

Does placement of a connective tissue graft improve the outcomes of coronally advanced flap for coverage of single gingival recessions in upper anterior teeth? A multi-centre, randomized, double-blind, clinical trial

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Cortellini P, Tonetti M, Baldi C, Francetti L, Rasperini G, Rotundo R, Nieri M, Franceschi D, Labriola A, Pini Prato G. Does placement of a connective tissue graft improve the outcomes of coronally advanced flap for coverage of single gingival recessions in upper anterior teeth? A multi-centre, randomized, double blind, clinical trial. *J Clin Periodontol* 2008; doi: 10.1111/j.1600-051X.2008.01346.x.

Abstract

Aims: This parallel-group, multi-centre, double-blind, randomized-controlled clinical trial was undertaken to compare the clinical outcomes and patient morbidity of coronally advanced flap (CAF) alone or in combination with a connective tissue graft (CAF+CTG) in single Miller Class I and II gingival recessions.

Material and Methods: Three centres enrolled 85 patients with one recession each. Surgery was performed elevating a pedicle flap; 42 sites randomly received a graft under the flap. Measurements were taken by blind and calibrated examiners. Outcome measures included recession reduction, complete root coverage (CRC), intra-operative and post-operative morbidity, dentine sensitivity, and side effects.

Results: No differences were noted in the intra-operative and post-operative patient-related variables between the two groups. Surgical time was significantly shorter in the CAF group. Recession reduction was not statistically different between the two groups, even though a model showed a tendency towards improved outcomes in sites treated with CAF+CTG (adjusted difference 0.33 mm, 95% CI = -0.06 to 0.72, $p = 0.1002$). Significantly greater probability of CRC was observed after CAF+CTG (adjusted OR = 5.09, 95% CI = 1.69–17.57, $p = 0.0033$). Dentine hypersensitivity improved in both the groups.

Conclusions: Both treatments were effective in providing a significant reduction of the baseline recession and dentine hypersensitivity, with only limited intra-operative and post-operative morbidity and side effects. Adjunctive application of a CTG under a CAF increased the probability of achieving CRC in maxillary Miller Class I and II defects.

Key words: clinical trial; periodontal surgery; gingival recession; root coverage

Accepted for publication 7 October 2008

Conflict of interest and source of funding statement

No conflict of interest declared by any of the Authors.

This study has been self-supported by the authors, the Accademia Toscana di Ricerca Odontostomatologica (ATRO), and the European Research Group on Periodontology, ERGOPERIO.

Recession of the gingival margin remains a highly prevalent problem for its impact on both aesthetics and dentine hypersensitivity (Röthlisberger et al. 2007, Hugoson et al. 2008). Even

though its aetiology still needs elucidation (Rajapakse et al. 2007), during the last three decades several surgical techniques have been proposed to treat gingival recessions, like the free gingival graft (Sullivan & Atkins 1968), the coronally advanced flap (CAF; Allen & Miller 1989), the CAF with a sub-epithelial or connective tissue graft (CAF+CTG; Langer & Langer 1985), and various regenerative procedures such as the use of non-resorbable barriers (Pini Prato et al. 1992), bio-resorbable barriers (Roccuzzo et al. 1996), enamel matrix derivative (EMD; Rasperini et al. 2000), or the application of a platelet-rich gel (Keceli et al. 2008) in combination with CAF. Although all these techniques have shown a consistent potential for root coverage, meta-analyses from several systematic reviews (Roccuzzo et al. 2002, Clauser et al. 2003, Oates et al. 2003, Cairo et al. 2008) revealed an ample degree of variability of clinical results. The outcomes of these reviews showed a greater recession reduction and a larger amount of roots completely covered following bilaminar techniques (CAF+CTG) as compared with regenerative procedures. More recently, several authors have proposed the application of EMD in combination with CAF, generally reporting improved outcomes with the combined approach (Del Pizzo et al. 2005, Spahr et al. 2005, Castellanos et al. 2006, Pilloni et al. 2006). A randomized-controlled study compared CAF+EMD *versus* CAF+CTG (McGuire & Nunn 2003). Authors reported that the latter resulted in a greater increase in the height of keratinized tissue (KT) at 24 months, but there was no difference in terms of root coverage. An acellular dermal matrix has also been recently proposed as a replacement for CTG in bilaminar techniques, with promising clinical outcomes in terms of root coverage (Harris 2000, Aichelmann-Reidy et al. 2001, Côrtes Ade et al. 2004, de Queiroz Côrtes et al. 2006, Joly et al. 2007, Andrade et al. 2008).

The CAF and the bilaminar techniques are actually perceived as the most reliable procedures, and, in recent years, several technical modifications have been developed to improve root coverage and the aesthetic result. Pini Prato et al. (2000) suggested that flap tension is a key issue in CAF procedures: tension-free flaps have higher chances to achieve complete root coverage (CRC). The same group (Pini Prato et al. 2005)

showed that CRC following CAF is influenced by the surgical positioning of the flap relative to the cemento-enamel junction (CEJ): when the flap was positioned 1–2 mm coronal to the CEJ, the probability for CRC increased. De Sanctis and Zucchelli (2007) proposed a modified CAF procedure consisting of a modification of the flap thickness and of the dimension of the surgical papillae during flap elevation. The same authors suggested a modification of the bilaminar technique (Zucchelli et al. 2003) with the adoption of a CTG of reduced size and minimal thickness, positioned immediately apical to the CEJ.

A direct comparison between CAF and CAF+CTG has been performed so far only in one randomized-controlled clinical trial (da Silva et al. 2004) on 11 patients. This study demonstrated a significant reduction of recession depth in both groups but did not show any difference between the two groups in terms of recession depth, pocket depth, and clinical attachment level both at baseline and at 6 months.

The aim of this multi-centre, double-blind, randomized-controlled clinical

trial was to compare the root coverage of the CAF alone with the CAF+CTG in the treatment of single Miller Class I and II gingival recessions.

Material and Methods

Experimental design

This was a parallel-group, randomized, double-blind, multi-centre clinical trial on treatment of gingival recessions (Fig. 1). Two different modalities for root coverage were compared: the CAF and the CAF with a CTG (CAF+CTG, bilaminar technique). Three centres were selected to participate in this study. Each centre was committed to enrol and treat 30 patients in a period of time between May 2005 and December 2006. Each patient (experimental unit) contributed with a recession. In case of patients presenting with multiple recessions, the deepest one was selected; in case of two or more recessions with the same depth, the selection was performed by tossing a coin. Early healing events were evaluated at weeks 1, 2, 3, and 4. Clinical outcomes were evaluated at 3 and 6 months. Patient

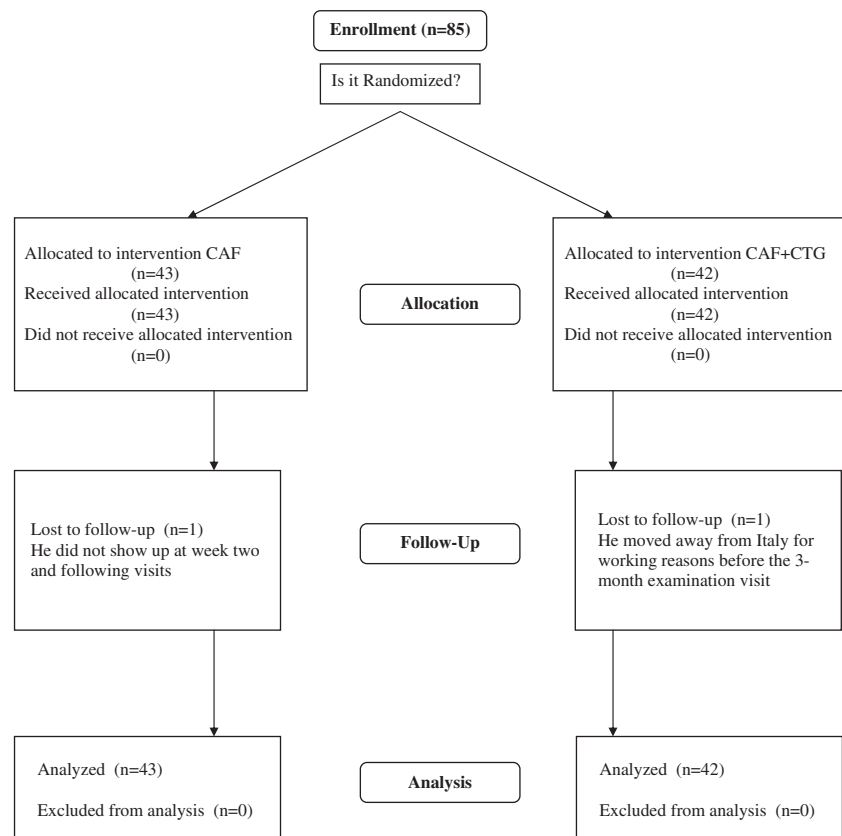


Fig. 1. Consort flowchart of the study.

questionnaires were collected immediately after surgery and at week 1.

Sample size

The study was powered to detect a minimum clinically significant difference (δ) in root coverage of 0.5 mm using $\alpha = 0.05$, a power = 80%, a σ of 0.46 mm obtained in a previous study from this group (Pini Prato et al. 2005), and considering Rec_{T0} (recession depth at time 0 or baseline) as a covariate. Calculations were performed using PS Power and Sample Size Calculation Software (version 2.1.30, February 2003, <http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize>) (Dupont & Plummer 1990). Fourteen patients per treatment arm and per centre were needed.

Investigator training

All participating investigators were required to attend two training and calibration meetings. The aims of the meetings were to review the objectives of the study and the protocol; standardize the case selection, the measurement techniques, and the surgical procedures; suggest rules for the compilation of the data collection sheets; explain study administration and communication procedures; and suggest detailed organizational strategies to optimize patient accrual and retention as well as patient and data management.

A calibration exercise was performed to obtain acceptable intra- and inter-examiner reproducibility for recession depth measurements to the CEJ and to a custom-made acrylic stent.

Study population

Patients in good general health, with no untreated periodontal disease, and presenting with gingival recessions were considered eligible for this clinical trial. To enrol a patient into the study, the following entry criteria had to be satisfied:

- Age ≥ 18 years.
- No relevant systemic condition or disease.
- Periodontal Screening and Recording score < 3 in all sextants.
- Full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS) $< 30\%$ (measured at four sites per tooth).

- Smoking ≤ 20 cigarette/day. Habitual pipe and cigar smokers were excluded from the study.
- Presence of at least one Miller Class I or II (Miller 1985) buccal recession ≥ 2 mm deep in each patient, limited to upper central and lateral incisors, canine, and first and second pre-molars.
- Teeth with prosthetic crown or restoration with the cervical edge at the CEJ area were excluded.
- Presence of a step ≤ 1 mm at CEJ level and/or presence of a root/crown abrasion, but with an identifiable CEJ, was accepted.
- Absence of a pulling frenum in the KT.
- No history of mucogingival or periodontal surgery at the experimental site.

Pre-treatment: modification of oral hygiene habits

All patients received oral hygiene instructions to modify the habits related to the aetiology of the recession at least 4 weeks before surgery. In particular, patients were instructed to use a power-driven toothbrush (Braun Triumph, Oral-B-Gillette, Milano, Italy), according to the manufacturer's instructions.

Patient entry (informed consent, patient registration, and randomization)

The study protocol was reviewed by the competent institutional review boards. Informed consent was obtained from all subjects entered into the study. In obtaining the informed consent and in the conduct of the study, the principles outlined in the Declaration of Helsinki on experimentation involving human subjects had to be adhered to. For patient protection, possible side effects of surgical therapy were handled according to the current standards of care in private practice.

Subjects were enrolled in the study by a Central Registrar located at the Clinical Research support infrastructure of the Department of Periodontology, Dental School, University of Florence. The Registrar verified the satisfaction of the entry criteria and randomly assigned each subject to a treatment group. Randomization was performed by computer-generated randomization tables. A balanced random permuted block approach was used to decrease the

chances of unbalances between test and control in terms of baseline recession depth and smoking. To ensure ability to perform a stratified analysis by centre, different randomization tables were constructed for each centre. Allocation concealment was obtained using a sealed coded opaque envelope containing the treatment to the specific subject.

A pre-surgical full-mouth professional prophylaxis appointment was scheduled during the 2 weeks before the scheduled surgical procedure.

The sealed envelope containing the treatment assignment was opened during the surgical procedure at the time that required the harvest and the application of the CTG (CAF+CTG) or its omission (CAF).

Clinical measurements

Clinical measurements and photographs (horizontal format 1:1) were taken at baseline, during and after surgery, at weeks 1, 2, 3, 4, and at months 3 and 6 examination visits by a blinded examiner in each centre.

Baseline measurements

At baseline, a stent was fabricated using Pattern Resin (GC[®], GC Italy, San Giuliano Milanese, Italy) material directly in the mouth. A reference point (slot) was carved on the stent at the mid-buccal area of the experimental tooth, to allow a reproducible periodontal probe positioning. The apical margin of the stent served as the measurement reference point: it was linear and positioned in the coronal third of the tooth, leaving the inter-dental papillae visible.

The following clinical measurements were taken by a blind examiner in each centre with a periodontal probe (PCP UNC 15, Hu-Friedy, Leimen, Germany):

- Probing depth (PD) on mesio-, mid-, and disto-buccal sites of the experimental tooth and the two adjacent ones.
- Recession depth (REC) on mesio-, mid-, and disto-buccal sites on the experimental tooth and the two adjacent ones. This measure was referred to the visible CEJ and it was considered equal to 0 whenever the gingival margin covered the CEJ. If a step was present, the measure was referred to the coronal

margin of the step. On the experimental tooth the mid-buccal recession was measured with the probe positioned according to the reference point of the stent.

- Presence of a step (≤ 1 mm at the CEJ level, according to entry criteria) and/or presence of a root/crown abrasion, but with an identifiable CEJ, was recorded.
- Distance from the apical reference point of the stent to the gingival margin of the experimental tooth.
- Distance from the apical reference point of the stent to the CEJ of the experimental tooth.
- Buccal recession width of the experimental tooth measured at the CEJ level with a caliper.
- Clinical attachment level of the three buccal sites was calculated as PD+REC (REC was considered equal to 0 whenever the CEJ was covered) on three buccal sites of the considered tooth and the two adjacent ones.
- KT width of the treated tooth and the two adjacent ones measured from the gingival margin to the mucogingival junction (MGJ), at the mid-buccal point. The MGJ was identified by means of Lugol staining.
- Dentine hypersensitivity was measured using the air evaporative stimulus (AES) essentially as described (Yates et al. 2004).

Intra-surgical and post-surgical measurements

After flap elevation and root debridement and at completion of surgery, the following measurements were taken:

- Distance from the CEJ (or the coronal part of a step, when present) at the mid-buccal point of the experimental tooth to the bone crest.
- Distance from the apical reference point of the stent at the mid-buccal point of the experimental tooth to the bone crest.
- Distance from the apical reference point of the stent at the mid-buccal point of the experimental tooth to gingival margin, after completion of sutures.
- Surgical chair-time.

Early healing records (weeks 1–4)

Exposure of the CEJ (or the coronal part of a step, when present) was evaluated at

the weekly recall examination visits for a period of 4 weeks, along with inflammation, swelling, dentine hypersensitivity, and any other post-surgical complication. The buccal local plaque score was also recorded.

Clinical measurements at 3 and 6 months

At 3 and 6 months, photographs (horizontal format 1:1) and the following clinical measurements were taken by the blind examiner in each centre:

- Buccal recession depth on the experimental tooth and the two adjacent ones, measured at the mesio-, mid-, and disto-buccal sites.
- Distance from the apical reference point of the stent to the gingival margin.
- PD at the mesio-, mid-, and disto-buccal sites of the considered tooth and the two adjacent ones (only at 6-month re-evaluation).
- KT, measured at the mid-buccal point from the gingival margin to the MGJ (using Lugol staining).
- Dentine hypersensitivity (AES).
- Buccal local plaque score of the experimental tooth and the two adjacent ones.

Evaluation of intra-operative and post-operative morbidity

Patient perception of intra-operative and post-operative morbidity was evaluated with a questionnaire given to patients immediately after surgery (hardship of the procedure and intra-surgical pain perception) and at the time of suture removal (post-operative pain, discomfort, use of anti-inflammatory tablets, interference with daily life, interference with job and interference with relationships, and tooth hypersensitivity). Questionnaires included dichotomous questions and the evaluation of the intensity of the given event on a visual analogue scale (VAS) of 100 mm (Cortellini et al. 2001, Tonetti et al. 2004).

Surgical procedure

The control group was treated with the CAF (Fig. 2), while the test group received the same CAF+CTG (Fig. 3). The same operator in each centre performed both procedures.

Local anaesthesia was performed trying to avoid over-infiltration in the KT. The surgical technique started in all the patients with two oblique, divergent releasing incisions extending beyond the MGJ. An intra-sulcular incision was performed at the buccal aspect of the involved tooth. A partial-thickness flap was raised extending beyond the MGJ, leaving the underlying periosteum in place. The papillae adjacent to the involved tooth were de-epithelialized. A gentle root debridement was performed using a sharp curette up to 1 mm from the bone crest.

The randomization opaque envelope was opened at this time and the clinician was instructed whether or not to harvest and apply a CTG under the flap.

In the cases treated with the test procedure, a 1–2-mm-thick CTG was harvested from the palate in the area between the second pre-molar and the second molar. The graft was positioned on the instrumented root surface immediately apical or at the level of the CEJ and was stabilized to the periosteum with four passing resorbable sutures (Dexon 5-0, Davis & Geck™, Wayne, NJ, USA) and a compressing sling suture. The wound on the donor site of the palate was also sutured.

Surgery was concluded by performing mesio-distal and apical extension of the partial-thickness dissection as needed in order to release any residual muscle tension and allow a passive coronal displacement of the flap. The flap was displaced coronally and sutured to cover the CEJ completely in the control group and both the graft and the CEJ in the test experimental sites. A sling suture was placed to stabilize the flap in a coronal position, followed by interrupted sutures on the releasing incisions with an apico-coronal direction, using gore-tex 6-0 sutures.

Post-surgical instructions

The following post-operative regime was prescribed to all patients. Post-operative pain and oedema were controlled with ibuprofen. Patients received 600 mg at the beginning of the surgical procedure and were instructed to take another tablet 6 h later. Subsequent doses were taken only if necessary to control pain. Patients with contraindications to NSAIDs received 500 mg acetaminophen at surgery and after 6 h. Patients were instructed to intermittently apply an ice bag on the operated

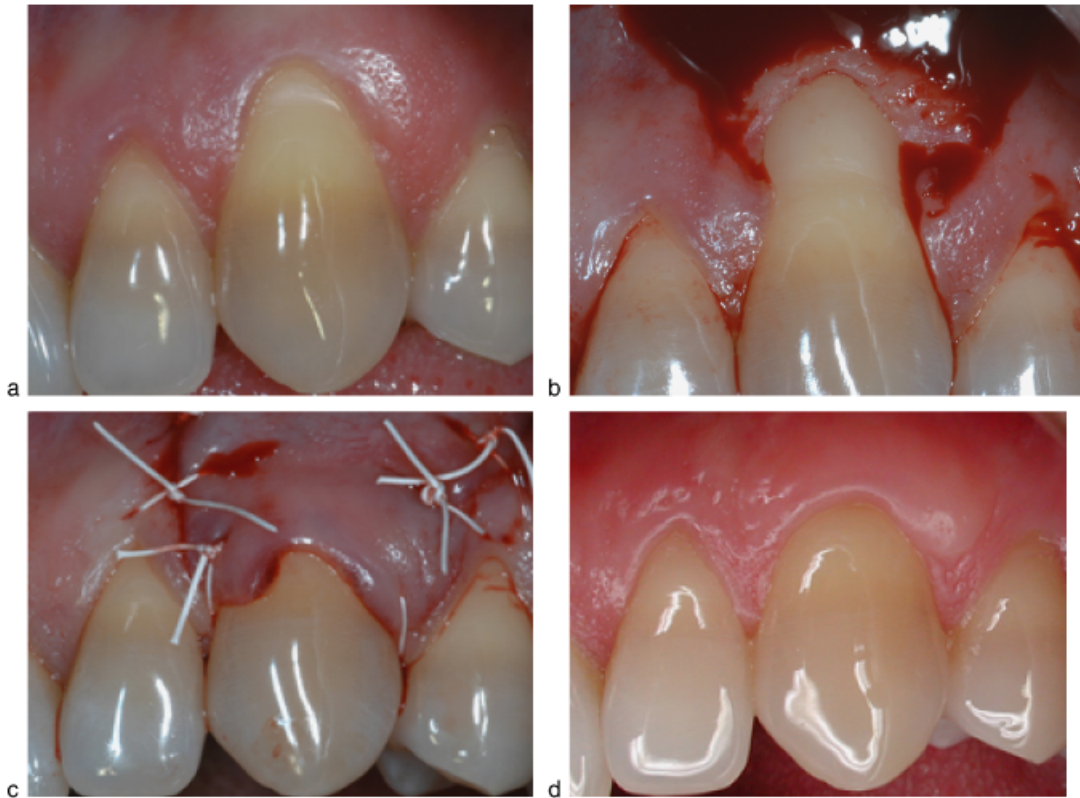


Fig. 2. (a) Pre-operative image of an upper left cuspid presenting with a Miller Class I recession and a shallow step at the cemento-enamel junction (CEJ). This recession was treated with a coronally advanced flap (CAF). (b) Exposed root surface after the elevation of the flap. (c) A pedicle flap has been sutured coronal to the CEJ. (d) Complete root coverage at 6-month examination.

area (5 min. yes, 5 min. no) for the first 2 h.

Patients were requested to discontinue toothbrushing and avoid trauma and food impaction at the surgical site. Smokers were reminded to limit (and possibly quit) smoking. A 1-min. rinse with 0.12% chlorhexidine digluconate was prescribed 3 times/day for the first 2 weeks.

Post-surgical controls and professional tooth cleaning

The sutures were removed after 7–9 days. Patients were instructed to brush with a post-surgical soft toothbrush (Gum Delicate Post-Surgical Toothbrush, Oral B-Gillette) for the following 2 weeks, resuming inter-dental cleaning. Three weeks after surgery, the patients were allowed to resume regular mechanical tooth cleaning of the treated areas using the power-driven toothbrush (Braun Triumph, Oral-B-Gillette), with the appropriate technique. Patients were recalled for controls (and prophylaxis as needed) at weeks 1, 2, 3, and 4

and at months 3 and 6. The modality of use of the power-driven toothbrush was reviewed at each appointment.

Subject protection

At each visit, the clinicians evaluated patients for any untoward effects. If a patient required any treatment during the course of the study, the necessary treatment was provided at the discretion of the clinician and according to the current standard of care.

Statistical analysis

Calibration of the three examiners was performed before the beginning of the study. The evaluation was performed on measurement of the gingival recession (CEJ – gingival margin) and on the measurement of the distance between the acrylic stent and the gingival margin. All analyses followed the intention-to-treat principle; in case of dropouts, the baseline clinical outcome measures were brought forward for the 3- and 6-

month evaluations. For wound-healing outcomes (swelling, inflammation, etc.), the last observation was carried forward.

Descriptive statistics were performed using mean \pm standard deviation for quantitative variables and frequencies and percentage for qualitative variables.

The primary outcome variable *recession reduction (rec red)* was measured as a difference between *stent-gingival margin_{T0}* and *stent-gingival margin_{T6}*. In the general linear model, the explicative variables were *therapy* (CAF+CTG versus CAF), *baseline recession (Rec_{T0})*, and *centre*. Interactions were permitted between *therapy* and *Rec_{T0}* and between *therapy* and *centre*. However, in case of a non-significant value of the interactions, they were discarded.

Two similar models were constructed for KT gain and CRC. For CRC, a logistic regression was performed.

Secondary variables were tested for difference between *therapy* and *centre* with a test for interaction effect, such as surgical time and patient-related factors (expressed in VAS values).

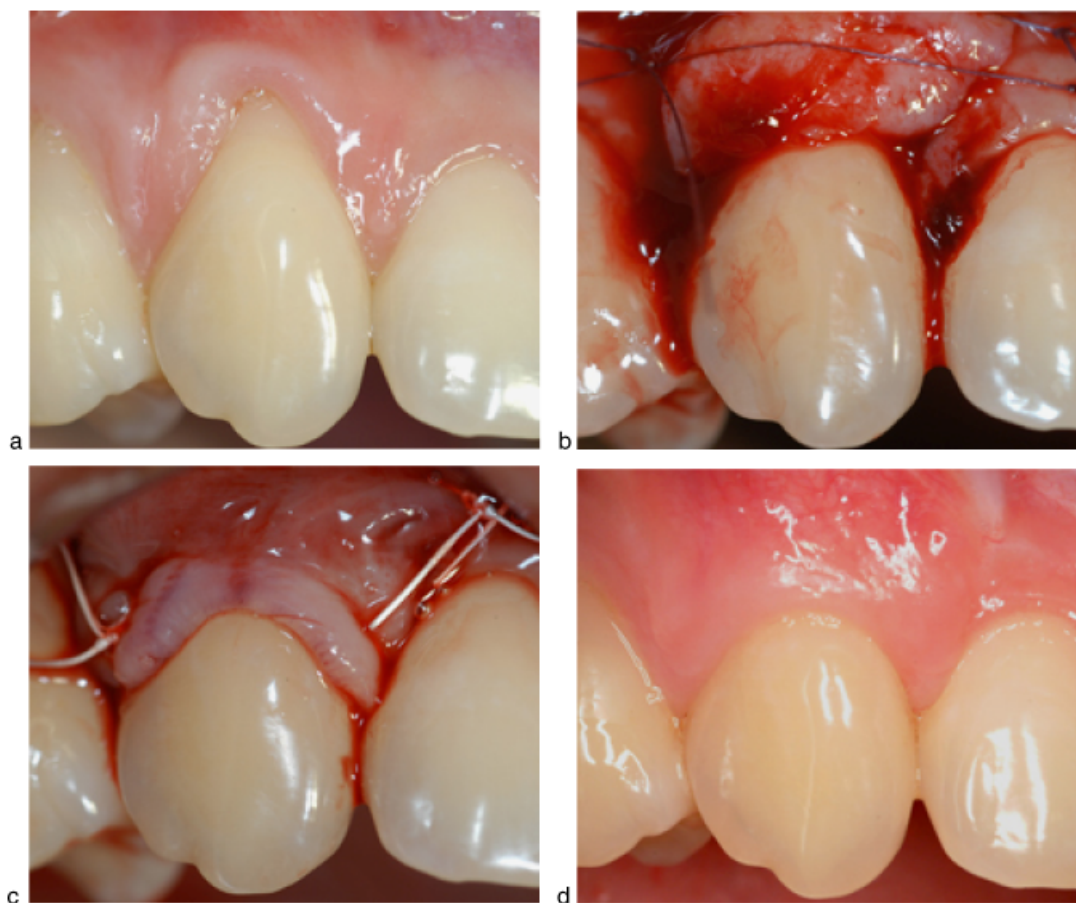


Fig. 3. (a) Pre-operative image of an upper right cuspid presenting with a Miller Class I recession. This recession was treated with a coronally advanced flap (CAF) plus a connective tissue graft (CTG). (b) The CTG has been sutured to cover the exposed root surface. (c) The pedicle flap has been coronally displaced and sutured to cover the graft and the cemento-enamel junction (CEJ). (d) At 6 months, the exposed root surface is completely covered.

Results

Experimental population and calibration of the examiners

A total of 85 patients participated in the study; two centres enrolled and treated the ideal number of 30 patients each (15 patients in the test and 15 in the control group), while one centre could enrol only 25 patients (13 in the CAF group) within the due experimental period of time (May 2005 to December 2006). There were two dropouts among the enrolled patients. One patient (centre 2 assigned to the CAF group) did not show up at week 2 and the following visits. Another patient (centre 3 assigned to the CAF+CTG group) before the 3-month examination visit moved away from Italy for working reasons.

The calibration of the three examiners resulted in an intra-class correlation coefficient of 0.87 for the measurement of the gingival recession (CEJ–gingival

margin) and of 0.96 for the measurement of the distance between the acrylic stent and the gingival margin. There was no statistically significant difference among the three examiners. Based on these results, changes in the gingival margin level were calculated using the acrylic stent as a reference point.

Patient and defect characteristics at baseline

Table 1 reports the baseline patient- and defect-related characteristics. The two groups were well balanced in terms of age, FMPS, and FMBS. There were more females in the CAF+CTG group. Most of the treated teeth in both groups were cuspids and most of the times the indication for surgery was aesthetics. One-third of the cases in both groups had formerly received an orthodontic treatment. Most of the treated recessions were classified as Miller Class I; about half of the experimental teeth in both

groups presented a root abrasion and <40% an identifiable CEJ step.

Baseline defect characteristics are reported in Table 2. No difference was noted for any of the collected measurements between the two groups.

Evaluation of the surgical procedure and of the post-operative period

Patient perception of the hardship of the two procedures was mild (Table 3) and there was no statistically significant difference between CAF and CAF+CTG, even if the latter resulted in higher average VAS values and in the report of one patient that reached the end of the VAS scale (100 mm). However, there was a significant difference among the centres: in particular, centre 2 reported higher values with respect to centres 1 and 3. Intra-operative pain was perceived by four patients in the CAF group and by eight patients in the other

one. VAS values were very low in both the groups.

Most of the cases ended surgery with the CEJ completely covered with the flap (about 1 mm coronal to CEJ on average, in both groups), even though

in five CAF-treated cases, and in three CAF+CTG-treated cases the CEJ remained visible at the end of surgery. In particular, centre 2 had a significantly lower GM₁ value in the CAF group with respect to the other centres.

The overall surgical chair-time was significantly shorter for CAF. There was no difference in the CAF surgical time among the centres, while there was a significant difference in the CAF+CTG approach. In particular, centre 2 declared a longer surgical chair-time than centre 1, and centre 1 longer than centre 3.

At the week 1 examination visit (Table 4), 10 patients reported some pain after CAF lasting for 1.1 days, and 18 after CAF+CTG (lasting for 1.4 days). Pain-related VAS values were very low and there was no statistically significant difference between the two groups and among the three centres. Fifteen patients from the CAF group and 18 from the test group took extra anti-inflammatory tablets (0.7 ± 1.2 and 1.3 ± 2.5 , respectively) in addition to the two compulsory ones given around surgery. There was no statistically significant difference between the two treatments and among the three centres in terms of consumption of anti-inflammatory tablets. Interference with daily life activities was reported by 30 patients for CAF and 26 patients for CAF+CTG, job interference by nine patients for CAF and 11 patients for CAF+CTG, and relationship interference by 18 patients for CAF and 14 for CAF+CTG. The related VAS values were rather low, and there was no difference between the two treatments and among the three centres for all these variables, with the exception of daily life interference, which was reportedly higher in VAS values for centre 1 with respect to centre 3.

Patient perception of swelling was greater in the group of patients treated with CAF+CTG. In general, patients from centre 2 reported more swelling in comparison with centre 3.

From a clinical point of view at week 1, clinicians noted suture disruption or loss in 33% of CAF-treated sites and in 19% of CAF+CTG-treated sites.

Table 1. Baseline patient-related characteristics

	CAF (n = 43)	CAF+CTG (n = 42)
Age [mean \pm SD (years), range]	37.8 \pm 8.4 (25–59)	35.0 \pm 8.7 (20–54)
Sex (female)	20 (47%)	28 (67%)
Smoking patients	9 (21%)	13 (31%)
Cigarette pack/year (mean \pm SD, range)	0.9 \pm 2.8 (0–15)	1.8 \pm 4.3 (0–19)
FMPS [mean \pm SD (%), range]	11.6 \pm 7.0 (1–25)	9.8 \pm 7.2 (0–29)
FMBS [mean \pm SD (%), range]	5.8 \pm 4.9 (0–18)	5.0 \pm 4.1 (0–16)
Type of tooth		
Incisor	3	1
Canine	26	20
Pre-molar	14	21
Indication		
Aesthetics	20 (47%)	15 (36%)
Dental hypersensitivity	4 (9%)	4 (10%)
Aesthetics+dental hypersensitivity	13 (30%)	14 (33%)
Other	6 (14%)	9 (21%)
Previous orthodontic treatment	14 (33%)	14 (33%)
Cervical abrasion		
Crown	1 (2%)	2 (5%)
Root	21 (49%)	16 (38%)
Crown+root	2 (5%)	5 (12%)
CEJ step	16 (37%)	15 (36%)
Miller class		
Class I	41 (95%)	38 (90%)
Class II	2 (5%)	4 (10%)

CAF, coronally advanced flap; CTG, connective tissue graft; FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score; CEJ, cemento-enamel junction.

Table 2. Baseline defect-related characteristics

	CAF (n = 43) mean \pm SD (range) in mm	CAF+CTG (n = 42) mean \pm SD (range) in mm
Recession depth	2.4 \pm 0.7 (2–5)	2.7 \pm 0.7 (2–5)
Recession width	3.8 \pm 0.7 (3–6)	3.8 \pm 0.8 (2–5)
PD	1.2 \pm 0.4 (1–2)	1.2 \pm 0.4 (1–2)
CAL	3.7 \pm 0.8 (3–6)	3.8 \pm 0.8 (3–6)
KT	3.2 \pm 1.3 (1–6)	2.7 \pm 1.2 (0–5)
CEJ-BC	5.0 \pm 1.5 (3–12)	4.9 \pm 1.0 (3–8)

CAF, coronally advanced flap; CTG, connective tissue graft; PD, pocket depth; CAL, clinical attachment level; KT, keratinized tissue width; CEJ-BC, distance between cemento-enamel junction and bone crest (intra-surgical measurement).

Table 3. Inferential statistic

	CAF (n = 43) mean \pm SD (range)	CAF+CTG (n = 42) mean \pm SD (range)	Difference CAF versus CAF+CTG p-value	Centre effect p-value	Interaction p-value
GM ₁ (mm)	0.7 \pm 1.1 (–1 to 3)	0.9 \pm 1.0 (–1 to 4)	0.3651	<0.0001	NS
Surgical time (min.)	28.1 \pm 5.4 (20–40)	45.5 \pm 12.0 (30–80)	<0.0001	<0.0001	<0.0001
Hardship of the surgery (VAS mm)	23.3 \pm 19.4 (0–81)	31.4 \pm 24.6 (3–100)	0.0811	0.0008	NS
Pain perception (VAS mm)	1.9 \pm 7.4 (0–44)	3.2 \pm 8.9 (0–46)	0.4770	0.3717	NS

Variables recorded during and immediately after surgery.

CAF, coronally advanced flap; CEJ, cemento-enamel junction; CTG, connective tissue graft; GM₁, position of the gingival margin at the end of the surgery with respect to the CEJ (positive number indicates coronal to the CEJ); VAS, visual analogue scale.

Table 4. Inferential statistic

	CAF (n = 43)	CAF+CTG (n = 42)	Difference CAF versus CAF+CTG p-value	Centre effect p-value	Interaction p-value
Pain (VAS mm)	7.9 ± 15.6 (0–50)	13.3 ± 20.3 (0–80)	0.1730	0.0969	NS
Anti-inflammatory tablets	0.7 ± 1.2 (0–5)	1.3 ± 2.5 (0–14)	0.1105	0.3031	NS
Daily-life interference (VAS mm)	24.4 ± 22.8 (0–80)	21.9 ± 22.7 (0–75)	0.5444	0.0010	NS
Job interference (VAS mm)	6.2 ± 14.2 (0–52)	7.3 ± 15.3 (0–50)	0.7141	0.2259	NS
Relationship interference (VAS mm)	15.9 ± 22.8 (0–75)	18.5 ± 29.4 (0–100)	0.6671	0.5927	NS
Swelling (VAS mm)	17.8 ± 19.9 (0–69)	32.2 ± 28.4 (0–100)	0.0068	0.0134	NS

Patient-related variables recorded 1 week after surgery with a questionnaire.

CAF, coronally advanced flap; CTG, connective tissue graft; VAS, visual analogue scale.

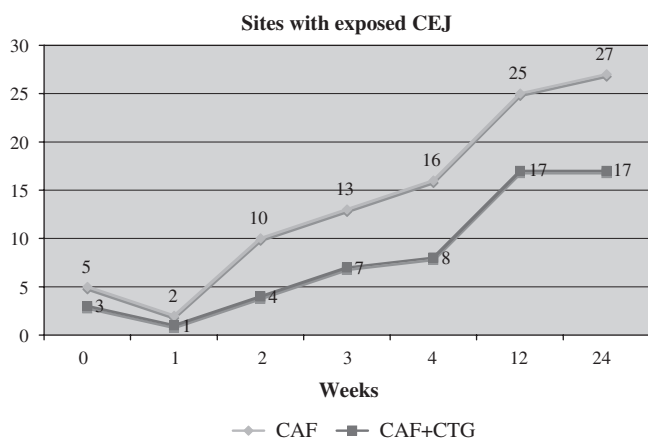


Fig. 4. Sites presenting an exposed cemento-enamel junction (CEJ). The baseline measure (time 0) was taken at the end of surgery.

Inflammation was present in 44% (CAF) and 43% (CAF+CTG) of the cases. It rapidly reduced to 26% (CAF) and 24% (CAF+CTG) at week 2, to 7% and 12% at week 3, and to 5% and 7% at week 4. The same trend was noted for swelling that, in the CAF-treated sites, was present in 28% of the cases at week 1, in 2% at week 2, in 2% at week 3, and in no cases at week 4. In the CAF+CTG-treated sites, from 36% at week 1, swelling dropped to 15% at week 2, and to 0% at weeks 3 and 4.

The CEJ was visible at the end of surgery in five (12%) of the CAF-treated cases and in three (7%) of the CAF+CTG-treated ones. At week 1, two CAF-treated sites and one CAF+CTG presented with an exposed CEJ. In the following 3 weeks, the cases that presented a visible CEJ increased constantly. The increasing number of sites with visible CEJ was greater in the CAF-treated sites; this trend was further confirmed at the 3- and 6-month examination visits (Fig. 4).

Three- and 6-month clinical outcomes

Clinical results at 3 and 6 months are reported in Table 5. Recession reduction was observed in all cases, with the exception of five (12%) cases treated with CAF and three (7%) cases treated with CAF+CTG. The additional use of a graft under the CAF resulted in a greater frequency of roots completely covered and provided a slight increase in KT, while the CAF-treated sites showed a slight loss of KT with respect to baseline.

A statistical model in which recession reduction (difference between baseline and 6 months, measured using the stent as the reference point) was the outcome variable explains 36% of the outcome variability in terms of therapy and baseline recession depth (Table 6). Recession reduction was greater in cases with a deeper baseline recession, and in cases treated with CAF+CTG (adjusted estimate 0.33 mm; 95% CI = -0.06 to 0.72) but the difference did not reach

statistical significance ($p = 0.1002$). There was also a significant centre effect, with centre 1 obtaining a greater recession reduction than the other two centres.

Table 7 reports a statistical model with 6-month CRC as the outcome variable. The model explains 28% of the variability in terms of therapy and baseline recession depth. The odds of obtaining CRC were 5.09 times greater with the additional use of a graft with respect to the CAF alone. Similarly, shallower baseline recessions had greater probability to be completely covered. There was also a significant centre effect, with centre 1 having the greatest chances to get the CRC and centre 2 the smallest.

An exploratory analysis reporting on CRC in smokers and non-smokers indicated that the adjusted OR of CRC after using CAF+CTG were 3.91 ($p = 0.0299$) among non-smokers and 48.32 ($p = 0.0200$) among smokers.

When change in KT width between baseline and 6 months was considered as the outcome variable (Table 8), the CAF+CTG provided a significant increase in KT on top of CAF alone (0.41 mm; 95% CI = 0.08–0.74). The amount of KT present at baseline was also significantly associated with the outcome: the greater the baseline KT, the greater the loss of KT (Table 8). There was a significant difference among centres, with centre 1 obtaining a greater amount of KT with respect to centre 3.

Discussion

The present randomized clinical trial was designed to test the added clinical benefit and the potential additional adverse events of the placement of a CTG under a CAF in the treatment of Miller Class I and II single gingival recessions.

Table 5. Descriptive statistics of variables recorded 3 and 6 months after surgery in mm (mean ± SD; range)

	CAF (n = 43) 3 months	CAF+CTG (n = 42) 3 months	CAF (n = 43) 6 months	CAF+CTG (n = 42) 6 months
Recession depth	0.8 ± 0.8 (0–3)	0.4 ± 0.7 (0–2)	0.8 ± 0.8 (0–3)	0.6 ± 0.9 (0–3)
Root coverage (mm)	1.6 ± 1.0 (–1 to 4)	2.1 ± 1.0 (0–4)	1.5 ± 1.1 (–2 to 4)	2.0 ± 1.0 (0–4)
PD	1.2 ± 0.6 (0–3)	1.1 ± 0.4 (0–2)	1.4 ± 0.6 (1–3)	1.4 ± 0.5 (1–3)
CAL	2.1 ± 1.2 (1–6)	1.6 ± 0.8 (1–3)	2.3 ± 1.2 (1–6)	2.0 ± 1.0 (1–4)
KT	2.9 ± 0.9 (1–4)	3.2 ± 0.9 (1–5)	3.0 ± 0.8 (1–5)	3.3 ± 0.9 (2–5)
KT difference	–0.3 ± 1.1 (–3 to 2)	0.5 ± 1.0 (–2 to 3)	–0.1 ± 1.2 (–3 to 2)	0.6 ± 1.1 (–2 to 3)
CRC (N and %)	18 (42%)	25 (60%)	16 (37%)	25 (60%)
Hypersensitivity	8 (19%)	5 (12%)	5 (12%)	5 (12%)

CAF, coronally advanced flap; CTG, connective tissue graft; root coverage (mm), recession reduction measured from the acrylic stent; PD, probing dept; CAL, clinical attachment level; KT, keratinized tissue width; KT difference, difference of KT width between 3 or 6 months and baseline; CRC, complete root coverage.

Table 6. Statistical model with recession reduction (6 months) measured with the stent as outcome variable

Term	Estimate	Standard error	p-value
Intercept	–0.06	0.36	
Therapy	0.33	0.20	0.1002
Rec _{T0}	0.64	0.14	<0.0001
Centre			0.0010

Therapy = CAF+CTG versus CAF.
R² = 0.36.

CAF, coronally advanced flap; CTG, connective tissue graft; Rec_{T0}, baseline recession.

Table 7. Statistical model with complete root coverage as outcome variable

	Adjusted odds ratio	95% CI (lower)	95% CI (upper)	p-value
Therapy	5.09	1.69	17.57	0.0033
Rec _{T0}	0.31	0.12	0.68	0.0031
Centre 1 versus 2	18.24			<0.0001
Centre 1 versus 3	6.70			
Centre 3 versus 2	2.72			

Therapy = CAF+CTG versus CAF.
R² (U) = 0.28.

CAF, coronally advanced flap; CTG, connective tissue graft; Rec_{T0}, baseline recession.

Both the test and the control procedures were effective in reducing the recession depth; 0.33 mm greater recession reduction was observed in the cases treated with the bilaminar technique (Table 6), but this difference did not reach statistical significance. These data confirm the outcomes of a previous small sample controlled study (da Silva et al. 2004) in which sites treated with CAF+CTG resulted in improved clinical outcomes with respect to CAF

Table 8. Statistical model with KT_{T6–T0} (difference in keratinized tissue between baseline and 6 months) as outcome variable

Term	Estimate	Standard error	p-value
Intercept	1.86	0.25	
Therapy	0.41	0.17	0.0145
KT _{T0}	–0.63	0.07	<0.0001
Centre			0.0220

R² = 0.61.

Therapy = CAF+CTG versus CAF.

CAF, coronally advanced flap; CTG, connective tissue graft; KT_{T0}, baseline keratinized tissue width.

alone, but the difference did not reach statistical significance. In the present clinical trial, however, the adjunctive application of a CTG under a CAF increased the probability of achieving CRC in Miller Class I and II defects (adjusted OR = 5.09, p = 0.0033).

The sites treated with a combination of CAF plus a graft resulted in a significantly higher number of recessions completely covered (60%) with respect to sites treated with CAF alone (37%, Table 5). The reported outcomes compare well with the existing body of evidence, setting the results of this clinical trial among the better ones in terms of CRC (Clauser et al. 2003, Kerner et al. 2008). When comparing results from different studies, however, it should be taken into account how and whether the ‘‘CRC’’ record were defined by the authors. The issue is especially relevant when teeth with large abrasions and/or deep steps involving the CEJ are included. In these instances, the CEJ is no longer detectable and the record of CRC becomes a guess. There is in fact a tendency to declare a root ‘‘completely covered’’ where the gingival margin reaches a position that the clinician ‘‘feels’’ as the maximum possible coverage obtain-

able in that specific case. This might in reality reflect the true maximum potential outcome, but still is not an ‘‘objective’’ measure. In this study, a site was declared ‘‘completely covered’’ when the CEJ or the coronal part of a step was not visible. A statistical model with CRC as the outcome variable (Table 7) explained 28% of the variability in terms of therapy and baseline recession depth: the adjusted odds of obtaining CRC were 5.09 times greater with the CAF+CTG with respect to the CAF alone. Similarly, shallower baseline recessions had a higher probability of being completely covered.

For both these variables (recession reduction and CRC), there was a significant centre effect: centre 1 generally performed better than the other two centres. The centre effect is a well-known factor described in flap surgery and periodontal regeneration (Tonetti et al. 1998, Cortellini et al. 2001, Tonetti et al. 2002). In the present study, the centre effect was significant in spite of the fact that surgery was performed by skilled periodontists, specifically trained and calibrated to perform the tested surgical approaches; in addition, the patient population was well balanced and carefully selected according to stringent entry criteria and randomization process, and the procedures were conducted within a common and tight protocol. The centre effect should be taken into account to explain, at least in part, the high degree of variability frequently observed when studies performed by different clinicians or groups of clinicians are evaluated and statistically analysed, as observed in the meta-analyses from three systematic reviews on root coverage procedures (Roccuzzo et al. 2002, Clauser et al. 2003, Oates et al. 2003). The CAFs with or without the use of a graft are

technique-sensitive procedures that require a specific and refined training and a high level of skills to be properly applied.

An explorative analysis was performed to evaluate the potential impact of cigarette smoking as a modifier of the treatment outcomes (Hyman 2006). Within the limits represented by the small numbers of smokers included in the study, the analysis demonstrated that if the odds of achieving CRC following the use of CAF+CTG were greater than after the use of CAF alone in the non-smokers (3.91, $p = 0.0299$), they were even greater in the smokers (48.32, $p = 0.0200$). This observation raises the hypothesis that the combined procedure may be a better choice to cover roots among smokers. A possible explanation for this finding may be that, as previously suggested, flaps raised for periodontal plastic surgery procedures may be exquisitely sensitive to the negative effect of cigarette smoking and particularly so whenever tissues are thin and positioned on an avascular surface. In this context, the application of the CTG could act as a 'protective' element: perhaps by allowing healing under the CAF and thus stabilizing the outcome in spite of the suffrance of the superficial layer. Further investigations are needed to better elucidate this issue.

Another relevant, although expected, difference between the two tested techniques was the change in KT between baseline and 6 months. Sites treated with the bilaminar technique resulted on average in a KT increase at 6 months, while the ones treated with CAF resulted in a slight loss (Table 5). Comparing the two procedures, the CAF+CTG provided a significant increase in KT on top of CAF alone (Table 8). There was a significant difference among centres, with centre 1 obtaining a greater amount of KT with respect to centre 3. This event might be explained, at least in part, by differences in the size of the CTG and in the accuracy of the surgical performances.

The differences noted between the two experimental procedures can be further explored and explained by analysing the soft tissue changes during the early healing phase. Both procedures were performed with a clear goal in mind: provide a complete coverage of the treated roots. To reach this objective, the best of clinical skill was applied in trying to obtain a tension-free pedicle flap, to position the flap margin coronal

to the CEJ, and to provide flap stability with the suturing technique (Pini Prato et al. 2000, 2005, Zucchelli et al. 2003). In spite of these efforts, the CEJ was visible in five CAF- and in three CAF+CTG-treated sites at the end of suture positioning. At week 1, only two CAF- and one CAF+CTG-treated sites revealed an exposed CEJ. The reduced number of sites with a visible CEJ could easily be explained by the slight inflammation (44% CAF and 43% CAF+CTG) and swelling (28% CAF and 36% CAF+CTG) noted at this time point of the healing period. Both inflammation and swelling rapidly dropped down to half of the positive cases at week 2 and further down at weeks 3 and 4. Along with the resolution of the post-operative inflammatory events, an increased number of sites with a visible CEJ were recorded at weeks 2, 3, and 4. At the 3- and 6-month examination visits, an exposed CEJ was recorded in 25 CAF- and 17 CAF+CTG-treated sites and 27 CAF- and 17 CAF+CTG-treated sites, respectively: this trend towards an increasing number of sites with exposed CEJ is clearly depicted in Fig. 4. The increasing exposure over time following both procedures could be explained by the tendency of the coronally advanced soft tissue to experience some contraction in the early healing phase: this is in agreement with previous similar observations (Pini Prato et al. 2005). Interestingly, there is a difference between the two procedures in favour of the bilaminar technique, where a CTG was positioned to increase the thickness of the covering soft tissue. The presence of a graft under the flap is associated with a reduced soft tissue contraction, resulting in a significantly greater amount of sites completely covered at 6 months.

The overall surgical chair-time was significantly shorter for CAF (about 17 min. on average, Table 3). This is easily explained by the additional time required to harvest, position, and suture the CTG in the CAF+CTG. The additional use of a graft also revealed a significant difference in the time devoted to graft handling among the different centres. In fact, while there was no difference in the CAF surgical time among the three centres, there was a significant difference in the CAF+CTG approach. In particular, centre 2 declared a longer surgical chair-time than centre 1, and centre 1 longer than centre 3. The prolongation

of the chair-time could potentially influence patient perception of the procedure's hardship and some of the post-operative clinical parameters, like inflammation and swelling. However, these potential correlations were not significant in the present study.

Patient perception of the hardship of the two procedures was mild and there was no statistically significant difference between CAF and CAF+CTG (Table 3). However, the latter resulted in higher average VAS values and there was a significant centre effect. VAS values were very low in both the groups and the differences were not statistically significant.

At the week 1 examination, in addition to cases with local signs of inflammation and swelling, already discussed above, three cases of haematoma were found in the group of patients treated with CAF, and five cases in patients treated with CAF+CTG. The limited amount of post-operative complications confirms the observations of a previous comparative study on the same techniques (da Silva et al. 2004).

Several patient-related variables were collected at the week 1 examination visit with a questionnaire (Table 4). More patients reported some pain after CAF+CTG. Pain-related VAS values were very low and there was no statistically significant difference between the two groups and among the three centres. The very limited post-operative pain experience was also underlined by the limited use of anti-inflammatory tablets in addition to the two doses given before and after the surgery. There was no statistically significant difference between the two treatments and among the three centres in terms of the consumption of anti-inflammatory tablets. Interference with daily life was reportedly very limited. The relative VAS values were rather low, and there was no difference between the two treatments and among the three centres for all these variables, with the exception of daily life interference, which was reportedly higher in VAS values for centre 1 with respect to centre 3.

Dentine hypersensitivity at the end of the follow-up period was a rare occurrence reported by five patients in each group. This has to be favourably compared with the baseline indications to surgery: about 40% of the experimental population in both groups requested therapy to treat hypersensitivity *per se* or as an additional issue to aesthetics,

the latter being the prevalent reason for a patient to require root coverage. Many patients, therefore, experienced an advantage in terms of root sensitivity reduction after therapy. The reduced prevalence of root sensitivity after root coverage confirms the outcomes of a previous study (McGuire & Nunn 2003).

The post-operative regimen adopted in this trial was designed to reduce direct trauma or any mechanical negative influence on the treated area. Three weeks after surgery, the patients were allowed to resume regular mechanical tooth cleaning of the treated areas using a power-driven toothbrush (Braun Triumph, Oral-B-Gillette) with the appropriate technique. Scientific background to use a powered toothbrush with a counter-rotation oscillating action was the consistent observation of greater effectiveness in removing plaque and reducing gingivitis with respect to manual toothbrushes (Robinson et al. 2007). In addition, controlled clinical trials demonstrated clinical safety to gingival or tooth tissues and no adverse reactions deriving from its use (Dentino et al. 2002, Vandekerckhove et al. 2004, Patters et al. 2005).

Within the limit of this study, the following conclusions can be drawn:

1. The additional use of a CTG results in a greater number of sites with CRC in upper anterior teeth.
2. Both treatments are equally effective in providing a consistent reduction of the baseline recession and of dentine hypersensitivity.
3. More sites treated with CAF experience soft tissue contraction in the early healing phase: this event is associated with more CEJ exposures in the first three post-surgical months.
4. The more limited amount of soft tissue contraction and thereby the greater amount of sites with CRC observed in the sites where a CTG is used could indicate the use of the bilaminar technique when the main goal of therapy is CRC.
5. In addition, if one of the therapeutic objectives is the increase of KT, the use of a free CTG under a CAF has to be considered.
6. The shorter chair-time and the relatively lower amount of post-operative swelling could indicate the use of a CAF alone when these issues are of primary value for the patient.

Acknowledgements

The authors would like to thank Dr. Gaia Pellegrini and Dr. Matteo Basso for having performed the clinical measurements in centres L. Francetti and G. Rasperini.

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Clinical Relevance

Scientific rationale for the study: The CAF alone or with the combined use of a free CTG is considered to be the most effective approach in the treatment of isolated gingival recessions. The combined approach includes the use of a CTG with a potential increase of technical difficulties and

intra-operative and post-operative patient morbidity.

Principal findings: Application of CAF and CAF+CTG to Miller Class I and II gingival recession resulted in significant clinical improvements in terms of root coverage. Placing an adjunctive CTG under a CAF increased the probability of obtaining CRC. Both procedures were well

tolerated and resulted in few adverse events.

Practical implications: The use of CAF+CTG can be suggested when the main goal of therapy is CRC and increase of KT. Whenever a shorter surgical chair-time is desired, a CAF alone could be the treatment of choice. Long-term observations are needed to confirm these results.