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Patient morbidity and root coverage outcome after subepithelial connective tissue and de-epithelialized grafts: a comparative randomized-controlled clinical trial

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Abstract

Aims: The aim of this randomized-controlled clinical trial was to compare the patient morbidity and root coverage outcomes of a coronally advanced flap (CAF) with connective tissue (CTG) or de-epithelialized gingival (DGG) grafts.

Methods: Fifty patients with one recession each were treated. In the control group, the CTG was harvested using the trap-door approach while in the test group the CTG resulted from the de-epithelialization of a free gingival graft.

Results: No statistically significant differences were demonstrated between groups in patients's pain killer consumption, post-operative discomfort and bleeding. Lower stress and better ability to chew were demonstrated in the CTG group. Analgesic consumption increased with increasing height of the graft and in the case of dehiscence/necrosis of the primary flap. Pain was negatively correlated with the residual thickness of soft tissue covering the palatal bone. A statistically greater increase in buccal soft tissue thickness was observed in the DGG group.

Conclusions: No differences were demonstrated in the post-operative pain and root coverage outcome in patients subjected to CAF with CTG or DGG.

Key words: connective tissue graft; esthetics; free gingival graft; gingival recession; mucogingival surgery

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A soft tissue graft is a withdrawal of soft tissue that is completely detached from its original donor site and placed in a prepared recipient bed (American Academy of Periodontology 2001). The palate is the most frequent donor site for intra-oral connective tissue (CTG) and epithelialized free gingival (FGG) grafts used for root coverage purpose.

Palatal fibromucosa is characterized by a dense connective tissue (lamina propria) covered by an orthokeratinized epithelium (Müller et al. 2000). A layer of fatty and glandular tissue (submucosa) of varied thickness is present between the palatal fibromucosa and the periosteum covering the palatal bone (Harris 2003). A remarkable variation in the histologic makeup of CTG has been observed (Harris 2003), in terms of both the thickness and the percentage of lamina propria and submucosa. The thickness of palatal fibromucosa varies from patient to patient and, in the same patient, from site to site of the palate (Müller et al. 2000). Palatal thickness (PT) can be clinically determined, at the time of anaesthesia, by penetrating with an endodontic reamer/needle perpendicular to the palatal bone plate (Studer et al. 1997, Paoloantonio et al. 2002, da Silva et al. 2004, Joly et al. 2007) The penetration depth can

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be measured, by using an endodontic silicon disk applied to the reamer/needle, as the distance between the silicon ring and the tip of the reamer/needle. An ultrasonic device has also been used to determine soft tissue thickness around teeth and implants (Eger et al. 1996, Müller et al. 1999, 2000). A study by Eger et al. (1996) showed that a needle and an ultrasonic device yield very similar results. In addition to the measurement of the distance between the external palatal surface and the palatal bone plate, from a clinical standpoint, it is even more important to evaluate the degree of the palatal soft tissues resistance to needle penetration. In fact, while the fibromucosa (epithelium and connective tissue) has a firm consistency and thus a resistance is felt during needle penetration, the fatty and glandular tissues do not offer opposition to the needle and resistance is felt only at the bone level. This evaluation is critical, particularly when connective tissue-harvesting procedures are chosen.

Different connective tissue-harvesting procedures with the purpose of achieving primary intention palatal wound healing have been described in the literature: the most common are the trap-door approach (Edel 1974) and the single-incision technique (Hürzeler & Weng 1999, Lorenzana & Allen 2000). These procedures have the following common characteristics: a primary split-thickness access flap elevation, the withdrawal of CTG and the complete closure of the palatal wound with the access flap. The primary objective of these techniques is to reduce patient morbidity by alleviating the post-operative course; however, they need an adequate thickness of the palatal fibromucosa to avoid desquamation of the undermined superficial flap due to compromised vascularization (Edel 1974, Langer & Langer 1985, Jahnke et al. 1993).

The FGG surgical wound heals by secondary intention within 2–4 weeks (Farnoush 1978) and has been consistently associated with greater discomfort for the patient due to post-operative pain and/or bleeding (Farnoush 1978, Jahnke et al. 1993, Del Pizzo et al. 2002). However, this technique is easy to perform and can be utilized even in the presence of a thin palatal fibromucosa.

The evidence in the literature evaluating differences in patient outcomes following the CTG and FGG, used for root coverage procedures, is minimal. Few prospective comparative studies (Del Pizzo et al. 2002, Griffin et al. 2006, Wessel & Tatakis 2008) reported poorer patient outcomes, specifically, a greater incidence of post-operative pain, for FGG compared with CTG procedures. No randomized study has been performed comparing both patient and root coverage outcomes after the use of CTG and de-epithelialized gingival (DGG) grafts for the treatment of gingival recessions.

The aim of the present randomized-controlled clinical study was to compare post-operative morbidity and root coverage outcomes in patients subjected to trap-door connective tissue (control group) and epithelialized (test group) graft-harvesting techniques for the treatment of gingival recession with the bilaminar procedure.

The primary objective of the study was to demonstrate the superiority in terms of the post-operative course and pain of the connective tissue-harvesting technique. The secondary goal was to compare the effectiveness, in terms of root coverage and increase in buccal gingival thickness (GT), of CTG, harvested with the trap-door approach or resulting from the de-epithelialization of a free gingival graft, used in combination with a coronally advanced flap (CAF) for the treatment of gingival recessions.

Material and Methods

Fifty subjects, 22 males and 28 females (age range 21–50 years, mean age 34.7 ± 6.0 years), were enrolled in the study. The patients were selected, on a consecutive basis, among individuals referred to the University of Bologna and the University of Siena, Dental School, in the period between February 2006 and March 2007. The study protocol, questionnaires and informed consent, in full accordance with the ethical principles of the Declaration of Helsinki of 1975, as revisited in 2000, were approved by the Institutional Review Board and received the approval by of the local ethic committee. All patients agreed to participate in the study and signed a written informed consent according to the above-mentioned principles. All participants met the study inclusion criteria: single or multiple Miller's Class I and II recession defects ($\geqslant 2$ mm in depth); presence of identifiable cemento-enamel junction (CEJ); presence of a step $\leqslant 1$ mm at the CEJ level and/or the presence of a root abrasion, but with an identifiable CEJ, were accepted; periodontally and systemically healthy; no contraindications for periodontal surgery and not taking medications known to interfere with periodontal tissue health or healing; no anti-inflammatory drugs or antibiotics for at least 6 months; and no periodontal surgery on the involved sites. Subjects smoking more than 10 cigarettes a day were excluded. Recession defects associated with caries or restoration as well as teeth with evidence of a pulpal pathology were not included. Molar teeth were also excluded.

Study design

The study was a double-centre (one in Bologna and the other one in Siena), double-blinded, randomized-controlled clinical trial, with a parallel design, comparing CAF with CTG or with DGG for the treatment of gingival recessions. In the control group, the CTG was harvested with the trap-door approach while in the test group, the CTG resulted from the de-epithelialization, using a scalpel blade, of a free gingival graft. Both types of grafts were harvested from the palate.

The study protocol involved a screening appointment to verify eligibility, followed by initial therapy to establish optimal plaque control and gingival health conditions, surgical therapy, evaluation of patient morbidity 1 week after the surgery, maintenance phase and post-operative clinical evaluation 1 year after the surgery.

Sample size

The study was powered to detect a minimum clinically significantly different pain killer consumption of 1800 mg using $\alpha = 0.05$, a power = 85%, a hypothesized within-group sigma of 2000 mg, obtained from the only previous randomized comparative studies (Wessel and Tatakis) with patient morbidity as the primary outcome. As a minimum, 24 patients per treatment arm would have been required.

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, and standardize the case selection, the measurement techniques and the surgical procedures.

Randomization

Patients were assigned to one of the two treatment groups using a computer-generated randomization table. All patients participated in the study with a single tooth. Twenty-five teeth were assigned to the control group and 25 teeth to the test group. In the case of patients presenting with multiple recessions, the deepest one was selected; in the case of two or more recessions of the same depth, the selection was performed by tossing a coin. Allocation concealment was achieved using a sealed coded opaque envelope containing the treatment of the specific subject. The sealed envelope containing treatment assignment was opened during the surgery immediately before the graft harvesting.

Initial therapy and clinical measurements

Following the screening examination, all subjects received a session of prophylaxis including instruction in proper oral hygiene measures, scaling and professional tooth cleaning with the use of a rubber cup and a low abrasive polishing paste. A coronally directed roll technique was prescribed for teeth with recession-type defects in order to minimize toothbrushing trauma to the gingival margin. Surgical treatment of the recession defects was not scheduled until the patient could demonstrate an adequate standard of supragingival plaque control.

All clinical measurements were carried out by a single masked examiner (M. M.) at baseline and 1 year after the surgery. MM did not perform surgery and was unaware of the treatment assignment. Before the study, the examiner was calibrated to reduce intra-examiner error ($\kappa > 0.75$) to establish reliability and consistency.

Full-mouth (FMPS) and the local plaque scores were recorded as the percentage of total surfaces (four aspects per tooth) that revealed the presence of plaque (O'Leary et al. 1972). Bleeding on probing was assessed dichotomously at a force of 0.3 N using a manual pressure-sensitive probe [(PCP-UNC 15 probe tip, Hu Friedy, Chicago, IL), equipped with a Brodontic spring device (Dentramar, Waalwijk, the Netherlands)]. Full-mouth (FMBS) and local bleeding scores were recorded as the percentage of total surfaces (four aspects per tooth) that revealed the presence of bleeding upon probing.

The following clinical measurements were taken 1 week before surgery and at the 1-year follow-up at the midbuccal aspect of the study teeth:

(1) gingival recession depth (RD), measured from the CEJ to the most apical extension of the gingival margin;
(2) probing depth (PD), measured from the gingival margin to the bottom of the gingival sulcus;
(3) clinical attachment level (CAL), measured from the CEJ to the bottom of the gingival sulcus;
(4) height of keratinized tissue (KTH): the distance between the gingival margin and the mucogingival junction (MGJ). The MGJ was identified by means of Lugol staining

All measurements were performed by means of the manual probe and were rounded up to the nearest millimetre:

(5) GT: determined 1.5 mm apical to the gingival margin with a short needle for anaesthesia and a 3-mm-diameter silicon disk stop. The needle was inserted perpendicular to the mucosal surface, through the soft tissues with light pressure until a hard surface was felt. The silicon disk stop was then placed in tight contact with the soft tissue surface with the coronal border overlapping the soft tissue margin. As the needle was located in the centre of the silicon disk, measurement of GT was performed 1.5 mm apical from the gingival margin. Once in the correct position, the disk was fixed with a drop of cyanocrylic adhesive; after careful removal of the needle, the penetration depth was measured with a calliper accurate to the nearest 0.1 mm (Paoloantonio et al. 2002, da Silva et al. 2004, Joly et al. 2007).

Intra-surgical measurement

After local anaesthesia, PT in the area selected for harvesting the graft was measured. The measurement was made at the mid palatal location about 1.5 mm apical to the gingival margin of the adjacent tooth, by means of the needle used for anaesthesia and a silicon disk stop. The needle was inserted perpendicular to the mucosal surface, through the soft tissues with light pressure until a hard surface was felt. The silicon disk stop was then placed in tight contact with the soft tissue surface and fixed by a drop of cyanocrylic adhesive; after careful removal of the needle, the penetration depth was measured with the calliper.

The thickness of the grafts was measured in both test and control groups just after being harvested (GRT) and just before being sutured (GRTs), after de-epithelialization (in the test group) and removal of fatty tissue when present. In the control group, the thickness of the primary flap (FT) was measured. All measurements were made 1.5 apical to the coronal border with the calliper. The width (GRW) (mesial–distal dimension) and the height (GRH) (apical coronal dimension) of the CTG were measured just before being sutured with the manual probe and rounded up to the nearest millimetre.

Graft measurements were performed by a different examiner (C. M.).

Patient morbidity

Post-operative pain was indirectly evaluated on the basis of the mean consumption (in mg) of analgesics (ibuprofene) (Wessel & Tatakis 2008, Sanz et al. 2009).

Patients' post-operative discomfort, bleeding, stress and inability to chew was evaluated with a questionnaire given to patients 1 week following surgery. The questionnaire included the evaluation of the intensity of the given event on a visual analogic scale (VAS) of 100 mm (Cortellini et al. 2001, 2009).

Discomfort was defined as the level of soreness/pain experienced by the patients during the first post-operative week due to the palatal wound. Bleeding was considered to be the prolonged haemorrhaging during the post-surgical week reported by the patients. Stress was evaluated based on the level of apprehension and fear experienced by the patients of jeopardizing the palatal

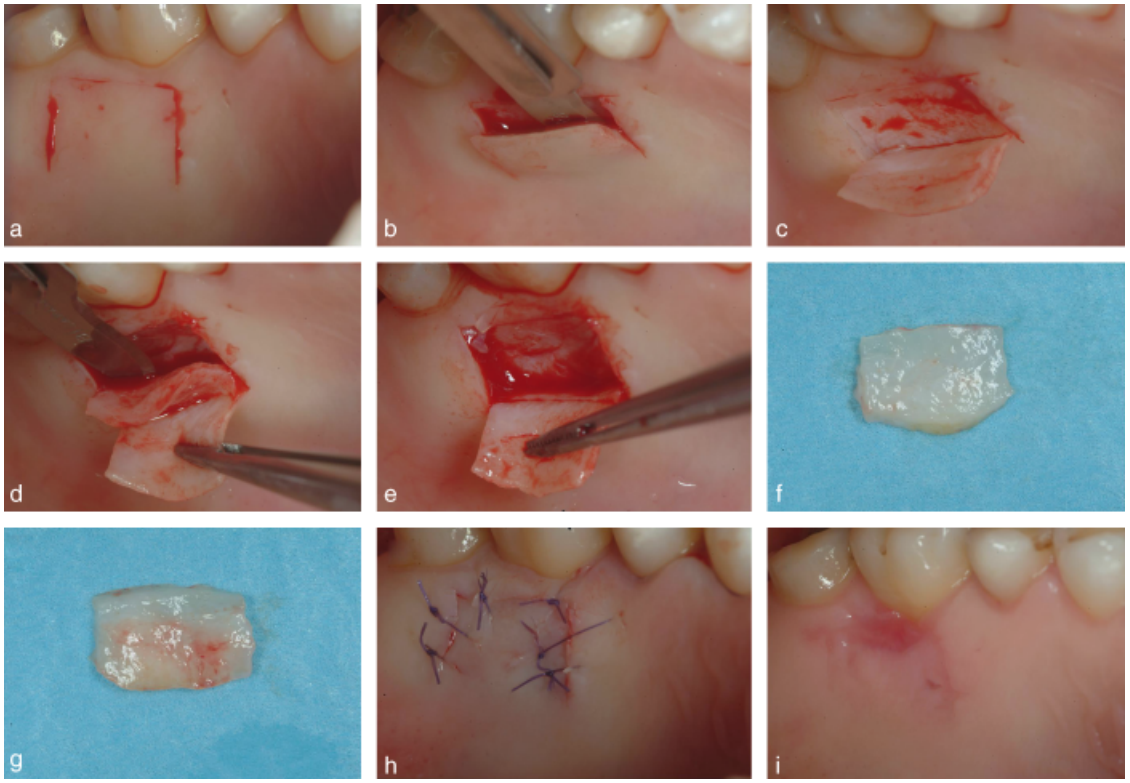

Fig. 1. The connective tissue graftharvesting technique. (a) The incision design of the primary access flap. (b) The blade proceeds apically parallel to the external palatal surface. (c) Split-thickness flap elevation. (d) The graft is being harvested. The blade proceeds parallel to the external surface of the graft in order to maintain a uniform thickness in the graft and to preserve as much soft tissue covering the periosteum as possible. (e) Soft tissue protecting the bone is left. (f) External surface of the graft facing the covering flap. (g) Internal surface of the graft facing the root surface. All fatty and glandular tissue has been removed. (h) Complete closure of the palatal wound has been achieved. (i) A slight dehiscence of the primary flap can be noticed 7 days after the surgery.

wound. Inability to chew was described as the level of variation of the patient's eating habits due to the presence of the palatal wound.

Surgical techniques

All surgeries were performed by two calibrated expert periodontologists (G. Z. and M. D. S.). G. Z. performed 26 surgeries (13 tests and 13 controls) and M. D. S. performed the remaining 24 (12 tests and 12 controls). During local anaesthesia (2% lidocain with epinephrine at a concentration of 1:100,000), the surgeon chose the harvesting site on the palate (pre-molar or molar) on the basis of the amount (PT) and quality (soft tissues resistance) of needle penetration. The surgeons were then informed as to which type of graft harvesting technique to perform, trap-door or epithelialized, by opening the envelope labelled, which contained the patient's number with the assigned treatment.

Control group

The surgical technique adopted for harvesting the CTG in the control group was a modification of the trap-door approach described by Edel (1974) (Fig. 1). In brief, one horizontal incision of the same length of the mesial–distal dimension of the graft was traced 1–1.5 mm apical to the gingival margin of the adjacent teeth. Two vertical releasing incisions were performed at the end of the horizontal incisions and were extended in the apical direction 1 mm more than the apical–coronal dimension of the graft. The primary flap was elevated split-thickness to maintain a uniform thickness throughout the flap. The horizontal incision of the graft was made along the horizontal incision of the flap with the blade almost perpendicular to the underlying bone. Once an adequate soft tissue thickness was obtained, the blade was rotated in order to be almost parallel to the external surface. The thickness of the graft was maintained uniform while proceeding apically with the blade. Care was taken not to remove the periosteum protecting the underlying bone. Once the graft was removed, the fatty tissue (yellow in colour) was eliminated. The primary flap was repositioned and interrupted single 6-0 sutures (Vicryl, Johnson & Johnson, Woluwe, Belgium) were made to achieve complete closure of the palatal wound.

Test group

A free (epithelialized) gingival graft was harvested in the test patients (Fig. 2). Two horizontal (the coronal incision was performed 1–1.5 mm apical to the soft tissue margin of the adjacent teeth) and two vertical incisions were traced to delimitate the area to be grafted. Along the coronal horizontal incision, the blade was oriented almost perpendicular to the bone plate and once an adequate soft tissue thickness was obtained, it was

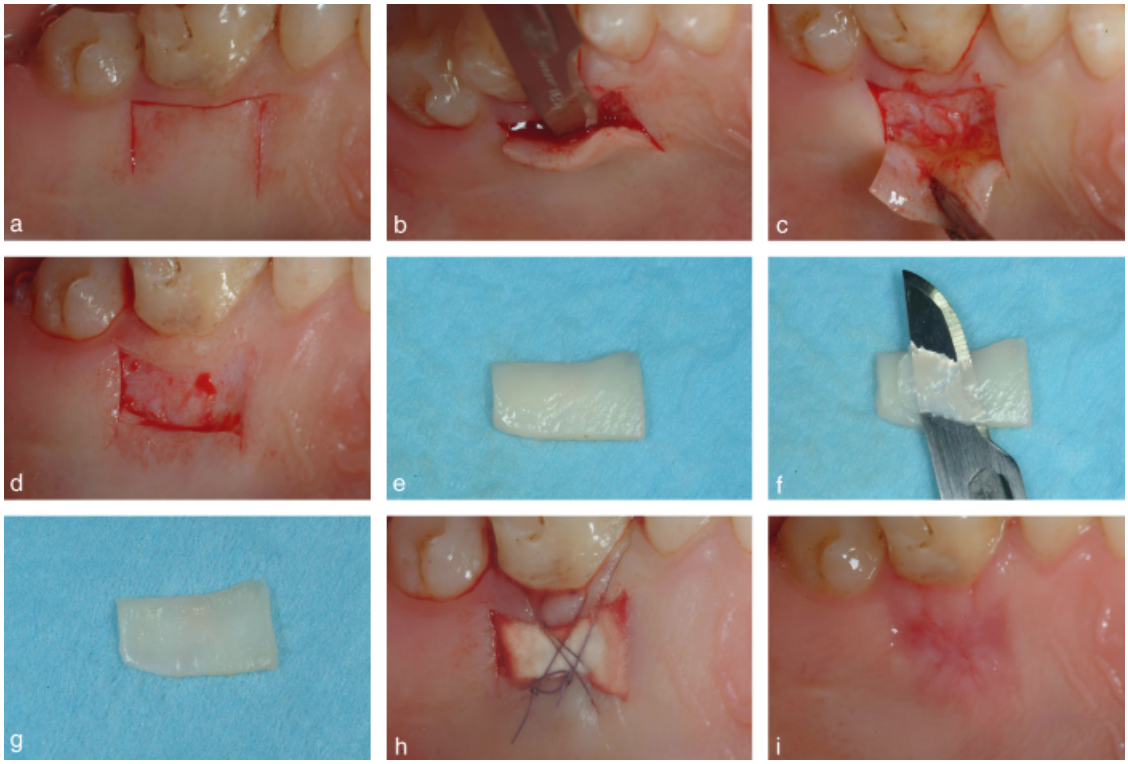

Fig. 2. The epithelialized gingival graft harvesting technique. (a) The incision design of the free gingival graft. (b) The blade proceeds apically parallel to the external palatal surface in order to maintain a uniform thickness in the graft. (c) Soft tissue thickness covering the periosteum has been preserved during the harvesting procedure. (d) Minimal bleeding after removal of the graft due to the superficial wound. (e) External surface of the graft before de-epithelialization. Note the reflection of the light due to the presence of the epithelium. (f) De-epithelialization made with the blade kept parallel to the external surface of the graft. (g) External surface of the graft after de-epithelialization. Note the different light reflection. (h) The palatal wound has been protected with equine-derived collagen maintained in situ with a sling mattress suture. (i) Secondary intention palatal healing 7 days after the surgery. Note the rapid tissue healing.

rotated in order to be almost parallel to the superficial surface. The thickness of the graft was maintained uniform while proceeding apically with the blade. Care was taken not to remove the periosteum protecting the underlying bone. Once the graft was separated, the fatty tissue (yellow in colour) was eliminated. The palatal wound was protected with equine-derived collagen (GABA Vebas, San Giuliano Milanese, MI, Italy) maintained in situ with compressive sling 5-0 sutures anchored to the soft tissue apical to the palatal wound area. The graft was de-epithelialized with a 15c blade. The graft was positioned on a sterile gauze or a surgical cloth and its surface was made wet with a saline solution. A light was oriented to be perpendicular to the graft. The different consistency (epithelium is harder and more rough while the connective tissue is softer and smoother) allowed removal of the epithelium when cutting with the blade kept parallel to the external surface. The different light reflection (the epithelium reflects more than the connective tissue) enabled to clinically distinguish when the epithelium was removed. The de-epithelialization of the graft and the control for epithelium removal were performed under magnification (4 ×) vision.

A bilaminar (CAF+CTG) technique (Zucchelli et al. 1998, 2003) was performed in both patient groups to accomplish root coverage. In brief, exposed root surfaces were mechanically treated with the use of curettes, a trapezoidal flap was raised split thickness and CTG were sutured at the level of the CEJ. The width of the graft was chosen according to the amount of tissue required to cover the exposed root and 3 mm of connective tissue mesial and distal to it. The height of the graft was based on the distance from the CEJ to the buccal bone crest. No attempt was made to cover the periosteum apical to the bone dehiscence. The remaining buccal soft tissue of the anatomic interdental papillae was de-epithelialized to create connective tissue areas to which the surgical papillae of the covering flap were sutured. The flap was coronally advanced, by cutting muscle insertions present in the thickness of the flap, and sutured with sling sutured anchored around the palatal cingulum of teeth with gingival recessions. At the time of suturing, the flap should cover the graft and the flap margin should be coronal to the CEJ of all teeth included in the flap design. No periodontal dressing was applied.

Surgical chair time was measured using a chronometer from the first incision to the last suture in both groups.

Post-surgical infection control

Post-operative pain and oedema were controlled with ibuprofen. Patients received 600 mg at the beginning of the surgical procedure. Subsequent doses were taken only if necessary to control pain. Patients had to record the quantity of analgesics taken during the first week post-surgery.

Patients were instructed not to brush their teeth in the treated area but to rinse with chlorhexidine solution (0.12%) three times a day for 1 min. One week

after the surgery, patients were recalled for a control visit and for the post-operative course evaluation. Fourteen days after the surgical treatment, the sutures were removed. Plaque control in the surgically treated area was maintained by chlorhexidine rinsing for an additional 1 week after suture removal. After this period, patients were again instructed in mechanical tooth cleaning of the treated tooth using an ultra-soft toothbrush and a roll technique for 1 month. During this month, chlorhexidine rinsing was used twice a day. Then the patient started to use a soft-toothbrush and chlorhexidine once a day for another month. All patients were recalled for prophylaxis 2 and 4 weeks after suture removal and, subsequently, once every 2 months until the final examination (12 months).

Data analysis

A statistical application software (SAS, version 6.09, SAS Institute, Cary, NC, USA) was used for the statistical analysis.

Descriptive statistics were expressed as mean ± SD.

Complete coverage was evaluated after 1 year by calculating the percentage of cases, in each treatment group, with the gingival margin at the level or coronal to the CEJ.

Percentage of root coverage was calculated after 12 months according to the following formula:

$$\frac{(\text{Baseline RD}) - (\text{12-month RD})}{(\text{Baseline RD})} \times 100$$

One-way ANOVA was used to evaluate differences between the test and the control groups regarding the mean age, mean baseline values of RD, CAL, PD, KTH, GT and the mean surgical chair-time

One-way ANOVA was also used to evaluate differences between the test and the control groups in PT, GRTs, GR width, GR length, depth of the withdrawal (WD) and residual soft tissue thickness (RTT) covering the palatal bone.

General linear models were fitted relating pain killer consumption (in mg), discomfort (VAS), bleeding (VAS), inability to chew (VAS) and stress (VAS) to the surgical procedures and the centres as predictive factors, and the interaction between surgical procedures and centres.

One-way ANOVA was used to evaluate the presence of any significant difference in pain, alarm, bleeding, chewing and discomfort, between the seven patients experiencing primary flap dehiscence/necrosis and the test patients and control patients who did not experience primary flap dehiscence/necrosis.

A general linear statistical model was fitted relating analgesic consumption to RTT, GR width, GR height and area (molar *versus* pre-molar) of the withdrawal.

General linear models were also fitted, and multiple regression ANOVA for repeated measures with a split-plot design was used to evaluate the existence of any significant difference regarding RD, CAL, PD, KTH and GT between techniques (CAF with CTG *versus* CAF with DGG), time (1 year *versus* baseline) and the interaction between techniques and time. In case of significance, the Bonferroni t test was applied as a multiple comparison test.

A logistic regression model was fitted to relate complete root coverage as the outcome variable and techniques (CAF with CTG *versus* CAF with DGG), including baseline RD as a confounding factor.

A multifactorial ANOVA was performed to evaluate the inter-group difference between GT increase at 1 year and GRTs with GRTs as a covariate.

A linear model was fitted to describe the relationship between pain killer consumption (in mg) and surgical time.

Results

Following the initial oral hygiene phase as well as at the post-treatment examinations, all subjects showed low percentages of plaque harbouring tooth surfaces (FMPS < 20%) and bleeding gingival units (FMBS < 15%), indicating a good standard of supragingival plaque control during the study period.

All 50 patients completed the study. In the control group, the mean age of the 25 patients (10 males and 15 females) was 32.2 ± 7.2 years (range 20–40). Ten maxillary (six canines and four pre-molars) and 15 mandibular (six canines, seven pre-molars and two lateral incisor) teeth with gingival recession were treated.

In the test group, the mean age of the 25 patients (12 males and 13 females) was 34.2 ± 6.8 years (range 22–46). Twelve maxillary (six canines, one lateral incisor and five pre-molar) and 13 mandibular (seven canines and six pre-molars) teeth with gingival recession were treated.

Intra-surgical measurements (Table 1)

The mean PT in the area of the withdrawal, in the control and test groups, were 3.1 ± 0.47 mm (range 2–4.5 mm) and 3.06 ± 0.46 mm (range 2–4 mm), respectively. The difference was not statistically significant ($F = 0.9$, p = NS). In the control group, 12 grafts were taken from the pre-molar area (mean PT was 3.32 ± 0.38 mm, range 3–4.5 mm) and the remaining 13 from the molar area (mean PT was 2.89 ± 0.50 mm, range 2–3.5 mm). In the test group, 10 grafts were taken from the pre-molar area (mean PT was 3.2 ± 0.53 mm; range 2.5–4 mm) and the remaining 15 from the molar area (mean PT was 2.96 ± 0.32 mm; range 2.5–4 mm).

In the *control* group, the mean thickness of the graft (GRT) immediately after being harvested was 1.34 ± 0.26 mm (range 1–1.8 mm). After removing the fatty and glandular tissues, the thickness of the CTG, at the time of suturing (GRTs), was 0.88 ± 0.17 mm (range 0.5–1.2 mm). The mean thickness of the primary flap was 0.72 ± 0.13 mm (range 0.5–0.9 mm). The mean height of the CTG (GRH) was 6.16 ± 0.89 mm (range 5–8 mm), while the

Table 1. Intra-surgical measurements

Parameters (in mm)	Control group	Test group	F	p
PT	3.1 ± 0.47	3.06 ± 0.46	0.9	NS
GRT	1.34 ± 0.26	1.32 ± 0.16	0.8	NS
GRTs	0.88 ± 0.17	0.83 ± 0.12	1.5	NS
GRH	6.16 ± 0.89	6.28 ± 0.97	0.2	NS
GRW	10.72 ± 0.84	10.96 ± 0.37	1.3	NS
WD	2.06 ± 0.27	1.32 ± 0.16	132.2	<0.01
RTT	1.04 ± 0.49	1.73 ± 0.47	25.6	<0.01

See text for abbreviations.
Significance was obtained from one-way ANOVA statistical analysis.

mean width (GRW) was 10.72 ± 0.84 mm (range 9–12 mm). Complete closure of the palatal wound was accomplished in all patients.

In the *test* group, the mean thickness of the epithelialized graft (GRT) was 1.32 ± 0.16 mm (range 1–1.6 mm). The mean thickness of the graft after de-epithelialization and removal of fatty tissue (GRTs) was 0.83 ± 0.12 mm (range 0.6–1 mm). The mean GRH was 6.28 ± 0.97 mm (range 5–8 mm), while the mean GRW was 10.96 ± 0.37 mm (range 10–12 mm).

No statistically significant difference was demonstrated between the test and the control groups in any of the considered dimensions of the CTG at the time of suturing: thickness ($F = 1.5$), width ($F = 1.3$) and height ($F = 0.2$). The depth of the withdrawal (WD), corresponding to the sum between the thickness of the primary flap and the thickness of the graft immediately after being harvested (FT+GRT = 2.06 ± 0.27 mm) in the control group, and to the thickness of the epithelialized graft (GRT = 1.32 ± 0.16 mm) in the test group, was statistically greater in the control group ($F = 132.2$, $p < 0.01$). In contrast, the difference between PT and WD, that is the RTT covering the palatal bone, was statistically lower in the control group ($F = 25.6$, $p < 0.01$). These data indicated that, in the control group, a greater depth was reached in the palate and a lower soft tissue thickness covering the bone was left during the harvesting procedure.

Surgical chair time

The overall surgical chair-time was significantly shorter for the test group. In particular, the average time needed for performing the CAF with DGG was 35.8 ± 3.4 min. (range 30–42 min.), while the mean time for completing the CAF with CTG was 45.0 ± 4.3 min. (range 38–55 min.). The difference was statistically significant ($F = 63.8$, $p < 0.01$). Surgical time was significantly correlated with pain killer consumption (in mg) ($F = 11.9$; $p < 0.01$; correlation coefficient 0.44): analgesic consumption increased on increasing the time needed to complete the surgery.

Patient morbidity (Table 2)

Healing was uneventful for all test patients. In seven (28%) control patients, a dehiscence/necrosis of the primary palatal flap occurred during the first healing period (7 days).

Table 2. Post-operative morbidity

Parameters	Control group	Test group	*F*	*p*
Pain killer (in mg)	2016 ± 1896.4	1656 ± 1532.2	0.5	NS
Discomfort (VAS)	2.65 ± 2.18	3.1 ± 1.99	0.1	NS
Bleeding (VAS)	2.9 ± 2.12	3.65 ± 1.89	1.2	NS
Inability to chew (VAS)	1.95 ± 1.87	3.85 ± 2.0	9.1	<0.01
Stress (VAS)	2.1 ± 1.25	4.5 ± 1.53	29.5	<0.1

See text for abbreviations.
Significance was obtained from General linear model statistical analysis.

The mean pain killer consumption (in addition to the 600 mg ibuprofen given before the surgery) in the control and the test groups was 2016 ± 1896.4 mg (range 0–5400 mg) and 1656 ± 1532.2 mg (range 0–4200 mg), respectively. The difference was not statistically significant ($F = 0.5$, p = NS). A separate analysis demonstrated statistically greater analgesic consumption in the seven patients experiencing primary flap dehiscence/necrosis (4028.5 ± 828.8 mg) than test patients with secondary intention palatal healing and control patients (1233.3 ± 1587, 8 mg) with primary intention palatal wound healing ($F = 9.3$, $p < 0.01$). In contrast, the difference in analgesic consumption between the test patients and the control patients experiencing primary intention wound healing was not statistically significant.

A general linear statistical model was fitted relating analgesic consumption to GRW, GRH, area (molar *versus* premolar) of the withdrawal and to the RTT covering the palatal bone. The R^2 statistic indicates that the model as fitted is highly significant and explains 54.9% of the variability in analgesic consumption. A significant relationship was found regarding RTT ($F = 14.5$, $p < 0.01$), GRH ($F = 23.1$, $p < 0.01$) but not regarding the area of the withdrawal ($F = 1.3$, p = NS) and the GRW ($F = 1.1$, p = NS). Pain killer consumption increased with increasing height of the withdrawal and by reducing the thickness of the soft tissue still covering the palatal bone.

Very limited post-operative morbidity was reported by both patient groups.

No statistically significant difference was demonstrated between the control and the test patients in terms of post-operative discomfort, ($F = 0.1$, p = NS) and bleeding ($F = 1.2$, p = NS)-related VAS values.

Statistically significant better results in terms of post-operative inability to chew ($F = 9.1$, $p < 0.01$)- and stress ($F = 29.5$, $p < 0.01$)-related VAS values were demonstrated in the control compared with the test patients.

Clinical parameters

The descriptive statistics for the clinical parameters measured at baseline and 12 months after surgery for both groups, as well as the mean differences within and between groups, are shown in Table 3. At baseline, there were no statistically significant differences between the two groups for any of the considered clinical parameters, indicating that the randomization process had been effective. In the control group, the mean RD was 3.4 ± 0.86 (range 2–5 mm) and the mean GT was 0.71 ± 0.15. In the test group, the mean RD and GT were 3.56 ± 0.86 (range 2–5 mm) and 0.75 ± 0.15, respectively.

One-year clinical outcome

RD

The results of fitting a general linear statistical model relating RD to techniques, time and the interaction between techniques and time showed a high R^2 statistic, indicating that the model as fitted is highly significant and explains 95.5% of the variability. A significant relationship was found regarding time (baseline *versus* 1 year) ($F = 904.9$, $p < 0.01$) in both groups, but not regarding the techniques ($F = 2.21$, p = NS). No statistically significant difference was demonstrated in the amount of root coverage (in mm) between the two bilaminar procedures.

The percentage of root coverage amounted to 96.2 ± 8.93% in the test group and to 92.28 ± 13.06% in the control group. Complete root coverage was achieved in 21 (84%) of the test and in 18 of the control (72%) treated defects. The results of fitting a logistic regression model, including baseline RD as a confounding factor, showed no

Table 3. Clinical parameters (mean ± SD) at baseline and 12 months post-surgery

Parameter (in mm)	Control group ($n = 25$)	Test group ($n = 25$)
RD		
Baseline	3.4 ± 0.86	3.56 ± 0.86
12 months	0.32 ± 0.55	0.16 ± 0.37
Difference	3.08 ± 0.70	3.4 ± 0.81
CAL		
Baseline	4.52 ± 0.87	4.72 ± 0.84
12 months	1.56 ± 0.65	1.52 ± 0.58
Difference	2.96 ± 0.78	3.2 ± 0.91
PD		
Baseline	1.12 ± 0.33	1.16 ± 0.37
12 months	1.24 ± 0.43	1.36 ± 0.48
Difference	0.12 ± 0.43	0.2 ± 0.5
KTH		
Baseline	1.36 ± 0.48	1.52 ± 0.50
12 months	3.28 ± 0.54	3.64 ± 0.48
Difference	1.92 ± 0.49	2.12 ± 0.52
GT		
Baseline	0,71 ± 0.15	0.75 ± 0.15
12 months	1.32 ± 0.22	1.55 ± 0.21
Difference	0.61 ± 0.16	0.80 ± 0.17

See text for abbreviations.

significant difference (χ^2 2.2, $p = $ NS) between the procedures.

CAL

The results of fitting a general linear statistical model relating CAL to techniques, time and the interaction between techniques and time showed a high R^2 statistic, indicating that the model as fitted is significant and explains 94% of the variability. A significant relationship was found regarding time (baseline *versus* 1 year) ($F = 651.2$, $p < 0.01$) in both groups, but not regarding the techniques ($F = 0.99$, $p = $ NS). No statistically significant difference was demonstrated in the amount of CAL gain between the two bilaminar procedures.

PD

The results of fitting a general linear statistical model relating PD to techniques, time and the interaction between techniques and time showed a high R^2 statistic, indicating that the model as fitted is significant and explains 68.9% of the variability. A significant relationship was found regarding time (baseline *versus* 1 year) ($F = 5.77$, $p < 0.05$) in both groups, but not regarding the techniques ($F = 0.36$, $p = $ NS).

KTH

The results of fitting a general linear statistical model relating KTH to techniques, time and the interaction between techniques and time showed a high R^2 statistic, indicating that the model as fitted is significant and explains 95% of the variability. A significant relationship was found regarding time (baseline *versus* 1 year) ($F = 784.5$, $p < 0.01$) in both groups, but not regarding the techniques ($F = 1.92$, $p = $ NS). No statistically significant difference was demonstrated in the increase in KTH between the two bilaminar procedures.

GT

The results of fitting a general linear statistical model relating GT to techniques, time and the interaction between techniques and time showed statistically significant differences considering both time ($F = 915.7$, $p < 0.01$) (baseline *versus* 1 year) and the interaction between techniques and time ($F = 16.2$, $p < 0.01$). A greater increase in GT was observed in the test compared with the control group.

The difference between GT increase at 1 year and graft thickness at the time of suturing was statistically significant ($F = 56.6$, $p < 0.01$) between the two groups independent of the thickness of the graft at the time of suturing.

Discussion

Sub-epithelium CTG is the most effective and predictable root coverage surgical procedure for the treatment of gingival recession (Roccuzzo et al. 2002, Cairo et al. 2008). CTG harvesting techniques are widely recommended so as not to expose patients to the more painful post-operative course associated with secondary intention palatal wound healing (Farnoush 1978, Jahnke et al. 1993, Del Pizzo et al. 2002, Griffin et al. 2006, Wessel & Tatakis 2008). Nevertheless, before performing a CTG harvesting technique, it is mandatory to evaluate the palatal anatomic characteristics and in particular the thickness of the palatal fibromucosa avoiding useless or even harmful surgical procedures. The primary access flap must include both epithelium and connective tissue that is critical for its viability. In some clinical situations, there is not enough connective tissue thickness for both the primary flap and the graft. Based on the clinical experience of the authors, this is true in the palatal pre-molar area of most of the patients, where, under a thin layer of connective tissue, there is a thick area of fatty and glandular tissue, as well as in the molar area of a few patients, where the entire palatal fibromucosa is not thick enough for obtaining a double layer (one for the flap and one for the graft) of connective tissue. In these situations, if the primary access flap is of a proper thickness, there is no connective tissue left for performing the graft. The risk lies in the incorporation of fatty and glandular tissue, inadequate for root coverage, instead of the connective tissue in the buccal aspect of teeth affected by gingival recession. Sullivan & Atkins (1968) emphasized the importance of removing all fatty tissue included in the graft that ''could function as a barrier both to diffusion and vascularization''. On the other hand, if the primary flap is too thin, it consists only or prevalently of epithelium and might result in necrosis/dehiscence during the first healing phase. As a result, the palatal wound heals by secondary intention. This eventful outcome was reported frequently in the literature when the trap-door approach was used as the CTG harvesting technique (Edel 1974, Broome & Taggart 1976, Jahnke et al. 1993, Harris 1997, Del Pizzo et al. 2002). The present study demonstrated a statistically significant greater analgesic consumption in the seven patients (28%) experiencing primary flap dehiscence/necrosis, with respect to test patients healed by secondary intention and control patients healed by primary intention. This is in

line with the opinion of several authors that indicated the sloughing of the primary flap as the main cause of marked post-operative discomfort following a trap-door approach (Edel 1974, Jahnke et al. 1993) and with the study by Harris (1997), which reported an association between extensive flap necrosis and post-operative pain. More specifically, the data of the present study indicated more analgesic intake by patients during secondary intention palatal wound healing as a result of flap necrosis/dehiscence than when it is the result of the withdrawal of an epithelialized graft. The reasons for this difference are unknown; it can be speculated that the more painful post-operative course might derive from sovra infection of the wound favoured by tissue necrosis and/or from the greater depth reached during the harvesting technique. In fact, when a CTG harvesting technique is performed, because some connective tissue has to be left to maintain the vitality of the primary flap, it is necessary to extend the dissection deeper into the palatal soft tissues.

The critical role of the depth of the withdrawal and in particular of the difference between PT and the depth of the withdrawal in influencing post-operative pain was one of the main results of the present study. The present study demonstrated that the RTT covering the palatal bone was negatively correlated with pain killer consumption. These data were statistically significant considering both the entire patient sample and the single treatment groups. In other words, patient analgesic consumption was greater in those patients in whom lower soft tissue thickness covering the bone was left during the harvesting procedure. Specifically, all patients consuming more than three analgesic tablets throughout the post-operative week had <2 mm thickness of soft tissue covering the palatal bone after the harvesting procedure.

It can be suggested that when 2 mm or more of soft tissue thickness can be left to cover the palatal bone, CTG harvesting techniques are preferred because primary intention wound healing results in very limited pain and a better post-operative course in terms of patient stress and ability to chew. Otherwise, if palatal soft tissue is not thick enough, connective tissue harvesting techniques are not recommended because of the risk of primary flap necrosis and/or the inadequacy of the graft due to the presence of fatty and glandular tissues instead of connective tissue. In this situation, harvesting an FGG that is subsequently de-epithelialized with the use of the blade is recommended.

When a FGG is harvested, a lower thickness of palatal fibromucosa is required to obtain both an adequate connective tissue graft and a residual thickness of soft tissue covering the bone. With the use of the blade, in fact, it is possible to clinically check (based on the difference in light reflection and tissue consistency) the removal of the epithelium and thus the most superficial connective tissue can be utilized in the graft. This approach allows for incorporating into the graft the portion of connective tissue closer to the epithelium. This tissue is denser, firmer, more stable and presumably more suitable for root coverage purpose (Harris 2003). This was confirmed by the present study data, which demonstrated a greater increase in GT at the buccal aspect of the test-treated patients despite the fact that no difference was found in the thickness of the graft at the time of suturing between the two treatment groups. Furthermore, the difference between GT increase at 1 year and graft thickness at the time of suturing was statistically significant ($F = 56.6$, $p < 0.01$) between the two groups, indicating that in the test group, almost the entire thickness of the graft became buccal GT at 1 year, while in the control group, a significant part of the graft thickness was lost during the healing period. It can be speculated that differences in the quality of the connective tissue between the two treatment groups were responsible for the different performance of the grafts during the healing phase. A negative aspect of the adopted de-epithelialization technique could be the remnant of some epithelium in the graft. However, the inclusion of some epithelium did not seem to affect the clinical results in terms of root coverage (Harris 2003).

The negative aspect of the epithelialized graft harvesting technique was reported to be the less favourable and more painful patient's post-operative course due to the secondary intention palatal wound healing (Farnoush 1978, Jahnke et al. 1993, Del Pizzo et al. 2002, Griffin et al. 2006, Wessel & Tatakis 2008). This does not seem to be confirmed by the present data. This study in fact failed to demonstrate any increase in terms of post-operative pain, discomfort and bleeding in patients subjected to the epithelialized graft harvesting procedure compared with patients undergoing the connective tissue harvesting technique. Furthermore, no statistically significant difference in pain killer consumption was demonstrated between control patients experiencing primary intention wound healing (thus excluding those with necrosis/dehiscence of the primary flap) and test patients healing by secondary intention. The reasons for the difference can only be speculated on; a possible explanation can be found in the surgical techniques and in particular in the dimensions of the graft or in the protection of the wound area with equine-derived collagen in the test group. In the studies comparing patients' post-operative outcomes after different graft-harvesting procedures (Farnoush 1978, Jahnke et al. 1993, Del pizzo et al. 2002, Griffin et al. 2006, Yen et al. 2007, Wessel & Tatakis 2008), no data are available on the thickness and height of the FGG. However, in some of these studies (Griffin et al. 2006, Yen et al. 2007) a periosteum elevator was used to free the graft from the underlying bone and the graft was extended apical to the buccal bone crest. It is conceivable that in the present study, shallower (in the apical–coronal dimension) and thinner FGG were harvested. The present study data demonstrated that the height and depth of the withdrawal and not the type (primary *versus* secondary) of palatal wound healing influence post-operative analgesic consumption. It can be speculated that in the studies reported in the literature, the more painful post-operative course in patients experiencing FGG procedures might be due to the greater height of the graft as well as the greater depth reached in the palatal soft tissue during the harvesting technique and not (or not only) due to the different type (primary or secondary) of palatal wound healing. A possible explanation is that by inserting the blade into the depth of the palatal soft tissue and/or towards the palatal vault (height of the withdrawal), the probability of severing a large-sized nerve/vessel increases, causing greater pain. In addition, it cannot be excluded that the limited patient morbidity in the test group of the present study can be ascribed to the protection of the secondary intention wound area with an equine-derived collagen matrix, which could have minimized post-operative discomfort and bleeding (Farnoush 1978,

Saroff et al. 1982). Nevertheless, Wessel & Tatakis (2008), despite using palatal stents to protect free gingival graft donor sites, reported a greater incidence of donor site pain compared with CTG.

Finally, a lack of statistical significance between groups in a trial designed to demonstrate superiority does not mean that equivalence exists between the two treatment techniques Gunsolley et al. 1998). In the present data, the lack of significance between the two groups might be due to the great within-group variability, which would have required a larger then expected sample size. A study, with a larger number of patients, is ongoing to confirm the present data.

Another finding of the present study was the statistically significant longer mean surgical chair time in patients undergoing the bilaminar procedure with the trap-door harvesting technique. Because the duration of the surgical grafting procedure has been correlated with post-surgical pain in both the present and the previous studies (Griffin et al. 2006, Cortellini et al. 2009), this might have contributed to balance pain killer consumption between the two patient groups. In other words, it can be hypothesized that longer surgical time and lower soft tissue thickness covering the bone in the trap-door control group might have balanced the secondary intention wound healing experienced by the test group in terms of post-operative pain suffered by the patients. The increased time to complete the CAF with CTG can be explained by the additional time required to harvest the graft (in particular, in dissecting the split thickness primary access flap) and to perform multiple interrupted sutures to achieve complete closure of the palatal wound.

Better results in terms of post-operative ability to chew and patient stress were demonstrated in patients subjected to the CTG harvesting technique. A possible explanation is that the open palatal wound may render the patient more anxious; hence, he/she avoids chewing for fear of jeopardizing wound healing. It is surprising that the difference in stress and ability to chew is significant also including control patients experiencing dehiscence/necrosis of the primary flap. It can be speculated that the presence of soft tissue closing the donor site, at least in first post-operative day/s, could minimize patient stress and help forget the presence of the wound palatal area. Nevertheless, this seems to be in contrast with the increased intake of anti-inflammatory drags in the patients with a failing trap-door approach. This rather controversial issue was also reported by Del Pizzo et al. (2002), and further studies are needed to clarify it.

The results of this study also indicated that both types of CTG can be successfully used under a CAF to cover gingival recession, with no statistically significant difference between them. One year post-treatment, 91.6% of the control gingival defects and 96.5% of the test gingival recessions were covered with the soft tissue. Furthermore, complete root coverage was achieved in 70% of the control and 85% of the test treated cases.

The only statistically significant difference in the clinical outcomes between the two treatment groups of the present study was the greater increase in the GT in the patients treated with the de-epithelialized graft. Any attempt to explain this difference is speculative in nature, but it might be related to the quality (better stability and less shrinkage) of the connective tissue resulting from the de-epithelialization of a free gingival graft with respect to that harvested with the trap-door approach.

Conclusions

No difference in post-operative analgesic consumption, discomfort and bleeding was demonstrated in patients subjected to CTG or epithelialized graft-harvesting techniques. However, the lack of difference could be ascribed by the considerable within-group variability. Studies with a larger number of patients are needed to confirm the present data.

Better results in terms of post-operative stress and ability to chew were demonstrated in patients undergoing the CTG harvesting technique.

Secondary intention palatal wound healing due to dehiscence/necrosis of the primary flap was associated with greater consumption of analgesics.

Pain killer consumption increased with increasing height of the withdrawal and decreasing RTT covering the palatal bone.

Both types of grafts were effective in root coverage and clinical attachment gain when associated with a CAF for the treatment of gingival recession.

A greater increase in GT was achieved in the test patients; this may be due to the better post-operative stability of connective tissue resulting from the de-epithelialization of free gingival grafts.

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Clinical Relevance

Scientific rationale for the study: Randomized studies comparing both patient morbidity and root coverage outcomes after the use of CTG and DGG for the treatment of gingival recessions are currently not available *Principal findings*: This study indicated no difference in post-operative pain, discomfort and bleeding between the two groups. Lower stress and better ability to chew were demonstrated in the CTG group. Pain increased with increasing height and depth of the withdrawal and in the case of necrosis of the primary palatal flap. A greater increase in buccal soft tissue thickness was achieved in the DGG group. *Practical implications*: In sites with thick palatal fibromucosa, a trap-door approach is more patient friendly, while with thin palatal tissues, there is a greater risk of a failure of the trap-door, thereby indicating a DGG.