiting gingival recession depth ≥ 2 mm were recruited and randomised to CAF with (test) or without (control) CMX respectively. Mean and complete root coverage, amount of keratinized tissue (KTw), gingival thickness (GThick) and patient reported outcomes (PROMs) were recorded at baseline, 3, 6, and 12 months.

**Results:** 24 patients providing 61 gingival recessions were analysed. After 1 year, gingival recession depth decreased from 2.3±0.7mm to 0.3±0.4mm in the CAF+CMX group (2.0±0.8mm meanRC), and from 2.6±1.0mm to 0.6±0.3mm in the control group (2.0±1.1mm meanRC). No difference was observed between the 2 groups (p= 0.2023). Nine-teen (63%) of the test and 16 (52%) of control defects showed complete root coverage (p= 0.4919). GThick greatly increased in the test group (0.5mm; 0.2 to 0.8 mm, 95% CI; p = 0.0057). No difference between the 2 groups was observed for KTw (p= 0.5668) and PROMs.

**Conclusion:** At 1 year, CAF+CMX provided similar root coverage to CAF alone, but a significant increase in gingival thickness.

**Key words**: gingiva, gingival recession, smiling, mucograft, randomized controlled trials, humans

**Clinical Relevance**

*Scientific rationale for the study*: The use of xenogenic collagen matrices (CMX) could improve the clinical performance of coronally advanced flap (CAF) procedures and be considered as a safe alternative treatment option to allograft material.

*Principal findings*: No statistically significant difference was observed between CAF alone or with a CMX in terms of gingival recession reduction and complete root coverage. A substantial increase in gingival thickness, however was found when CAF was combined with CMX.

*Practical implications*: CMXs could be mainly indicated as an adjunct to CAF in clinical cases with thin gingival phenotype and/or in procedures to increase gingival thickness.

## INTRODUCTION

In patients with a sufficient amount (≥2mm) of keratinized tissue, the Coronally Advanced Flap (CAF) has been shown, to be a very effective treatment of single and multiple recessions in terms of aesthetic results and patients' morbidity (Pini Prato et al. 2014; Chambrone et al. 2018). Although CAF is a safe and predictable approach for achieving root coverage, there is strong evidence supporting the combination of this technique with autologous connective tissue graft to achieve complete root coverage (CRC) in gingival recessions without interproximal attachment loss and non carious cervical lesions, with long term stability (Graziani et al. 2014; Chambrone et al. 2015, 2018; Tonetti & Jepsen 2014; Pini Prato et al. 2018 a,b).

The free gingival graft (FGG) technique has been confirmed as a predictable method to create and retain new keratinized tissue up to 27 years after treatment (Agudio et al. 2009). This procedure, however has limited indications in aesthetic areas due to compromised results obtained by virtue of using epithelialized grafts ("patch-like area"). The use of free connective tissue grafts (CTG) conversely provides higher predictability in achieving complete root coverage and improved colour matching (Chambrone et al. 2015, 2018; Pini Prato et al. 2018 a,b). Both surgical techniques are associated with variable but important patient morbidity due to the need of creating a palatal wound (donor site) and limited by the amount and quality of soft tissue available.

The use of soft tissue substitutes (STS) in Mucogingival Surgery has received increasing attention by researchers and clinicians because of the need of identifying easier and less invasive techniques in reconstructive surgery. STS could also be used without limitation in terms of size, shape and homogeneous thickness and a larger variety exists already on the market. Several studies have been conducted with the aim of investigating efficacy and safety of STS when compared to soft tissue autografts (Thoma et al. 2010; Rotundo et al. 2012; Ahmedbeyli et al. 2014; Tonetti et al. 2018). There is some evidence supporting the use of CAF with acellular dermal matrix (ADM) in achieving greater CRC and thicker periodontal phenotype changes (Ahmedbeyli et al. 2014; Chambrone et al. 2015). Ethical concerns and the risk of disease transmission have however greatly limited the use of these substitutes because they are obtained from human cadavers. A recent systematic review and meta-analysis compared clinical outcomes including width of keratinized tissue (KT) around teeth, when living cultured cells as STS were compared to FGG procedures. Data analyses showed that STS resulted in some increase in the width of KT, but inferior (1.39mm) to what could be achieved with FGG (Dragan et al. 2017). The use of xenogenic collagen matrices (CMX) could be considered as valid alternative treatment option to standard autologous free grafting procedures with the aim of

reducing patient morbidity (no donor site is required) and improving safety (no risk of transmission of human diseases) (Sanz et al. 2009).
CMX present with a bilayer structure: a spongious portion (predominant) and a compact layer. The spongious part is meant to facilitate cellular ingrowth and in turn improve neo-angiogenesis and wound healing resulting in greater root attachment and gingival thickness. Indeed Ghanaati et al. (2011) reported that such bilayered matrices elicit favorable soft tissue reactions enhancing soft tissue ingrowth and are associated with good clinical outcomes.

The use of CMX in the management of gingival recessions has shown promising clinical outcomes and it is considered a valid alternative to the CTG (Sanz et al. 2009; Herford et al. 2010; Schmitt et al. 2013). A recent systematic review (Atieh et al. 2016) appraised the comparative evidence on the the use of CMX with CTG, CAF and FFG for the treatment of gingival recessions associated with variable amounts of KT in terms of clinical parameters and patient- related outcomes. Authors concluded that there was limited evidence to support the use of CMX in achieving greater root coverage, gingival recession reduction and gain in KT when compared to CTG plus CAF. Limited and inconclusive evidence, however supported the notion that CMX may improve aesthetic satisfaction, reduce postoperative morbidity and shorten operating times. Indeed only two comparative studies using CAF alone vs CAF+CMX were identified. They reported opposite results in terms of clinical outcomes in single and multiple recessions respectively and with an unclear risk of bias (Jepsen et al. 2013; Cardaropoli et al. 2014).

The aim of this study was to test the clinical efficacy of CMX when combined to CAF for the treatment of multiple gingival recessions in terms of root coverage, KT augmentation and patient-reported outcomes when compared to CAF alone.

## MATERIALS AND METHODS

### Trial design

This is a single-centre, superiority, assessor-blind clinical trial, with balanced randomisation and two parallel groups design (Schulz et al. 2010). Ethical approval was obtained by the local authority (Azienda USL 3 Pistoia, prot. 24/CESM 19.11.2012) and the study protocol was registered on ClinicalTrials.gov (Identifier n°. NCT03833765). All study participants gave informed consent and all study procedures were performed according to the Declaration of Helsinki on experimentation involving human subjects.

### Participants

Eligible patients were recruited in a private office between January 2013 and January 2016 and the the last follow-up visit of this study (after 1 year) was completed in July 2017. All eligible participants were recruited in this study based on the following inclusion and exclusion criteria:

*Inclusion Criteria*

1. Must be 18 years or older
2. Diagnosis of gingival recessions in the upper teeth from central incisor to first molar
3. Gingival recessions on at least 2 adjacent teeth with a minimal depth of 2mm and detectable cemento-enamel junction (CEJ) (abrasion step <1mm)
4. Able to comply with study-related procedures such as demonstrating good oral hygiene and attending all follow-up procedures
5. Full Mouth Plaque (FMPS) and Bleeding (FMBS) Score <20%
6. Able to fully understand the nature of the proposed surgical procedure and to provide signed consent

*Exclusion Criteria*

1. Current Smokers
2. Pregnancy

3. Patients with uncontrolled diabetes
4. Medical contraindications for dental and/or surgical treatment
5. History of malignancy, radiotherapy or chemotherapy for malignancy within the past 5 years
6. Medications or treatments known to affect mucosal wound healing (e.g. steroids, large doses of anti-inflammatory drugs, anticoagulation drugs)
7. Diseases which affect connective tissue metabolism (e.g. collagenases)
8. Allergy to collagen
9. Drug or alcohol abuse
10. Participation in another clinical trial in the last 6 months
11. Untreated periodontitis
12. Gingival recessions on second/third molar teeth or on malpositioned teeth
13. Presence of abrasion ≥ 1 mm or cervical restoration, with non-detectable CEJ

**Intervention**

*Pre-Surgical Phase*

All participants underwent a comprehensive periodontal examination performed by a single calibrated examiner who recorded gingival recession depth, probing pocket depth (PD), Full Mouth Plaque Score (FMPS) and Full Mouth Bleeding Score (FMBS). Prior to surgical therapy, all patients received detailed dental hygiene instructions on correct (not-traumatic) toothbrushing procedures and a course of professional dental cleaning (supragingival scaling and polishing).

*Surgical Procedure*

A single operator (RR), with more than 20 years of experience in mucogingival surgery, performed all surgical interventions. According to Zucchelli & De Sanctis (2000), an envelope split-full-split thickness flap without vertical incisions was performed. The horizontal incision was extended to include one tooth on each side of the teeth with gingival recession, in order to facilitate the planned

coronal repositioning of the flap tissue over the exposed root surfaces. The procedure was timed with a start at the first incision. Oblique interdental/papillary incisions were carried out keeping the blade parallel to the long axis of the teeth in order to dissect split-thickness the surgical papillae. Gingival tissue apical to the exposed roots was raised as full-thickness whilst the most apical portion of the flap was elevated in a split-thickness fashion. Interdental papillae were then de-epithelialized using a microscissor. All exposed root surfaces were mechanically treated with the use of curettes, avoiding the connective attachment area near the bone crest. A sharp dissection into the vestibular lining mucosa was then carried out to eliminate any muscle tension on the flap. Only at this point of the surgical procedure the envelope was opened to reveal group allocation. In the test group, CMX (Geistlich Mucograft®, Geistlich Pharma AG, Wolhusen, Switzerland) was prepared and securely placed onto all root recession defects according to manufacturers' instructions including:

1. CMX was cut into the right dimensions, measured with a probe, and its measurements recorded.

2. CMX was placed from the CEJ to the bone crest on the recipient bed using single sutures, 7/0 PGA sutures.

3. The matrix was rehydrated with blood, in order to reconstitute and maintain the maximal thickness possible.

4. The flap was closed at or slightly coronal to the CEJ with sling sutures using resorbable PGA 6/0 and limiting any compression of the matrix.

In the control group, no biomaterials was used and sling sutures were performed to achieve precise adaptation of the buccal flap on the exposed root surfaces and to stabilize every single surgical papilla over the de-epithelialized anatomic papillae.

Two clinical cases, one for each group are reported in Figure 1(a-d) and 2 (a-d).

**Postoperative Care**

Following the procedure, all study participants were instructed to rinse twice daily with chlorhexidine mouth rinse (0.12%) for 3 weeks. No toothbrushing was allowed in the treated area for 21 days, and thereafter they were instructed to use only an ultrasoft toothbrush. Anti-inflammatory therapy

(Ibuprofen 600mg, one before the surgery and one after 6 hours) and additional analgesics were prescribed according to individual needs and each patient was instructed to keep record on a daily basis of the medications used (number and dosage) and pain intensity using a dedicated questionnaire. All patients were reviewed after 14days when all sutures were removed.

**Outcomes Assessment**

The main objective of this RCT was to compare CAF with CMX (test group) versus CAF alone (control group) in the treatment of multiple adjacent recession defects. Based on a hypothetical superiority effect of the CMX (test group), the following endpoints were considered:

*Primary Endpoint*: Mean gingival recession reduction difference at 12 months post treatment between study groups.

*Secondary Endpoints*:

1) Percentage of complete root coverage after 6 and 12 month between study groups

2) Thickness of soft tissue over the root after 6 and 12 month between study groups

3) Recession width after 6 and 12 month between study groups

4) Keratinized Tissue (KT) width between study groups.

5) Patients reported outcomes

*Periodontal Clinical Measurements*

A single calbrated examiner (GL) recorded the following variables on the mid buccal surfaces of the treated teeth using a calibrated North Carolina University probe, and rounding measurements to the nearest 0.5mm:

1. Distance between CEJ and the free gingival margin (*Rec depth*)
2. Distance between incisal margin to the free gingival margin (*IM-GM*)
3. *Recession width (Rec width)*, represented by the horizontal measurement of gingival recession at the CEJ level

4. *Bleeding on Probing (BOP),* recording the presence/absence of bleeding after probing (Ainamo & Bay 1975)

5. *Plaque Index (PI),* recording the presence/absence of plaque on tooth surface (Ainamo & Bay 1975)

6. *Probing Pocket Depth (PD),* measured using a periodontal probe between GM and the end of the gingival sulcus

7. *Clinical Attachment Levels (CAL),* calculated as PD+Rec depth

8. *Keratinized tissue (KTw) width,* assessed with the visual and functional method by means a probe as a distance between GM and mucogingival junction (MGJ)

9. *Keratinized tissue (KT thick) thickness,* measured by the use of a needle pierced through the center of a circular-shaped silicon marker of 3 mm in diameter. The edge of the marker (k-file nr. 10) was positioned at the soft tissue margin providing a distance from the margin of 1.5 mm. The distance between the tip of the needle and the silicon marker was assessed by the use of a magnifying glass and a Dentsply Maillefer silicon stops AO197 was used. In addition, a digital calliper C041 0-150 mm (Kennon Instruments) with a sensibility of 0.01 mm was used (da Silva et al. 2004; Santamaria et al. 2008)

10. Duration of the surgery (in minutes) from the first incision to the last suture.

All periodontal clinical measurements were recorded before the surgery (baseline) and at each follow-up visits: 3, 6 and 12 month after mucogingival surgery.

A set of frontal and lateral digital pictures were taken at baseline, after flap elevation, after material application, after suturing, and at 7 and 14 days, 1, 3, 6 and 12 months post-op in all participants.

*Patient Reported Outcomes*

A preliminary questionnaire was given at baseline to all patients to assess their aesthetic concerns, discomfort while brushing and hypersensitivity using a linear scale (0=no problem to 10=major problem). Following the surgery, all participants were instructed to complete a daily diary for 7 days, to record patient reported experience measures (PREMs). In the diary, patients were asked to record post-surgery sequelae, pain and discomfort, chewing function, interference with daily activities, use of medications more than the prescription. Diaries were collected 7 days after surgery.

At the 6 and 12 months follow-up visits, two additional questionnaires were administered to all patients in order to ascertain discomfort, chewing function, tooth cleaning procedures, dental hypersensitivity and esthetic outcomes following the surgical procedure.

*Calibration session*

One calibrated examiner (LG) performed all clinical periodontal measurements. For *recession depth* and *incisal margin-gingival margin* measurements, an intra-rater agreement study was performed. A set of 20 recessions were evaluated twice with a two-hour interval between the measurements. The examiner was considered reliable if the intraclass coefficient of correlation was greater than 0.70.

**Sample Size**

The following 2 hypotheses were considered: H0, there was no difference in mean recession reduction between CAF+CMX and CAF alone; H1, there was a difference in mean recession reduction between CAF+CMX and CAF alone.

The following variables were used in order to calculate the sample size:

- Endpoint: Normal. Difference in reduction in gingival recession (root coverage) between baseline and 1 year
- Clinical important difference (Effect size d): set at 1 mm
- Assessing variability: From a previous study (Woodyard et al. 2004) describing envelope type CAF procedures for the management of multiple recessions, a standard deviation of recession reduction of 0.93 mm was recorded.
- Mean number of treated teeth per patient (2.92) was obtained from a previously published study (Pini Prato et al. 2010)
- Intraclass correlation coefficient (ICC) of the cluster sites (recessions nested into patient) of 0.35) was derived from a previously published study (Pini Prato et al. 2010)
- Allocation ratio: 1:1
- Type I error: $\alpha = 0.05$
- Type II error: $\beta = 0.10$, corresponding to a Power of 90%
- Accounting for an estimated 10% drop-out.

The sample size calculation using a formula for cluster design was based on the detection of a 1 mm difference in gingival recession reduction between study groups (standard deviation of 0.93 mm) (Woodyard et al. 2004) with a two-side 5% significance level, a power of 90%, a mean number of

treated teeth per patient of 2.92, an intraclass correlation coefficient of 0.35, resulting in 12 patients needed per treatment group.

**Randomization and Allocation Concealment**

The patients were randomized to one of the two groups: CAF + CMX (test group) and CAF alone (control) on a 1:1 ratio. The randomization code was computer-generated by an independent researcher not involved in any clinical evaluations (MN). A blocked randomization approach was used: 24 patients were divided in 12 patients per treatment group. The center received 24 sequentially numbered and sealed opaque envelopes containing the group allocation. The envelope was opened by the investigator only after flap elevation as above mentioned.

**Blinding**

The surgeon and the patients were aware of the treatment allocation whilst the clinical examiner was masked to the treatment allocation.

**Data analysis**

Descriptive statistics were performed using mean and standard deviation for quantitative data and frequency and percentage for qualitative data. All unintentional side effects in each group were reported. For quantitative variables mixed models were performed with the treatment (CAF alone versus CAF+CMX) as a fixed explicative variable and patient as a random variable. Baseline value was used as a covariate. Interaction terms between treatment and the covariate were used only if statistically significant.

Complete root coverage outcome was analysed with a multilevel logistical model at two level (patient and tooth). For patient-reported outcomes including study experience measures t-tests were applied. Estimates for the treatment effect, standard errors, p-values and 95% confidence intervals are provided. The statistical software used was MLwiN 2.21 Centre for Multilevel Modelling, University of Bristol.

**RESULTS**

The CONSORT flow-chart diagram showing for each group, the numbers of participants who were randomly assigned, received the intended treatments, and analysed for the primary outcome is reported in Figure 3.

**Clinical Outcomes**

Twenty-four patients providing 61 gingival recessions were enrolled for the present study and randomized to receive the allocated intervention (CAF or CAF+CMX). All study participants completed the study by attending the 1 year follow-up and their characteristics are described in Table 1. Similar number (N=30 in the test and N=31 in the control) and severity (mean depth of 2.3±0.7mm for the test and 2.6±1.0mm for the control) of gingival recessions were recruited in this study.

Gingival recession depth, measured as CEJ-GM distance, decreased from 2.3±0.7mm to 0.3±0.4mm in the CAF+CMX group, with a root coverage gain of 2.0±0.8mm (Table 2) and a mean percentage root coverage of 87±19 % (Table 3). In the control (CAF alone) group, CEJ-GM measurement decreased from 2.6±1.0mm to 0.6±0.3mm, with a root coverage gain of 2.0±1.1mm (Table 3) and a mean percentage root coverage of 75±30 % (Table 3). The adjusted difference between treatments was 0.3mm (95%CI from -0.2 to 0.8) but it did not reach statistical significance (p= 0.2023) (Table 3). A total of 19 (63%) of the test and 16 (52%) of the control defects exhibited CRC (OR=1.67; 95%CI from 0.39 to 7.13) (Table 3).

No difference in KTwidth between the study groups was observed (p= 0.5668). KTwidth decreased after 12 months from 3.3±1.5mm to 2.7±1.2 mm in the CAF+CMX group, with a mean KT width loss of 0.6±1.7mm. In the control group, KT width decreased from 3.5±1.8mm to 2.5±1.1mm, with a mean reduction of 1.1±1.3mm. (Table 3).

A statistically significant increase of 0.5 mm (0.2 to 0.8 mm, 95% CI, p=0.0057) in GThick was observed between study groups. GThick increased from 1.4±0.7mm to 1.7±0.7mm in the test group, with a mean increase of 0.2±0.7mm. However, in the control group, GThick decreased from 1.5±0.6mm to 1.2±0.5mm, with a mean thickness loss of 0.3±0.7mm, after 12 months.

No statistically significant *treatment x covariate* interactions were observed (Table 3).

A reduced surgical time in the CAF-alone procedure compared t CAF-CMX was found (mean difference of 11.2 minutes, 95% CI from 6.8 to 15.7min, p<0.0001) (Table 4).

**Patient-Reported Outcomes and Experience Measures**

No substantial differences in all patient-reported outcomes (esthetics, brushing discomfort, dental hypersensitivity, post-surgery sequelae, pain and discomfort, chewing function, interference with daily activities, use of medications) were observed between the study groups (Table 4).

## DISCUSSION

In the present randomized study, performed on multiple recessions and 12 months follow-up period no difference in mean recession reduction and CRC between CAF+CMX and CAF alone is reported. This is in line with the available evidence on the use of CMX for the management of missing or reduced gingiva. A recent systematic review (Atieh et al. 2016) focussed on the efficacy of CMX in comparison with CTG, CAF and FGG for the treatment of gingival recessions and/or sites with insufficient KT in terms of root coverage, recession reduction, gain in KT, changes in probing pocket depth (PPD) and clinical attachment level, operating time, and and patient- related outcomes (aesthetic satisfaction, postoperative morbidity and discomfort). Due to the limited number and to the moderate quality of the examined trials, the review reported no evidence in support of the effectiveness of CMX in achieving greater root coverage, recession reduction and gain in KT compared to CTG plus CAF. These findings have been recently confirmed by a multicenter RCT (Tonetti et al. 2018). In this study superior short-term results in treating gingival recessions compared with CAF alone were reported. In terms of patient-related outcomes, the benefit of using CMX appeared limited. When considering studies aimed to test the adjunctive effect of CMX to CAF alone the review included only 2 studies at unclear risk of bias: one peformed on localised recessions (Jepsen et al. 2013) and the other on multiple recessions (Cardaropoli et al. 2014). The overall meta-analysis showed no differences between CMX and CAF in the percentage of complete root coverage (RR 0.86; 95% CI 0.66–1.11; $p = 0.24$), even though CMX showed a higher mean percentage of root coverage (mean diff. 9.26 %; 95% CI 3.55 to 14.97; $p = 0.001$), recession reduction (mean diff. 0.35 mm; 95% CI 0.03 to 0.66; $p = 0.03$) and gain in KT (mean diff. 0.36 mm; 95% CI 0.04 to 0.69; $p = 0.03$) when compared to CAF alone. In contrast, the use of CMX was associated with greater gingival thickness (mean diff. 0.55 mm; 95% CI 0.39 to 0.70; $p < 0.0001$) and this was also observed in this trial.

Similar results, but on localized gingival recessions, were reported by a secondary analysis performed at 1 year (Stefanini et al. 2016) on patients recruited from a previous study (Jepsen et al. 2013) with an initial 6 months duration. Authors suggested stable clinical outcomes over the additional 6 months in terms of mean root coverage (76.3% for CAF + CMX and 75.0% for CAF) and complete root

coverage (36% for CAF + CMX and 31% for CAF). Further evidence on the stability of the obtained clinical outcomes after 6 months and 12 months year for both procedures, was further reported in a third subgroup analysis after 3-years follow-up (Jepsen at al. 2017). In the present study despite greater estimates of CRC were observed, a worsening trend of the clinical results obtained was however noted between 6 and 12 months especially in the control group although this difference resulted statistically not significant. This could be attribuited to the different flap design (single vs multiple) used and possibly due to different healing phases followed by the 2 different surgical approaches.

This study results are in contrast with the ones observed by Cardaropoli et al. (2014). At this stage it might be difficult to discuss these differences as the study reported better clinical outcomes favouring the combined treatment (93.2% mean RC and 72% CRC versus 81.5% mean RC and 58% CRC, respectively) after 1 year follow-up. Long-term (beyond 1 year) and properly designed randomized and multicentre studies should be performed to provide a more definitive answer.

In terms of *gingival thickness*, results obtained by the analyses of the present study are in line with the ones obtained by Cardaropoli et al. (2014) and Stefanini et al. (2016). A significant increase in gingival thickness using CMX+CAF compared to CAF alone has been consistently reported. Disagreement is highlighted in terms of *keratinized tissue width* obtained however between the present and the 2 previous studies. Indeed while the present study showed no difference in KT width observed between groups (both test and control treatments showing a reduction), the other 2 studies, performed on multiple and single recessions, a KT width increase was reported favouring the use of CMX. Possible mechanisms explaining these tissue volumetric/phenotipic changes could be due to reduced blood supply of the flap during the initial healing phase as reported by Pini Prato et al. (2011, 2012). Further research using better and more objective tools to monitor soft tissue healing should be performed.

With regard to patient-reported outcomes, evidence from the present study suggests no difference in terms of esthetics, brushing discomfort, dental hypersensitivity, post-surgery sequelae, pain and discomfort, chewing function, interference with daily activities, use of medications between patients who underwent a CAF+CMX versus CAF alone procedure. These results confirms the ones observed after tretament of single recessions by Stefanini et al. (2016). To the best of our knowledge no other evidence is available, hence it would be sensible to include appropriate and sufificnet number of PROM measures in future long-term clinical studies on multiple gingival recession treatment.

Some limitations of the present study should be performed including the single-centre design and narrow spectrum of gingival recessions included for treatment (a relatively shallow initial mean recession depth of 2.5mm). This could impact on the external validity of the study results. Nevertheless a rigorous study design, appropriate sample size and statistical analysis, a single experienced operator and no loss to follow-up represent the main strenghts of this study results.

In conclusions from this study comparing the clinical efficacy of CAF plus CMX versus CAF alone for the treatment of multiple adjacent gingival recessions we report that: i) CAF+CMX provided similar root coverage to CAF alone after 1 year including similar patient-reported and clinically assessed outcomes; ii) a significant increase in gingival thickness was obtained when CMX was combined to CAF. CMX could represent a valid and predictable tool for clinician when dealing with gingival deformities and thin phenotypes.

**REFERENCES**


Agudio, G., Nieri, M., Rotundo, R., Franceschi, D., Cortellini, P., Pini Prato, G.P. (2009). Periodontal conditions of sites treated with gingival augmentation surgery compared to untreated contralateral homologous sites: a 10- to 27-year long-term study. Journal of Periodontology, 80, 1399-405.

Ahmedbeyli, C., Ipçi, Ş.D., Cakar, G., Kuru, B.E., Yılmaz, S. (2014). Clinical evaluation of coronally advanced flap with or without acellular dermal matrix graft on complete defect coverage for the treatment of multiple gingival recessions with thin tissue biotype. Journal of Clinical Periodontology, 41, 303-310.

Ainamo, J., Bay, I. (1975). Problems and proposals for recording gingivitis and plaque. International Dental Journal, 25(4), 229-235.

Atieh, M.A., Alsabeeha, N., Tawse-Smith, A., Payne, A.G.T. (2016). Xenogeneic collagen matrix for periodontal plastic surgery procedures: a systematic review and meta-analysis. Journal of Periodontal Research, 51, 438–452

Cardaropoli, D., Tamagnone, L., Roffredo, A., Gaveglio, L. (2014). Coronally advanced flap with and without a xenogenic collagen matrix in the treatment of multiple recessions: a randomized controlled clinical study. International Journal of Periodontics and Restorative Dentistry, 34 (Suppl.), s97–s102.

Chambrone, L., Salinas Ortega, M.A., Sukekava, F., Rotundo, R., Kalemaj, Z., Buti, J., Pini Prato, G.P. (2018). Root coverage procedures for treating localised and multiple recession-type defects. Cochrane Database Systematic Reviews, October 2, 10:CD007161.

Chambrone, L., Tatakis, D.N. (2015). Periodontal soft tissue root coverage procedures: a systematic review from the AAP Regeneration Workshop. Journal of Periodontology, 86, (2 Suppl), S8-51.

da Silva, R.C., Joly, J.C., de Lima, A.F., Tatakis, D.N. (2004). Root coverage using the coronally positioned flap with or without a subepithelial connective tissue graft. Journal of Periodontology, 75, 413-419.

Dragan, I.F., Hotlzman, L.P., Karimbux, N.Y., Morin, R.A., Bassir, S.H. (2017). Clinical outcomes of comparing soft tissue alternatives to free gingival graft: a systematic review and meta-analysis. Journal of Evidence Based Dental Practice, 17, 370-380.

Ghanaati, S., Schlee, M., Webber, M.J., Willershausen, I., Barbeck, M., Balic, E., Görlach, C., Stupp, S.I., Sader, R.A., Kirkpatrick, C.J. (2011). Evaluation of the tissue reaction to a new bilayered collagen matrix in vivo and its translation to the clinic. Biomedical Materials, 6, 015010.

Graziani, F., Gennai, S., Roldan, S., Discepoli, N., Buti, J., Madianos, P., Herrera, D. (2014). Efficacy of periodontal plastic procedures in the treatment of multiple gingival recessions. Journal of Clinical Periodontology, 41 (Suppl. 15), S63–S76.

Herford, A.S., Akin, L., Cicciu, M., Maiorana, C., Boyne, P.J. (2010). Use of a porcine collagen matrix as an alternative to autogenous tissue for grafting oral soft tissue defects. Journal of Oral Maxillofacial Surgery, 68, 1463–1470.

Jepsen, K., Jepsen, S., Zucchelli, G., Stefanini, M., de Sanctis, M., Baldini, N., Greven, B., Heinz, B., Wennstrom, J., Cassel, B., Vignoletti, F., Sanz, M. (2013). Treatment of gingival recession defects with a coronally advanced flap and a xenogeneic collagen matrix: a multi-center randomized clinical trial. Journal of Clinical Periodontology, 40, 82–89.

Jepsen, K., Stefanini, M., Sanz, M., Zucchelli, G., Jepsen, S. (2017). Long-Term Stability of Root Coverage by Coronally Advanced Flap Procedures. Journal of Periodontology, 88(7), 626-633.

Moreira, A.R.O., Santamaria, M.P., Silvério, K.G., Casati, M.Z., Nociti Junior, F.H., Sculean, A., Sallum, E.A. (2016). Coronally advanced flap with or without porcine collagen matrix for root coverage: a randomized clinical trial. Clinical Oral Investigation, 20, 2539-2549.

Pini Prato, G.P., Cairo, F., Nieri, M., Franceschi, D., Rotundo, R., Cortellini, P. (2010). Coronally advanced flap versus connective tissue graft in the treatment of multiple gingival recessions: a split-mouth study with a 5-year follow-up. Journal of Clinical Periodontology, 37(7), 644-650.

Pini Prato, G., Rotundo, R., Franceschi, D., Cairo, F., Cortellini, P., Nieri, M. (2011). Fourteen-year outcomes of coronally advanced flap for root coverage:follow-up from a randomized trial. Journal of Clinical Periodontology, 38, 715–720.

Pini Prato, G.P., Franceschi, D., Cortellini, P., Chambrone, L. (2018). Long-term evaluation (20 years) of the outcomes of subepithelial connective tissue graft plus coronally advanced flap in the treatment of maxillary single recession-type defects. Journal of Periodontology, 11, 1290-1299.

Pini Prato, G.P., Magnani, C., Chambrone, L. (2018). Long-term evaluation (20 years) of the outcomes of coronally advanced flap in the treatment of single recession-type defects. Journal of

Periodontology, 89, 265-274.

Pini-Prato, G., Nieri, M., Pagliaro, U., Giorgi, T.S., La Marca, M., Franceschi, D., Buti, J., Giani, M., Weiss, J.H., Padeletti, L., Cortellini, P., Chambrone, L., Barzagli, L., Defraia, E., Rotundo, R. (2014). Surgical treatment of single gingival recessions: clinical guidelines. European Journal of Oral Implantology, 7, 9-43.

Pini-Prato, G., Rotundo, R., Cairo, F., Cortellini, P., Nieri, M. (2012). Long-term 8-year outcomes of coronally advanced flap for root coverage. Journal of Periodontology, 83, 590–594.

Rotundo, R., Pini-Prato, G. (2012). Use of a new collagen matrix (Mucograft) for the treatment of multiple gingival recessions: case reports. International Journal of Periodontics and Restorative Dentistry, 32, 413-419.

Santamaria, M.P., Suaid, F.F., Casati, M.Z., Nociti, F.H., Sallum, A.W, Sallum, E.A. (2008). Coronally positioned flap plus resin-modified glass ionomer restoration for the treatment of gingival recession associated with non-carious cervical lesions: a randomized controlled clinical trial. Journal of Periodontology, 79, 621-628.

Sanz, M., Lorenzo, R., Aranda, J.J., Martin, C., Orsini, M. (2009). Clinical evaluation of a new collagen matrix (Mucograft prototype) to enhance the width of keratinized tissue in patients with fixed prosthetic restorations: a randomized prospective clinical trial. Journal of Clinical Periodontology, 36, 868–876.

Schmitt, C.M., Tudor, C., Kiener, K., Wehrhan, F., Schmitt, J., Eitner, S., Agaimy, A., Schlegel, K.A. (2013). Vestibuloplasty: porcine collagen matrix versus free gingival graft: a clinical and histologic study. Journal of Periodontology, 84, 914–923.

Schulz, K.F., Altman, D.G., Moher, D., for the CONSORT Group. (2010). CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. Annals of Internal Medicine, 152.

Stefanini, M., Jepsen, K., de Sanctis, M., Baldini, N., Greven, B., Heinz, B.,Wennstrom, J., Cassel, B., Vignoletti, F., Sanz, M., Jepsen, S., Zucchelli, G. (2016). Patient-reported outcomes and aesthetic evaluation of root coverage procedures: a 12-month follow- up of a randomized controlled clinical trial. Journal of Clinical Periodontology, 43, 1132–1141.

Thoma, D.S., Jung, R.E., Schneider, D., Cochran, D.L., Ender, A., Jones, A.A., Görlach, C.,

Uebersax, L., Graf-Hausner, U., Hämmerle, C.H. (2010). Soft tissue volume augmentation by the use of collagen-based matrices: a volumetric analysis. Journal of Clinical Periodontology, 37, 659-666.

Tonetti, M.S., Jepsen, S.; Working Group 2 of the European Workshop on Periodontology (2014). Clinical efficacy of periodontal plastic surgery procedures: consensus report of Group 2 of the 10th European Workshop on Periodontology. Journal of Clinical Periodontology, 41 Suppl 15, S36-43.

Tonetti, M.S., Cortellini, P., Pellegrini, G., Nieri, M., Bonaccini, D., Allegri, M., Bouchard, P., Cairo, F., Conforti, G., Fourmousis, I., Graziani, F., Guerrero, A., Halben, J., Malet, J., Rasperini, G., Topoll, H., Wachtel, H., Wallkamm, B., Zabalegui, I., Zuhr, O. (2018). Xenogenic collagen matrix or autologous connective tissue graft as adjunct to coronally advanced flaps for coverage of multiple adjacent gingival recession: Randomized trial assessing non-inferiority in root coverage and superiority in oral health-related quality of life. Journal of Clinical Periodontology, 45, 78-88.

Woodyard, J.G., Greenwell, H., Hill, M., Drisko, C., Iasella, J.M., Scheetz, J. (2004). The clinical effect of acellular dermal matrix on gingival thickness and root coverage compared to coronally positioned flap alone. Journal of Periodontology, 75, 44–56.

Zucchelli, G., De Sanctis, M. (2000). Treatment of multiple recession-type defects in patients with esthetic demands. Journal of Periodontology, 71, 1506-1514.

## FIGURE LEGENDS

**Figure 1.** Coronally Advanced Flap with CMX – Test group: a) baseline; b) surgery; c) suturing; d) 1 year after surgery.

**Figure 2.** Coronally Advanced Flap alone - Control group: a) baseline; b) surgery; c) suturing; d) 1 year after surgery.

**Figure 3.** CONSORT Flow Diagram

**TABLES**

**Table 1. Baseline descriptive statistics. Mean and standard deviation are reported for quantitiative variables; frequency (percentage) is reported for the qualitative variables.**

| Variable | CAF N=12 Pat | CMX N=12 Pat |
|---|---|---|
| **Age (years)** | 38.1 (7.3) | 31.4 (4.9) |
| **FMPS** | 14.2 (6.1) | 7.4 (5.6) |
| **FMBS** | 10.6 (5.4) | 10.4 (6.1) |
| **Gender (% Female)** | 10 (83%) | 9 (75%) |
| **Recession (total)** | 31 | 30 |
| **Incisor** | 3 (10%) | 3 (10%) |
| **Canines** | 10 (32%) | 8 (27%) |
| **Premolars** | 17 (55%) | 17 (57%) |
| **Molars** | 1 (3%) | 2 (7%) |
| **Rec Width mm** | 3.6 (0.7) | 3.2 (0.7) |
| **IM-CEJ mm** | 8.9 (1.2) | 8.9 (1.5) |
| **IM-GM mm** | 11.6 (1.5) | 11.2 (1.7) |
| **CEJ-GM mm (Rec)** | 2.6 (1.0) | 2.3 (0.7) |
| **GThick mm** | 1.5 (0.6) | 1.4 (0.7) |
| **KTwidth mm** | 3.5 (1.8) | 3.3 (1.5) |
| **Plaque (%)** | 0 (0%) | 0 (0%) |
| **BoP (%)** | 0 (0%) | 2 (7%) |
| **PD mm** | 1.5 (0.5) | 1.5 (0.5) |
| **CAL mm** | 4.2 (1.3) | 3.8 (0.6) |

**Table 2. Periodontal Clinical variables at baseline, 3, 6, and 12 months**

| | Baseline | | 3 months | | 6 months | | 12 months | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | **CAF N=12 Pat n=31 Rec** | **CMX N=12 Pat n=30 Rec** | **CAF N=12 Pat n=31 Rec** | **CMX N=12 Pat n=30 Rec** | **CAF N=12 Pat n=31 Rec** | **CMX N=12 Pat n=30 Rec** | **CAF N=12 Pat n=31 Rec** | **CMX N=12 Pat n=30 Rec** |
| **CEJ-GM mm (Rec)** | 2.6 (1.0) | 2.3 (0.7) | 0.5 (0.9) | 0.2 (0.4) | 0.5 (0.8) | 0.3 (0.5) | 0.6 (0.3) | 0.3 (0.4) |
| **Rec Width mm** | 3.6 (0.7) | 3.2 (0.7) | 1.0 (1.7) | 0.7 (1.2) | 1.2 (1.7) | 0.8 (1.2) | 1.5 (1.7) | 0.7 (1.0) |
| **GThick mm** | 1.5 (0.6) | 1.4 (0.7) | 1.5 (0.8) | 1.4 (0.6) | 1.4 (0.6) | 1.6 (0.8) | 1.2 (0.5) | 1.7 (0.7) |
| **KT width mm** | 3.5 (1.8) | 3.3 (1.5) | 2.8 (2.1) | 3.5 (1.5) | 2.6 (1.8) | 2.8 (1.0) | 2.5 (1.1) | 2.7 (1.2) |
| **Plaque (%)** | 0 (0%) | 0 (0%) | 1 (3%) | 5 (17%) | 4 (13%) | 5 (17%) | 0 (0%) | 4 (13%) |
| **BoP (%)** | 0 (0%) | 2 (7%) | 0 (0%) | 0 (0%) | 1 (3%) | 1 (3%) | 1 (3%) | 0 (0%) |
| **PD mm** | 1.5 (0.5) | 1.5 (0.5) | 1.1 (0.4) | 1.5 (0.8) | 1.2 (0.6) | 1.5 (0.6) | 1.3 (0.5) | 1.4 (0.6) |
| **CAL mm** | 4.2 (1.3) | 3.8 (0.6) | 1.6 (1.1) | 1.8 (0.8) | 1.7 (1.0) | 1.8 (0.7) | 1.9 (1.0) | 1.7 (0.6) |
| **CRC (%)** | 0 (0%) | 0 (0%) | 22 (71%) | 22 (73%) | 20 (65%) | 20 (67%) | 16 (52%) | 19 (63%) |

**Table 3. Mixed model with Patient random effect and covariate with the value at baseline at 1 year of follow-up and between baseline and end of follow-up.**

| Variable | CAF N=12 Pat n=31 Rec | CMX N=12 Pat n=30 Rec | Adjusted Difference | 95%CI | P-value |
|---|---|---|---|---|---|
| **CEJ-GM T1 mm** | 0.6 (0.3) | 0.3 (0.4) | | | |
| **Diff T1T0 CEJ-GM mm** | 2.0 (1.1) | 2.0 (0.8) | 0.3 | -0.2; 0.8 | 0.2023 |
| **Rec Width T1 mm** | 1.5 (1.7) | 0.7 (1.0) | | | |
| **Diff T1T0 Rec Width mm** | 2.1 (1.6) | 2.4 (1.2) | 0.5 | -0.5; 1.5 | 0.2919 |
| **GThick T1 mm** | 1.2 (0.5) | 1.7 (0.7) | | | |
| **Diff T1T0 GThick mm** | -0.3 (0.7) | 0.2 (0.7) | 0.5 | 0.2; 0.8 | 0.0057 |
| **KT T1 mm** | 2.5 (1.1) | 2.7 (1.2) | | | |
| **Diff T1T0 KT mm** | -1.1 (1.3) | -0.6 (1.7) | 0.2 | -0.6; 1.1 | 0.5668 |
| **PD T1 mm** | 1.3 (0.5) | 1.4 (0.6) | | | |
| **Diff PD T1T0** | 0.2 (0.6) | 0.1 (0.7) | -0.1 | -0.5; 0.3 | 0.7331 |
| **CAL T1 mm** | 1.9 (1.0) | 1.7 (0.6) | | | |
| **Diff CALT1T0** | 2.2 (1.5) | 2.1 (0.8) | 0.3 | -0.4; 0.9 | 0.4073 |
| **Plaque*** | 0 (0%) | 4 (13%) | | | 0.0525 |
| **BoP*** | 1 (3%) | 0 (0%) | | | 1.0 |
| **CRC** | 16 (52%) | 19 (63%) | | | 0.4919 |
| **Diff T1T0 CEJ-GM%** | 75 (30) | 87 (19) | 11 | -8; 30 | 0.2578 |

* Fisher exact test

**Table 4. Inferential statistics (t-tests) on *chair-time* and *patient-reported outcomes and experienced measures.***

| Variable | CAF N=12 | CMX+CAF N=12 | Difference | 95%CI | P-value |
|---|---|---|---|---|---|
| **Chair-time (min)** | 36.1 (4.6) | 47.3 (5.8) | 11.2 | 6.8; 15.7 | <0.0001 |
| **VAS 1d** | 1.5 (1.9) | 2.7 (2.7)* | 1.2 | -0.8; 3.2 | 0.2192 |
| **VAS 2d** | 1.5 (2.5) | 1.4 (2.0)* | -0.1 | -2.1; 1.8 | 0.8876 |
| **VAS 3d** | 1.5 (2.8) | 0.7 (1.6)* | -0.8 | -2.8; 1.2 | 0.4341 |
| **VAS 4d** | 1.2 (2.5) | 0.5 (1.3)* | -0.6 | -2.4; 1.1 | 0.4661 |
| **VAS 5d** | 0.9 (2.0) | 0.4 (0.9)* | -0.6 | -1.9; 0.8 | 0.4069 |
| **VAS 6d** | 0.4 (0.9) | 0.2 (0.4)** | -0.2 | -0.9; 0.4 | 0.4933 |
| **Pain Day (day 7)** | 1.2 (2.2)* | 0.7 (0.9)* | -0.5 | -1.9; 1.0 | 0.5307 |
| **Swelling (day 7)** | 1.7 (1.6)* | 3.6 (2.7)* | 1.9 | -0.0; 3.9 | 0.0552 |
| **Chewing (day 7)** | 1.1 (2.3)* | 0.8 (2.1)* | -0.3 | -2.2;1.7 | 0.7762 |
| **Hemorrhage (day 7)** | 0.1 (0.3)* | 0.3 (0.9)* | 0.2 | -0.4; 0.8 | 0.7329 |
| **Painkillers (n° days)** | 2.9 (1.7) | 2.7 (2.3) | -0.2 | -1.9; 1.6 | 0.8239 |
| **Satisfaction (1 year)** | 9.1 (1.6)* | 9.3 (1.5) | 0.2 | -1.1; 1.6 | 0.7092 |
| **Esthetics (1 year)** | 8.8 (2.0)* | 9.3 (1.0) | 0.4 | -0.9; 1.8 | 0.5094 |
| **Brushing (1 year)** | 1.2 (2.4)* | 0.1 (0.3) | -1.1 | -2.6; 0.4 | 0.1361 |
| **Hypersensitivity (1 year)** | 0.7 (1.1)* | 1.7 (2.2) | 1.0 | -0.5; 2.5 | 0.1765 |

* N=11; ** N=10