SURGICAL TREATMENTS OF MULTIPLE GINGIVAL RECESSIONS: STATE OF THE ART and WOUND HEALING MODULATION

Ph.D. thesis

Dr. med. dent. Sofia Aroca

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1. INTRODUCTION

1.1 Etiology

Several factors are associated with marginal gingival tissue recession and its etiology is complex. Gingival recession is a very common feature in populations with high standards of oral hygiene where the buccal root surface is exposed (Wennström 1996). A « wedge-shaped » defect at the crevicular area is a very common feature on one or several teeth (Sagnes & Gjermo 1976) and traumatic tooth - brushing may be a main causative factor for the development of recessions. In a 5- year study it has been shown that the percentage of affected sites increased with the level of oral hygiene education (Daprile et al. 2007).

The mechanism leading to gingival recession is not well understood. Some authors suggest that tooth abrasion, which is usually observed with recession, may play a key role in the apicalization of gingival margin (Litonjua et al. 2003). Others claim that the mechanism is inflammatory in nature (Susin et al. 2004) while in cases of periodontal disease, marginal tissue recession may be observed on all surfaces of the tooth.

The following risk factors have been postulated to play a role in the etiology of gingival recession (Wennström 1996). Tooth malposition, path of eruption, tooth shape, profile and position in the arch, alveolar bone dehiscence, muscle attachment and frenal pull, periodontal disease and treatment, iatrogenic restorative or operative treatment, improper oral hygiene methods (e.g. tooth brushing, floss, interproximal brush), other self-inflicted injuries (e.g. oral piercing). The most important factor increasing the risk of gingival recession may be a thin gingival biotype where a delicate marginal tissue is covering a non-vascularized root surface (Müller et al. 1998).

Marginal tissue recession is due to the displacement of the soft tissue margin apical to the cemento-enamel junction (Wennström 1994) with exposure of the root surfaces to the oral cavity. This tissue recession is frequently associated with aesthetic impairment.

1.2 Frequency / Prevalence

More than 50 percent of the population has one or more sites with gingival recession of 1 mm or more. The prevalence of gingival recession was found in patients with both good and poor oral hygiene (Kassab & Cohen 2003). Patients with high standards of oral hygiene show loss of attachment, marginal tissue recession in buccal surfaces (Källestål et al. 1990, Löe et al.1992), whereas when they are affected by periodontal disease or after periodontal treatment, all tooth surfaces can present marginal tissue recession (Baelum et al. 1992, Miller 1987)

Gingival recession has been linked to ethnicity. Non-hispanic blacks have the highest prevalence and extent of gingival recession, while Mexican Americans and non-Hispanic whites display a lessened and similar prevalence and extent of gingival recession (Albandar & Kingman 1999). While no difference between whites and non-whites was found with a different study design(Susin et al. 2004).

Gingival recession is a common condition and its extent and prevalence increase with age (Albandar & Kingman 1999, Kassab & Cohen 2003, Litonjua et al. 2003, Susin et al. 2004).

A gender component to gingival recession has been reported in several studies, with males exhibiting greater levels of recession than females (Albandar & Kingman 1999, Susin et al. 2004).

Tobacco smoking and prevalence of gingival recession has been discussed by several authors and their association is controversial. Some studies have shown that smokers have significantly more extensive recessions than non-smokers (Susin et al. 2004). While other studies have shown, no correlation between smoking and gingival recession (Müller et al. 2002, Slutzkey & Levin 2008).

1.3 Classification

A clinical classification of gingival recession in four categories according to the relationship with the muco-gingival line and interproximal bone is widely accepted and a predictive value for root coverage in each class has been proposed (Miller 1985).

In Class I: recession type defect the marginal tissue recession does not extend to the mucogingival junction and there is no loss of interdental periodontium and one hundred percent of exposed root coverage can be expected as a surgical outcome.

In Class II: recessions the defect extends beyond the mucogingival junction without interdental tissue loss and also full coverage of exposed root surfaces can be anticipated.

Class III: marginal tissue recession extends to or beyond the mucogingival junction, interdental bone and soft tissue loss are apical to the cemento enamel junction but coronal to the bottom of the recession and partial root coverage can be expected.

Class IV: marginal tissue recession extends beyond the mucogingival junction with loss of interdental bone and soft tissue loss apical to the base of the recession and surgical root coverage cannot be anticipated.

The main advantage of Miller's classification is its simplicity and its use in communication. This classification is used in most studies, even if there is a lack of important criteria such as biotype, root prominency, supporting bone.

1.4 Localized gingival recession

Historically, indications for treatment of gingival recession consisted in halting progressive recession, enhancing plaque control, preserving a band of keratinized gingiva, decreasing frenum pull and preventing postorthodontic and postprosthetic marginal tissue recession. Attempts have occasionally been made to cover denuded roots for cosmetic purposes and to decrease root sensitivity and one of the main concerns of clinicians has always been predictability.

The objectives were then modified not only to arrest and cure a disease process but, if possible, to regenerate any lost tissue. Therefore the goal of root coverage is now to obtain full root coverage of a denuded root and this will imply blending of the mucosa and or keratinized gingiva and reduced root sensitivity without any residual periodontal pocket.

Successful treatment of recession-type defects is based on the use of predictable periodontal surgery (PPS) procedures. As first proposed by Miller in 1988, the term PPS comprises different surgical techniques intended to correct and prevent anatomical, developmental, traumatic or plaque disease-induced defects of the gingiva, alveolar mucosa or bone (Wennström 1996).

Several surgical procedures have been proposed since the beginning of the 20th century. Younger in 1902, Harlan in 1906 and Rosenthal in 1911 (Baer & Benjamin 1981*)* first described the use of pedicle or free soft tissue grafts to cover denuded root surfaces. These techniques were abandoned for a long time up to the end of the 1950-es. From these decades different surgical procedures have been described in order to improve clinical parameters such as recession depth, clinical attachment level and width of keratinized gingiva. Laterally repositioned flaps, free gingival grafts, coronally advanced flaps and subepithelial connective tissue grafts (Grupe & Warren 1956, Björn 1963, Grupe 1966, Nabers 1966, Sullivan & Atkins 1968, Bernimoulin et al. 1975, Patur 1977).

Plastic periodontal procedures used for root coverage are usually classified as pedicle soft tissue graft and free soft tissue grafts

1.4.1 Lateral sliding flap

The Lateral sliding flap, is one of the oldest plastic periodontal techniques (Grupe & Warren 1956). A full-thickness flap was mobilized on the adjacent tooth and the flap was then positioned laterally and sutured to cover the exposed root surface. In order to minimize the risk of recession and dehiscence on the donor site due to the exposure of the buccal bone plate, the technique was modified. On the donor site, which is an adjacent area of keratinized tissue, the pedicle flap is elevated by split thickness dissection with a submarginal incision (Staffileno 1964).

Many other modifications of this technique have been proposed and these are the follows: double papilla flap (Cohen & Roos 1968), the oblique rotational flap (Pennel et al. 1965), the rotation flap (Patur 1977), and the transpositioned flap (Bahat et al. 1990).
The blood supply, that nourishes the flap over the avascular root surface, is supplied by the wide base of the flap and the underlying periosteum surrounding the denuded roots. The advantages of this technique are as follows: excellent tissue and color blend, good vascularisation of the flap. The disadvantages are: unpredictable root coverage, limited success in case of wide localized or multiple recession type defects, risk of root exposure of the donor tooth, the adjacent donor site must have enough keratinized tissue.
The mean root coverage achieved with this technique is about 64% (Wennström 1996)
The predictability of complete root coverage is of 40-50%.

1.4.2 Autogenous free gingival graft

The autogenous free gingival graft was introduced by Björn(1963) and Nabers(1966) with the objective to increase the width of keratinized gingiva. Because this graft retains none of its own blood supply and blood vessels it was not originally intended to cover denuded roots. However, several modifications have improved root coverage by means of this procedure, (Miller 1985, Holbrook & Ochsenbein 1983) but too few comparative studies are available.
The mean percentage of root coverage is 72%. The predictability of complete root coverage ranges from 0% to 90%, with an average of 57% (Wennström 1996).

1.4.3 Coronally advanced flap

As an alternative to lateral sliding flap, a coronally positioned flap (CAF) to cover root surfaces may be used (Allen & Miller 1989). This technique was first described by Norberg 1926), Harvey (1965), Brustein (1970) and Restrepo (1973) (Wennström 1994). This procedure is based on the coronal shift of soft tissue apical to the denuded root surface (Allen & Miller 1989; Pini Prato et al. 2000). A great advantage of this procedure is its applicability for the treatment of multiple recession type defects. Tarnow (1986) suggested a semilunar approach in shallow single recessions (Tarnow 1986).
The mean percentage of root coverage is 83%. The percent of teeth with complete root coverage (CRC) ranges from 24% to 95% when used alone.
This approach of the coronally advanced flap may also be used in combination with connective tissue graft (Wennström & Zuchelli 1996), barrier membranes (Pini Prato et al. 1992), enamel matrix derivatives (Rasperini et al. 2000), acellular dermal matrix (Harris 1998) or other. A systematic review (Cairo et al. 2008) shows that only the connective tissue graft and the enamel matrix derivative under CAF procedure enhance the probability of obtaining complete root

coverage and to improve recession reduction in Miller's Class I and II recession defects. However, when the CAF is combined with CTG (bilaminar technique), it is considered to be the gold standard.

Is it important to notice that the total coverage of denuded roots remains a problem for most clinicians despite new surgical techniques, because the avascular nature of the root surface hampers the ability of most graft to survive. Consequently, with a wider area of root exposure, the difficulty is increased for the clinician.

1.5 Multiple recession type defects

In multiple adjacent recession type defects (MARTD) the avascular surface is more extensive. Furthermore some anatomical characteristics such as thin biotype, decreased keratinized tissue (KT) width, root prominence and root proximity make much more difficult the choice of surgical treatment compared to localized gingival recession type defects.

The predictability of treatments aimed to provide root coverage in cases of localized gingival recessions (LGR) has been reviewed extensively in several systematic reviews (Roccuzzo et al. 2002, Cheng et al. 2007, Oates et al. 2003) of Miller's Class I and II recession type defects (Miller 1983). However scientific literature is sparse regarding the treatment of MARTD and randomized control trials (RCTs) are needed to identify the indication for each surgical technique and any prognostic factors (Chambrone et al. 2009).

Both localized and multiple gingival recessions may be a concern for patients for a number of reasons. In addition to root hypersensitivity, erosion and root caries, aesthetic considerations may also come into play (Wennström 1996), particularly in those patients who have a high lip smile line.

Recently, new techniques have been suggested for the surgical treatment of multiple adjacent recession type defects (MARTD). These are mainly derived from the coronally advanced flap (CAF) (Zucchelli & De Sanctis 2000), a supraperiosteal envelope technique (SET) in combination with a subepithelial connective tissue graft (CTG) (Allen 1994), or its evolution as a tunnel technique (Azzi & Etienne 1998, Zabalegui et al. 1999, Tozum & Dini 2003). The main goal of these plastic periodontal surgery procedures is to obtain root coverage and optimal aesthetic appearance with complete root coverage and blending of the mucosa and/or gingiva. So as increase the efficacy of the root coverage treatment, reduce the morbidity of the technique and improve clinical outcomes, proposals have been made for the addition of biological factors such as; enamel matrix derivative (EMD) (Ito et al. 2000, Pilloni et al. 2006); platelet rich plasma (Petrungaro 2001); platelet rich fibrin (PRF) (Aroca et al. 2009).

Platelet Rich Plasma (PRP) is a fraction of plasma which provides a rich source of growth factors (Kawase et al. 2005) and may enhance initial stabilization and revascularization of the flap and grafts (Petrungaro 2001). PRP is prepared with an anticoagulant to avoid platelet activation and degranulation. Thereafter, it must undergo two centrifugation processes. PRP is then mixed with bovine thrombin and calcium chloride at the time of application (Dohan et al. 2006). In a pilot study on the treatment of Miller's Class I recessions (Miller 1985), the application of PRP with a CAF root coverage procedure provided no clinically measurable enhancement. However, positive benefits from the use of PRP were better gingival index and wound healing index values, as well as increased gingival thickness (Huang et al. 2005).

The autologous Platelet Rich Fibrin clot (PRF) was used initially in implant surgery in order to improve bone healing (Choukroun et al. 2000). In spite of a lack of scientifically proven clinical benefit, the homogeneous fibrin network that is obtained is considered by the promoters of the technique to be a healing biomaterial and is commonly utilized in implant and plastic periodontal surgery procedures (Choukroun et al. 2006) to enhance bone regeneration and soft tissue wound healing. Compared to PRP, there are few references in the literature to the biological properties of PRF. However, it contains platelets, growth factors and cytokines that may enhance the healing potential, not only of bone, but also of soft tissues (Soffer et al. 2003).

PRP and PRF differ in their preparation protocols. PRF is used without any addition of anticoagulant and is centrifugated once.

The use of acellular dermal connective tissue allograft (ADM) instead of CTG has been proposed to support the gingival margin and change the gingival biotype (Henderson et al. 2001, Mahn 2001), eventually with the combination with bioabsorbable membranes (Cangini et al. 2003). Improved outcomes have also been claimed with the use of microsurgical techniques (Zuhr et al. 2007), vertical releasing incisions (Mahn 2001), an extension of the CTG (Ribeiro et al. 2008), and full coverage of the grafted soft tissue or substitute (Azzi & Etienne 1998, Mehlbauer & Greenwell 2005).

Case studies have been published for the treatment of multiple Miller's Class I and II recession defects (Carvalho et al. 2006, Chambrone & Chambrone 2006, Dembowska & Drozdzik 2007, Henderson et al. 2001, Tozum & Dini 2003, Tozum 2006, Zabalegui et al. 1999, Zucchelli & De Sanctis 2000, Zucchelli & De Sanctis 2005, Zucchelli & De Sanctis 2007, Murata et al. 2008, Berlucchi et al. 2005). Comparisons have been made between surgeons with various degrees of experience (Georges et al. 2009) and limited randomized controlled trials have compared surgical alternatives (Aroca et al. 2009, Henderson et al. 2001).

Surgical treatment of MARTD Miller's Class III defects is more challenging mainly due to loss of interproximal bone and soft tissues. There are additional anatomical characteristics that are of paramount importance when compared to Miller's Class I and II recessions. These include increased avascular surfaces, increased root prominence, reduced periosteal bed and, sometimes, deeper periodontal pockets.

Classification of root recession indicate the difficulty in obtaining favourable surgical outcomes, especially in cases of Class III and IV recession type defects (Miller 1983, Wennström 1996). Treatment techniques similar to those for Miller's Class I and II recessions are suggested for class III recessions (Cueva et al. 2004, Remya et al. 2008). When optimal MARTD root coverage is considered, we are dealing mostly with case reports where the envelope technique, in combination with a subepithelial CTG has been used (Sato et al. 2006), or a tunnel technique (Azzi et al. 2001, Zabalegui et al. 1999). This combined technique, with a subepithelial CTG, may improve interproximal soft tissue support, while the clinical benefit of adding EMD could not be evaluated in these limited cases (Ito et al. 2000). There is a lack of evidence for changes in the dimensions of papillae but an increased thickness of marginal gingiva (Muller et al. 1998) and significant gain in the width of keratinized tissue (KGW) has been observed (Carvalho et al. 2006).

One advantage of the SET procedure is that of preserving the continuity of the gingival papillae by creating a pouch to contain a CTG, which is slightly exposed over the recession (Allen 1994a). The pouch is created by separating the alveolar mucosa, from the bone underlying the papilla, to a position beyond the muco-gingival line, by blunt dissection using curettes. By positioning this pouch and tunnel coronally, it is possible to completely cover the CTG (Azzi & Etienne 1998). In the case of Miller's class III and IV recession type defects, this coronally advanced modified tunnel (CAMT) technique can be further improved by gently separating the entire interproximal papilla from the bone, which allows an even more coronal positioning in order to cover the defect (Azzi & Etienne 1998).

EMD, obtained from porcine embryogenesis, is an amelogenin derivative (Hammarstrom 1997) that has been developed to promote periodontal regeneration. A systematic review on EMD has shown this regenerative potential in the treatment of intrabony defects (Esposito et al. 2005) and an additional gain of clinical attachment has been shown when compared to open flap debridement alone (Esposito et al. 2003, Pagliaro et al. 2008, Sculean et al. 2008). Less recession has been found with EMD when compared to guided tissue regeneration technique for the treatment of intrabony defects (Sculean et al. 1999). Its biological potential was then tested with a coronally advanced flap for the treatment of gingival recessions. However, there is conflicting data for root coverage of Miller's Class I and II recession type defects, with or without EMD. The addition of EMD to a CAF has been seen to be beneficial in some studies (Pilloni et al. 2006, Spahr et al. 2005), while others do not find any difference between the two groups (Del Pizzo et al. 2005, Hagewald et al. 2002, Modica et al. 2000). A CAF-EMD combination has been associated with improved root coverage when compared to a CAF-CTG (Nemcovsky et al. 2004), while a contrary result was found after a 2-year follow-up study (Moses et al. 2006). According to a systematic review, one of the benefits of EMD may be to improve CAF predictability (Cheng et al. 2007).

Histological results support these clinical conclusions. Using EMD, evidence of periodontal regeneration was first described for the treatment of artificially created buccal dehiscence (Hammarström et al. 1997). New cementum, new periodontal ligament and new bone were reported after recession defects had been treated with a CAF-EMD combination (McGuire & Cochran 2003) and also with CAF-CTG-EMD (Rasperini et al. 2000) but a more limited regeneration with the latter combination has been found in four biopsies (Carnio et al. 2002).

Multiple adjacent recession type defects (MARTD) present a further challenge since:

- in order to minimize patient discomfort and to improve clinical outcomes , several recessions must be treated in a single surgical session.
- the management of Class III recession defects, combined with interproximal bone loss and cervical recession presents a complex challenge to the periodontist for the regeneration of soft tissues and bone.

2. AIM OF THE STUDIES

Studies are scarce on our capacity to modify gingival biotype after treatment of MARTD.
Most studies are concerned only with percentage of root coverage. CAF technique has become popular due to the simplicity of the technique and the excellent post-operative wound healing.
We choose to determine whether the addition of an autologous fibrin clot to a CAF will improve root coverage of multiple Miller's Class I or II gingival recessions when compared to a CAF alone. We have also decided to use suspended sutures on both test and control sides. The change in biotype thickness was evaluated by measuring the thickness of marginal gingiva or mucosa, with an endodontic probe. Changes in keratinized tissue width were also measured at 6 Months.

Our second project concerned Class III Miller's recessions. In that situation there is some bone loss and papilla loss. Case series or case reports have shown with SET or various tunnel techniques our ability to obtain root coverage with Class I and II Miller's recession type defects. A CAMT technique with subepithelial CTG was our control and on test side we evaluated the effect of an EMD application. Parameters for root coverage were monitored, but also the potential of EMD to stimulate a gain in papilla.

3. MATERIALS AND METHODS

3.1 Treatment of multiple Miller's class I and II gingival defects with and without PRF

3.1.1 Experimental design

Twenty patients were recruited, based on the following inclusion criteria:

1) At least three multiple Miller's Class I and II recession defects together with similar contralateral lesions.
2) Systemically healthy subjects.
3) age: 18 years old.
4) A full mouth plaque index <20%
5) A signed informed consent form.

Smokers (≤ 20 cig/day) were included

Patient exclusion criteria were as follows:

a) Inflammatory periodontal disease.
b) Previous surgical attempt to correct gingival recession.
c) Systemic disease or severe immune deficiency.
d) Coagulation defect or current anticoagulation treatment.
e) Addiction to drugs.
f) Subjects unable or unwilling to complete the trial.
g) Lack of linguistic skills or psychiatric disorders or decline to sign the informed consent.
h) Pregnant women.
i) Molar or premolar teeth with furcation involvements.

A total of 67 recession-type defects were treated. Twenty subjects, 15 females and 5 males aged 22 to 47 years (mean age 31.7 years) were enrolled. Fifteen patients had maxillary recessions, four with mandibular and one patient had both maxillary and mandibular recessions allowing test and control on the upper arch and also on the mandibular arch. Therefore, a total of 21 pairs of treatment (test and control) were performed. Full mouth scaling and prophylaxis were scheduled one month before surgery. CAF was performed on both sides of the mouth, either in conjunction with a PRF membrane (test side) or without (contralateral control side).

The following clinical values were recorded at baseline, then at 1, 3 and 6 months post-operatively:

- Gingival recessions (GR) were measured from the cemento-enamel junction (CEJ) to the gingival margin at the mid-buccal point of the teeth involved, using a periodontal probe

At baseline and 6 months after surgery the following parameters were recorded:

- Keratinized gingival width (KGW) was measured from the mucogingival junction (MGJ) to the gingival margin.
- Recession width (RW) was measured at the CEJ.

- Probing pocket depth (PD), clinical attachment level (CAL) and gingival - mucosal thickness (GTH).

GTH was measured 3 mm below the gingival margin (GM) in the attached gingiva or the alveolar mucosa, using a number 15 endodontic reamer with a silicone disk stop. The mucosal surface was pierced at a 90° angle with slight pressure until hard tissue was reached. The silicone stop on the reamer was then slid until it was in close contact with the gingiva. After removal of the reamer, the distance between the tip of the reamer and the inner border of the silicone stop was measured to the nearest 0.1 mm with calipers (Paolantino 2002).
All clinical measurements were performed by the same two investigators. They had previously been calibrated by assessing 30 recession defects on five patients with an interval of 72 hours between assessments. Calibration was accepted when 90% of measurements of recession agreed to within 0.5 mm for the periodontal probe (Pilloni et al. 2006) and 90% agreed to within 0.2 mm for measurements of gingival thickness with the endodontic file.
Adverse effect for patient comfort, tooth sensitivity and aesthetics were evaluated by interviewing patients at 1 and 6 months after surgery.

3.1.2 Surgical procedure

The recession defects were randomly assigned to the following treatments:

Modified coronally advanced flap alone

Modified coronally advanced flap with PRF

Before surgery all patients were given a single dose of 4 mg betamethasone and one tablet of 0.25 mg of alprazolanum in order to minimize post-operative oedema and anxiety. After local anaesthesia both surgical operations (test and control) were performed during one single surgical session by the same practitioner (SA). Test and control side were determined by tossing a coin.
Intravenous blood was collected in four 10 ml vials without anticoagulant just prior to surgery and immediately centrifuged at 3000 rpm for 10 minutes. The fibrin clot forms in the middle part of the tube. The upper part contains acellular plasma and the bottom part the red corpuscles (Fig. 1) (Dohan et al. 2006).

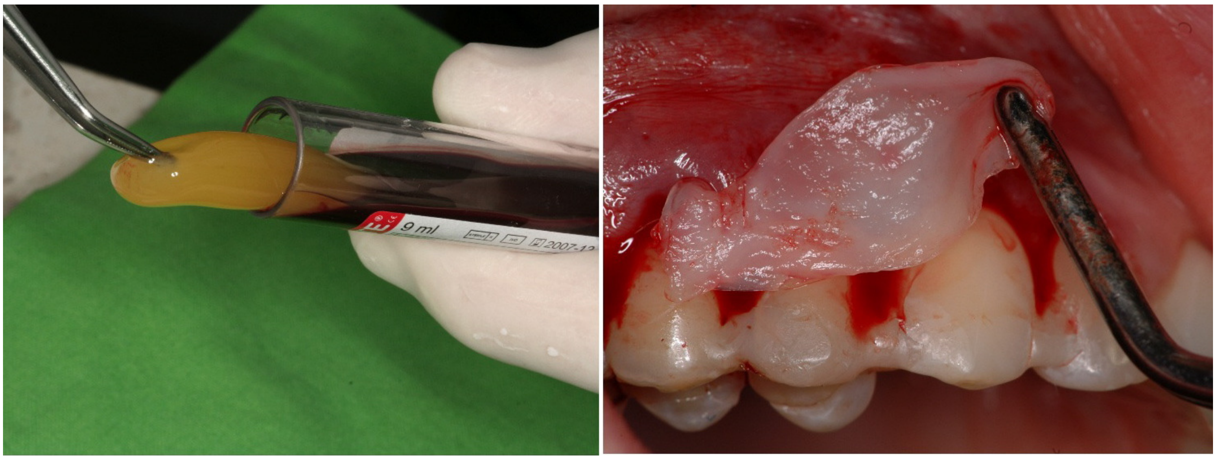


Figure 1. *Plasma-rich fibrin clot being applied after centrifugation*

The fibrin clot was easily separated from the lower part of the centrifuged blood and spread on a sterile gauze. A dry gauze was then folded over the PRF, which was stored in a refrigerator (4°C) until used.

Test surgery was performed first in order to minimize the delay before using the fibrin clot.

Recession defects were thoroughly scaled using Gracey curettes. No root conditioning was used. A modified coronally advanced flap (MCAF) technique (Zucchelli & De Sanctis 2000) was undertaken using a modified suturing technique. The flap design was as follows: Submarginal incisions were made in the interdental areas and intrasulcular incisions around those teeth with recession defects. Split-full-split flap incisions were performed in a coronal-apical direction. Gingival tissue adjacent to the root defect and the interproximal bone was raised full thickness, while the most apical portion of the flap was split-thickness to allow a coronal repositioning of the flap without tension. All papillae were de-epithelialized to create a connective tissue bed. On the test sites, the previously prepared fibrin clot was positioned over the recession defects just below the CEJ.

The gingival flap was then repositioned to totally cover the fibrin clot with its margin located on the enamel on both the test and control sides. They were held in that position with horizontally suspended sutures around the contact points (Fig.2) (Azzi et al. 1998).

Figure 2.

Test procedure. A) Preoperative view of maxillary right anterior teeth (test side). B) PRF membrane over the recession defects. C) CAF maintained in a coronal position with suspensory sutures around the contact points.

Stabilization of the blood clot was achieved by the application of gentle pressure on each side for 3 minutes.

Individualized oral hygiene instructions were given to each subject at the first appointment and a full mouth supragingival scaling and polishing were performed one month before the root coverage surgical procedure.

The study was performed according to a split-mouth design. In each patient, one side of the mandible (or maxilla) served as control and the opposite side as test. Treatment allocation was performed by the toss of a coin immediately prior to surgical procedure (Fig. 3). The same experienced practitioner performed both operations (SA) (at test and control sites) during a single surgical session.

Figure 3.

Results of treatment of multiple recession-type defects on the same patient (test and control). Test side. View at baseline (A) and at I (B) , 3 (C), and 6 months (D). Control side. View at baseline (E), and at I (F), 3 (G), and 6 months (H)

3.1.3 Statistical methods

The statistical analysis was performed using commercially available software. A subject-level analysis was performed for each parameter. Mean values and standard deviations (mean ± SD) for the clinical variables were calculated for each treatment. The method of Kolmogorov and Smirnov was used to confirm that the data was sampled from a Gaussian distribution. The significance of the difference within each group and between groups before and after treatment was evaluated with the paired samples t test. Differences were considered statistically significant when the p value was less than 0.05.

3.2 Treatment of multiple Miller's class III gingival recession type defects

3.2.1 Experimental design

Twenty subjects (mean age 31.7 years), with multiple Miller's Class III recession type defects (Miller 1983), representing 139 recession type defects, were enrolled in the study after having signed informed consents.

Criteria for subject selection were: 1) the presence of at least three adjacent gingival recessions (defect depth > 2 mm with at least one defect ≥ 3mm) on both sides of the maxillary or mandibular arch. 2) No systemic diseases that could influence the outcome of the therapy. 3) A full mouth plaque score (FMPS) of < 20% (O'Leary et al. 1972). 4) Non-smoker. 5) Not pregnant.

3.2.2 Surgical procedure

The coronally advanced modified tunnel (CAMT) technique used in the study has already been described (Azzi & Etienne 1998). Briefly, after local anesthesia, the root coverage procedure, based on a modified tunnel design, was performed as follows: Root planing of the exposed root surface was performed with Gracey curettes. Composite stops were placed at the contact points to prevent collapse of the future suspended sutures into the interproximal spaces. Ethylenediaminetetraacetic (EDTA) was applied on the test sites, as recommended by the EMD manufacturer. No chemical was used on control sites. Initial sulcular incisions and flap separation were then made with a tunnel knife–elevator instrument (Zuhr et al. 2007). The muco-periosteal dissection was extended beyond the muco-gingival junction and under each papilla so that the flap could be moved in a coronal direction without tension. Muscle fibers and any remaining collagen bundles on the inner aspect of the flap alveolar mucosa were cut with extreme care using Gracey curettes in order to avoid perforation of the flap and to obtain a passive coronal positioning of the flap and the papilla.

Preparation of the donor site was done immediately after completion of the tunnel. Dense connective tissue was harvested from the tuberosity using a distal wedge technique (Azzi & Etienne 1998). Two parallel incisions were made in the retromolar area and the more palatal incision was curved at a distance of approximately 2 mm from the tooth and continued towards the mesial aspect of the second or first molar depending on the required size of the graft. The donor site was sutured with a cross-mattress suture at the tuberosity and, when needed, interrupted sutures were used to approximate the papillae. Alternatively, when the tuberosity size was limited, a single incision was made on the palate (Hurzeler & Weng 1999) between the distal aspect of the canine and the mesial aspect of the second molar. In all cases an adequate size of connective tissue graft was obtained and the graft was trimmed to achieve a thickness of 1.0-1.5 mm with a N° 15 blade. Immediately after the graft was taken, pressure was applied to the donor area. Afterwards, the donor site was sutured with modified horizontal mattress sutures (Borghetti & Monnet-Corti 2000). The graft was then inserted under the CAMT at the sites of recession, and retracted laterally by sutures towards each end of the tunnel in the same manner as the original SET (Allen 1994b). After positioning the CTG laterally, the site was rinsed with saline solution to remove any clot. EMD was applied to the test sites only.

In all cases the flaps were maintained in a similar coronal position, slightly coronal to the cemento-enamel junction (CEJ), with suspended sutures around the contact points (Azzi & Etienne 1998). These horizontal mattress sutures will pull the flap coronally over the CTG if there is a trend for the CTG to be exposed, or they will move both the flap margin and the CTG coronally if there is a tendency for the graft to slide apically towards the mucogingival line.

Post-surgically, all patients were given analgesics (3 X 250 mg acidum nifluminicum) for 3-4 days and antibiotics (3 x 300 mg Dalacin-C) for 5 days in accordance with University regulations for implantable biological materials. Patients were informed not to brush their teeth in the operated areas until suture removal two weeks later. They were instructed to rinse their mouths with a 0.12% chlorhexidine solution twice a day for one minute for 3 weeks. Fifteen days after surgical treatment, all patients were checked and instructed in mechanical tooth cleaning of the operated areas using a soft toothbrush and a roll technique. All patients were recalled at 28 days, then at 3, 6 and 12 months for evaluation. Each time, clinical measurements were performed and subjects received one session of prophylaxis, including reinforcement of oral hygiene, supragingival debridement, and tooth polishing. The study was completed after 1 year.

The following clinical parameters were assessed at baseline, 6 months and 1 year postoperatively: Plaque Index (Löe 1967), Gingival Index (GI) (Löe & Silness 1963), probing depth (PD), gingival recession (REC) and clinical attachment level (CAL). Additionally, the width of the keratinized gingiva (KGW), measured as the distance from the mucogingival junction (MGJ) to the gingival margin, the width of the recession defect (RW) and the distance between the contact point and the top of the papilla at the mesial aspect of the tooth (DCP) were recorded. PD, REC, CAL, KGW measurements were made at the mid-buccal point of the teeth involved. The same blinded calibrated examiner (TK) undertook all the probing measurements using a Hu-Friedy periodontal probe. The CEJ was used as a reference point for these measurements, except in those cases where the CEJ was not visible; in which case, the margin of a restoration was used as a reference point.

At 28 days and at 3 months, only the measurements for REC and DCP were recorded. Any patient concerns regarding discomfort, tooth sensitivity or aesthetic appearance, or any other complaints during the study period were also recorded.

Figure 4.

Results of test and control procedures after treatment of multiple Miller's class III recessions: On the test group: (a) a radiograph showing inter-dental bone loss; (b) baseline view; (c) post-operative view: the modified tunnel/connective tissue graft is sutured after enamel matrix derivative (EMD) application and maintained in a coronally position by suspended sutures around the contact point; (d) clinical view at 28 days; (e) 3 months; (f) 6 months; (g) 1 year. On the control group: (h) a radiograph showing inter-dental bone loss; (i) baseline view; (j) post-operative view: sutures of the modified tunnel/connective tissue graft without EMD application; (k) at clinical view at 28 days; (l) 3 months; (m) 6 months; (n) 1 year.

3.2.3 Statistical methods

Sample size calculation

Using root coverage percentage as the primary outcome variable and assuming that the standard deviation of the differences in the paired measurements would not exceed 30%, the sample size for paired continuous data was calculated to be eighteen subjects per treatment group. This would provide 80% power to detect a true difference of 20% between test and control. To allow for possible dropouts, twenty patients were finally recruited.

Statistical analysis was performed using commercially available software. A subject-level analysis was performed for each parameter. Mean values and standard deviations (mean ± SD) for the clinical variables were calculated for each treatment. The method of Kolmogorov and Smirnov was used to confirm that the data were sampled from a Gaussian distribution. The significance of the difference within each group and between groups before and after treatment was evaluated with the paired samples t test. Ordinal data (PI, GI) were analyzed using the Wilcoxon matched-pairs test. Differences were considered statistically significant when the p-value was less than 0.05.

4. RESULTS

4.1 Treatment of multiple Miller's class I and II gingival defects with and without PRF

There were no statistically significant differences between the recession-type defects in the two groups at baseline. Figure 3. shows the clinical improvements achieved by both procedures.

All patients completed the study and expressed improvement as far as root sensitivity was concerned. Sloughing of the flap, without infection, occurred in one patient, resulting in a recession defect without any aesthetic complaint. Two patients were moderate smokers (<10 cigarettes/day) and they did not show any altered wound healing.

At one month, both treatments resulted in significant improvement in the percentage of root coverage. This amounted to 81.0 ± 16.6 % and 86.7 ± 16.6 % respectively for test and control groups (Table 1). The difference between the two groups was not statistically significant. At three months, there was a slight decrease of root coverage in the test group and the difference between the two groups became statistically significant (76.1 ± 17.7 % in the test group and 88.2 ± 16.9 % in the control group). At six months, when compared with the three months data, there was a statistically significant increase in root coverage in the control group (up to 91.5 ± 11.4 %). No statistically significant differences were observed at the test sites over the same time period. Therefore, at six months, the difference of root coverage between the two groups was statistically significant. The observed values were 80.7± 14.7 % and 91.5 ± 11.4 %, for test and control sites respectively (Table 1).

Full root coverage was achieved on 74.62% of the control sites compared with only 52.23% on the test sites (Table 2).

A threshold of 0.5 mm remaining recession defect may be considered to be a clinically satisfactory aesthetic outcome. This value represents a 17% lack of coverage (test sites) and 20% (control sites), when applied to the mean baseline values. In the present study, this value was obtained for 64.17% and 88.05% respectively of the test and control sites.

At patient level, at six months, the test procedure resulted in a lower percentage of root coverage than the control procedure for 15 patients. For these patients, the mean percent of root coverage was 74.1% ± 12.1% for the test side versus 92.4% ± 11.8% for the control side. Only two patients showed worse results with the control procedure. On four patients, 100% root coverage was obtained for both control and test procedures. Eleven patients showed 100% of root coverage in the control group whereas only four patients obtained this optimal result with the test procedure.

The best results were found at anterior maxillary sites where the control procedure resulted in 100% root coverage and test procedure resulted in 91.1% ± 18.8% root coverage (Table 1).

Worst results were obtained for maxillary molars with only 86.3% ± 17.6% root coverage for the control procedure and 70.9% ± 19.9% for the test procedure. These differences are statistically significant (Table 1).

A better and significant reduction of RW was achieved at 6 months with control treatment (66.2% ± 37.5% reduction for the test sites versus 82.4% ± 33% for the control sites) (Table 1).

Both procedures resulted in a significant CAL gain at 6 months from 4.23 ± 1.56 mm to 1.76 ± 0.97 mm and from 3.93 ± 1.43 mm to 1.37 ± 0.62 mm, for test and control group respectively (Table 3). Although there were no significant differences between the two groups at baseline, a statistically significant difference was found at 180 days.

We also observed a statistically significant decrease of PD in the two groups from baseline to 6 months. However, the difference between the two groups at six months was not statistically significant (Table 3).

A significant decrease of KGW was observed from baseline to six months in both groups. However, there were no statistically significant differences between the two groups, at baseline and at six months (Table 3).

A significant increase in GTH between baseline and 6 months was observed only in the test group (from 1.1 ± 0.4 mm to 1.4 ± 0.5 mm) (Table 3).

Finally, no significant difference in terms of root coverage was observed with a threshold of GTH ≤ 0.5 mm and ≤ 1 mm within the test and the control group.

The Kolmogorov-Smirnov test of baseline data showed a homogeneous distribution of the data ($P<0.05$).

No patient needed to be excluded from the study, nor had significant complications.

As far as root sensitivity was concerned, all patients expressed improvement.

Table 1.

	Test (mean ± SD)	Control (mean ± SD)	*P* Value
% of root coverage at 28 days	81.0 ± 16.6	86.7 ± 16.6	0.1189 [ns]
% of root coverage at 90 days	76.1 ± 17.7	88.2 ± 16.9	0.0173*
% of root coverage at 180 days	80.7 ± 14.7	91.5 ± 11.4	0.0039*
% of root coverage at 180 days for maxillary anterior teeth	91.1 ± 18.8	100	0.0474*
% of root coverage at 180 days for maxillary posterior teeth	70.9 ± 19.9	86.3 ± 17.6	0.0030*
% of recession width reduction at 180 days	66.2 ± 37.5	82.4 ± 33	0.0091*

ns No statistically significant difference * Statistically significant difference ($p<0.05$)

Table 2. Individual recession and root-coverage results

Treatment pair	n recessions / patient	Recessions in mm								% of root coverage at 180 days		Complete root coverage	
		Baseline		28 days		90 days		180 days					
	T/C	T	C	T	C	T	C	T	C	T	C	T	C
1	3/3	2.3	2.0	0.2	0.1	0.2	0.2	0.3	0.2	87	90	1/3	2/3
2	3/3	4.3	2.8	1.3	0.7	2	0.8	1.5	0.8	68.1	71.4	0/3	1/3
3	3/3	4.3	2.7	1.8	0.2	1.8	0	1.8	0.2	58.1	92.6	0/3	2/3
4	3/3	3.3	2.5	1	0	1.3	0	0.8	0	75.8	100	0/3	3/3
5	3/3	3.7	3.7	1	0.5	1.2	0.5	1	0.3	73	91.9	2/3	2/3
6	3/3	3.7	3.5	1.7	1.8	1.3	1.8	1.5	1.2	60.5	63.6	0/3	0/3
7	3/3	2.0	2.2	0.7	0.2	1.2	0	0.7	0.5	68.2	77.3	1/3	1/3
8	3/3	3.5	3.0	1.7	0.3	2	0.7	1.5	0	57.1	100	0/3	3/3
9	3/3	2.8	2.0	0	0	0	0	0	0	100	100	3/3	3/3
10	3/3	4.0	3.0	0.8	1	0.7	1.7	0.5	0.8	87.5	71.4	2/3	2/3
11	3/3	3.0	3.3	0.5	0.3	0.8	0.7	1	0	66.7	100	1/3	3/3
12	3/3	2.2	1.2	0	0	0.3	0	0	0	100	100	3/3	3/3
13	3/3	3.0	2.7	0.3	0	0.2	0	0	0	100	100	3/3	3/3
14	3/3	1.8	1.5	0	0	0.2	0	0	0	100	100	3/3	3/3
15	3/3	1.7	1.3	0.7	0.7	0.3	0.3	0.2	0.2	88.2	84.6	2/3	1/3
16	3/3	5.0	4.0	0.5	1.2	0.7	0.3	0.3	0.2	94	95	2/3	2/3
17	3/3	2.0	1.7	0.2	0.2	0.2	0	0.3	0	85	100	2/3	3/3
18	3/3	2.5	3.8	0.2	0.8	0.8	0.1	0.3	0.8	88.9	78.9	1/3	0/3
19	4/4	1.5	2.0	0	0	0	0	0.4	0	75	100	2/4	4/4
20	5/5	2.7	2.8	0.4	0	0.6	0.3	0.5	0	81.5	100	4/5	5/5
21	4/4	1.0	1.0	0	0	0.1	0	0.1	0	90	100	3/4	4/4
Mean		2.9	2.5	0.6	0.4	0.8	0.4	0.6	0.2	80.7	91.5		
± SD		1.1	0.9	0.6	0.5	0.6	0.5	0.6	0.4	14.7	11.4		
% of recession with 100% root coverage at 180 days												52.23%	74.62%

T= Test sites C= Control sites

T=test sites; C= control sites

Table 3. Mean ± SD of PD, CAL, Height of keratinized gingival, and Tissue Thickness (mm) of the operated
Sites at baseline and 6 months postoperatively

	Test (mean ± SD)	Control (mean ± SD)	*p* Value
PD (mm)			
Baseline	1.41 ± 0.65	1.44 ± 0.6	0.6725^{ns}
6 months	1.17 ± 0.41 (*p* value 0.0103*)	1.14 ± 0.34 (*p* value 0.0003*)	0.5593^{ns}
CAL (mm)			
Baseline	4.23 ± 1.56	3.93 ± 1.43	0.0628^{ns}
6 months	1.76 ± 0.97 (*p* value < 0.0001*)	1.37 ± 0.62 (*p* value < 0.0001*)	0.0004*
KGW (mm)			
Baseline	2.78 ± 1.08	2.85 ± 1.23	0.5760^{ns}
6 months	2.54 ± 0.85 (*p* value 0.0299*)	2.37 ± 0.89 (*p* value 0.0013*)	0.1446^{ns}
GTH (mm)			
Baseline	1.1 ± 0.4	1.1 ± 0.3	0.7653^{ns}
6 months	1.4 ± 0.5 (*p* value 0.01222*)	1.1 ± 0.3 1.2 (*p* value 0.5774 ^{ns})	0.0036*

ns No statistically significant difference *** Statistically significant difference (*p* <0.05)**

4.2 Treatment of multiple Miller's class III gingival recession type defects

Ten patients had defects in the maxillary arch and the same number had defects in the mandibular arch. Ten subjects had sites involving only anterior teeth (5 on the maxilla and 5 on the mandible). Seven subjects had sites that also involved bicuspids, (2 in the maxilla and 5 in the mandible) and three subjects had sites involving maxillary bicuspids and molars.

The values of the clinical parameters at baseline, six months and at one year are shown in table 4. No statistical difference was observed within and between groups for PI, GI, PD or KGW values between baseline, 6-month and I-year measurements.

Both treatment groups showed significant post-surgical improvement in the coverage of gingival recession and CAL gain, when compared with baseline.

At the subject level, for the test sites, the mean recession depth decreased significantly from 3.5 ± 1.5 mm (baseline) to 0.6 ± 0.9 mm (28 days) and to 0.8 ± 1.1 mm (1 year), with slight variations for measurements at the other time intervals. The corresponding results for the control sites were; 3.2 ± 1.4 mm, 0.6 ± 0.8 mm, and 0.6 ± 0.9 mm (Fig.5). Also, both treatments resulted in a significant CAL gain (3.11 mm and 2.86 mm for test and control groups respectively). REC coverage and CAL gain were not significantly different between the two groups. Statistically significant decreases in RW and DCP measurements were observed between the baseline, the 6-month and 1-year data, but these results were not statistically different between the two treatment groups (Table 4).

When the results were expressed as a percentage of root coverage at 1 year, both treatments resulted in a root coverage of 82% and 83% for test and control groups respectively (Table 5). After one year, the gain in the vertical height of the papilla (as measured by the reduction of the DCP distance), when expressed as a percentage, was 58.6% and 59.2% for test and control groups respectively. Mean mesial and distal probing were respectively 1.9±0.7mm, 2.0±0.7mm at baseline for the test group, 2.0±0.6mm, 2.1±0.6mm for the control group. These values were not statistically different between groups and no significant differences were also found at one year between groups and baseline measurements (table 6).

At 28 days, complete root coverage (100%) was observed in 8 (38%) and 7 (33%) of the test and control group surgeries respectively. At the 1-year assessment, complete root coverage was observed in 8 of the surgeries in each of the two groups. Eight of the surgeries in each group resulted in coverage in the 99%-75% range at 28 days, but only 6 at one year for the test group. The distribution of surgeries according to the percentage of root coverage is shown in Table 7. Among those patients with 100% root coverage on the control side at I year, 5 showed similar results on the test side.

Figure 5. *Mean change (± SD)in recession from baseline to 28, 90, 180, 365 days, The data for each group were calculated at the patient level. There was no statistical difference between groups when evaluated with the paired sample t-test*

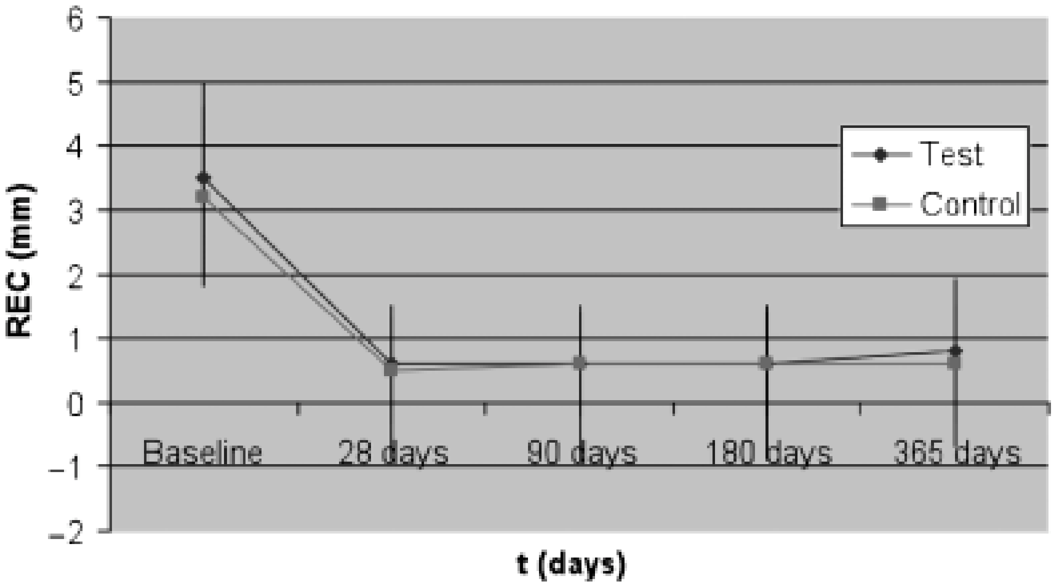


Table 4. *Mean and SD of all evaluated parameters in the operated patients at baseline, 6 and 12 months postoperatively. Plaque index (PlI), gingival index (GI), recession defects (REC), clinical attachment level (CAL), pocket depth (PD), width of keratinized gingival (KGW), width of recession defect (RW), distance the contact point and the top of papilla at the mesial aspect of the tooth (DCP).*

PlI (1-3)	Baseline	6 months postoperatively	1 year postoperatively	P value
Test (n=20)	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.3	>0.999
Control (n=20)	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	>0.999
P value	>0.999	>0.999	>0.999	

GI (1-3)	Baseline	6 months postoperatively	1 year postoperatively	P value
Test (n=20)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.2	>0.999
Control (n=20)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	>0.999
P value	>0.999	>0.999	>0.999	

REC (mm)	Baseline	6 months postoperatively	1 year postoperatively	P value
Test (n=20)	3.5 ± 1.5	0.6 ± 0.9	0.8 ± 1.1	<0.001
Control (n=20)	3.2 ± 1.4	0.6 ± 0.8	0.6 ± 0.9	<0.001
P value	0.51	>0.999	>0.999	

CAL (mm)	Baseline	6 months postoperatively	1 year postoperatively	P value
Test (n=20)	4.8 ± 1.9	1.7 ± 1.0	1.9 ± 1.1	<0.001
Control (n=20)	4.7 ± 1.7	1.8 ± 0.9	1.9 ± 1.0	<0.001
P value	0.858	0.735	>0.999	

PD (mm)	Baseline	6 months postoperatively	1 year postoperatively	P value
Test (n=20)	1.4 ± 0.7	1.1 ± 0.4	1.2 ± 0.4	0.264
Control (n=20)	1.5 ± 0.7	1.2 ± 0.5	1.3 ± 0.5	0.294
P value	0.645	0.478	0.478	

KGW (mm)	Baseline	6 months postoperatively	1 year postoperatively	P value
Test (n=20)	2.5 ± 1.4	2.7 ± 1.0	2.6 ± 1.2	0.805
Control (n=20)	2.6 ± 1.3	2.8 ± 1.1	2.7 ± 1.2	0.797
P value	0.812	0.759	0.788	

RW (mm)	Baseline	6 months postoperatively	1 year postoperatively	P value
Test (n=20)	3.8 ± 1.5	1.2 ± 1.7	1.3 ± 1.7	<0.001
Control (n=20)	3.6 ± 1.4	1.3 ± 1.6	1.3 ± 1.8	<0.001
P value	0.657	0.845	>0.999	

DCP (mm)	Baseline	6 months postoperatively	1 year postoperatively	P value
Test (n=20)	2.9 ± 1.4	1.7 ± 1.5	1.7 ± 1.2	0.004
Control (n=20)	2.7 ± 1.3	1.6 ± 1.3	1.6 ± 1.2	0.007
P value	0.634	0.819	0.788	

Table 5. Mean and SD of the root coverage percentage and complete root coverage in the operated patients at 12 months postoperatively.

	Root coverage (%)	Complete root coverage
Test (n=20)	82 ± 25	8/20
Control (n=20)	83 ± 26	8/20
P value	0.90	1.0

Table 6. Mean and SD of mesial and distal probing depth (PD) at baseline and in the operated patients at 12 months postoperatively.

PD mesial

	baseline	1 year post op	P value
Test (n=20)	1.9 ± 0.7	1.8 ± 0.7	0.101
Control (n=20)	2.0 ± 0.6	2.1 ± 0.6	0.497
P value	0.264	0.064	

PD distal

	baseline	1 year post op	P value
Test (n=20)	2.0 ± 0.7	1.9 ± 0.6	0.121
Control (n=20)	2.1 ± 0.6	1.9 ± 0.6	0.071
P value	0.556	0.655	

Table 7. Frequency of root coverage between the groups after 28 days and 1 Year postoperatively.

Mean root coverage per surgery	100%		99 – 75%		74 – 50%		49 – 0%	
Time of evaluation	28 days	-1Year	28 days	- 1Year	28 days	- 1Year	28 days	- 1Year
Test Group (n=20)	8	8	8	6	2	4	2	2
Control Group (n=20)	7	8	8	8	4	3	1	1

5. DISCUSSION

5.1 Treatment of multiple Miller's class I and II gingival defects with and without PRF

Treatment of multiple gingival recessions may be a concern with patients with a high lip smile and the surgical challenge increase from the treatment of Miller Class I and II recession defects, to the treatment of class III and IV with interproximal bone loss. Suspended sutures around the contact point have been selected in our study to allow comparison with the study on class III recessions defects. One of the objectives of these suspended sutures was to obtain a stabilisation of the flap margin at the CEJ during the first two weeks of wound healing. In this study two patients were smokers (<10 cigarettes/day) and since they showed an uneventful wound healing we have not considered the impact of cigarette smoking on root coverage stability (Silva et al. 2007).

A six months post-operative measurement period is sufficient to evaluate the gingival margin stability after a coronally advanced flap (Cheng et al. 2007) and in the present study a mean 92,22% root coverage was obtained on the control site, which may be compared to the 1 year mean root coverage of 97.1%, since we don't have six months data, with a CAF and sling sutures (Zucchelli & De Sanctis 2000). Complete root coverage up to the CEJ of 88% was obtained at one year on maxillary recessions (Zucchelli & De Sanctis 2000), against 74.62% in our study at six months. In the reference study recession defects were exclusively on the maxilla, from tooth 15 to 25, while we have included maxillary recessions (from 1st right molar to 1st upper left molar) and mandibular recessions (from 2nd right molar to 2nd left molar). Other variables can be pointed out, our mean recession number of treated teeth was 3.19 (range 3 to 5) against 3.4 (range 2 to 5) and only 38% of recessions in our control group had recessions ≥ 3 mm against 91%.

Using the control procedure, if mandibular recessions and maxillary first molars are excluded, the remaining 6 patients will show mean root coverage of 100% on 20 recessions defects. The five patients with recession defects on mandibular teeth showed instead mean root coverage of 91.42% on 17 recessions. Mean root coverage for maxillary molars was 86 % on 27 recessions. These reduced root coverage have been reported on molars and for mandibular teeth (Chambrone & Chambrone 2006) for multiple recessions defects after a CAF-CTG combination treatment. The study design does not allow a direct comparison between sling sutures and suspended sutures to promote full coverage but improved results with non-molars maxillary teeth and suspended sutures may be due to a more coronal stabilization of the flap margin, during initial wound healing.

The aim of the present study was to evaluate a plastic periodontal surgery procedure with or without the adjunction of a PRF membrane and to our knowledge, this is the first randomized split mouth controlled study on this topic. Considering the beneficial aspects induced by the

PRF membranes and claimed for bone regeneration / soft tissue healing in implantology, we could have expected an improvement of root coverage, but the adjunction of a PRF membrane to a CAF has not improved root coverage results at 6 months. A statistical significant difference was in favour of the control group due to an increase in root coverage percentage between 3 and 6 months (87.15% and 92.22% respectively), which was not observed in the test group. It seems that, for this time period, interposing a 0.5 mm width PRF membrane, between an avascular surface and the flap, is not beneficial for improved root coverage and for the survival of the marginal gingiva. A similar detrimental effect of the addition of a platelet derivate in a CAF-PRP combination was not observed at six months when compared to CAF alone (Huang et al. 2005) or with a CAF-CTG-PRP combination when compared to a CAF-CTG alone (Keceli et al. 2008). On both test and control sites of the present study a careful release of flap tension has been performed and reduced root coverage in the test group may be due to differences in biological properties between PRP and PRF, or to the physical status (gel like versus membrane like) between the two biological products.

Controversies arise then with the promoters of the technique. They are claiming a greater efficiency in a rapid wound Healing and gingival biotype changes due to a better conservation of growth factors after centrifugation and the use of several layers of PRF « membranes ». These controversies are discussed in a reply and are shown in annex 1 (Aroca & Etienne 2009)

One parameter of importance to consider when covering a graft is to appreciate flap thickness. The initial thickness of the flap and the type of dissection will alter more or less the connective tissue microcirculation and the interposition of PRF may limit the collateral circulation which is essential for a thin flap to revascularize and heal (Hwang & Wang 2006). If sites having an initial GTH threshold ≤ 0.5 mm are compared to those > 0.5 mm, the mean root coverage is 76.5%±33.4% and 81.6%±22.6% for the test group versus 97.1%±7.5% and 92.0±16.8% for the control group. By increasing the thresholds to ≤ 1 mm and > 1 mm (Anderegg et al. 1995; Allen & Miller 1989), we obtain a root coverage of 81.8%±26.5% and 78.1%±19.9% for the test group versus 92.8%±16.1 and 92.0%±14.7% for the control group. The importance of soft tissue thickness for root coverage with CAF has been stressed in systematic review (Cheng et al.2007; Hwang & Wang 2006) on single recessions but limited information is available for multiple recessions (Paolantonio 2002). In the present study the different threshold of gingival thickness are not associated with any significant difference of root coverage within each group which is in contradiction with other investigators (Baldi et al. 1999) who find a mean root coverage of 64,3% for 7 recessions with flap thickness ≤ 0.5 mm and a full coverage obtained only with flap thickness > 0.8 mm. These differences may be due to the use of two releasing incisions in the quoted study, for the treatment of at least 2 mm recession defects and measurements were performed at three months. In the present study an envelope flap was used and in spite of avascular root surfaces of at least three recessions we have obtained a full coverage of 6 recessions of the initial 7 recessions associated with marginal gingival thickness ≤ 0.5 mm (85,7%) on the control site, and a full coverage of 7 recessions of the 12 initial recessions associated with marginal thickness ≤ 0.5 mm (58%) for the test sites.

There was a clear trend towards an increased thickness of the gingival margin of the test site and at 6 months this difference was statistically significant. The clinical benefit of an enlargement is still controversial (Wennström & Zuchelli 1996), a thin gingival biotype increases the likehood of gingival recession (Baker et al. 1998; Müller et al.1998), is more prone to a deleterious effect of a hard tooth brushing technique (Müller et al. 1998) and thick gingival tissue is associated with more predictable surgical results (Borghetti & Gardella 1990; Huang et al. 2005; Müller et 1998), resists to trauma and promotes creeping attachment. However, even if thick tissue seems to improve clinical results a systematic review failed to establish conclusively a minimum thickness requirement (Hwang & Wang 2006). The absolute mean gain in GTH for the test group in the present study is limited (0.21 mm) and cannot be positively compared with a mean GTH gain ≥ 1.22 mm after a CAF-CTG combination (Paolantonio 2002, Joly et al. 2007) but the protocol of measurement differs. In the present study we have a constant evaluation of thickness at 3 mm below the gingival margin and the compared studies have measurements at the middle of the apico-coronal width of keratinized tissue which represent a mean distance around 2 mm from the gingival margin at 6 months. Future studies are needed to evaluate if the GTH gain of 37 % at 6 months for test group in our study is of clinical value and/or is associated with an improved esthetic outcome without soft tissue enlargement as observed with the bilaminar technique. The soft tissue increase in the present study may be the result of a gingival and periodontal ligament fibroblasts proliferation due to the influence of growth factors from PRF or to a spacing effect below the gingival margin of the PRF membrane.

Surprisingly we have not observed any gain of keratinized gingiva in the test and control group on the contrary to studies with CAF alone (Silva et al. 2007), CAF-PRP combination (Huang et al. 2005) or to a CAF-platelet concentrated grafts (Cheung & Griffin 2004). However, the 6-month time frame adopted in our study may not be appropriate to observe a significant creeping attachment when a PRF membrane is interposed under the flap, because the length of time for this observation may vary among mucogingival techniques (Matter 1980; Harris 1997). Both treatments resulted in a statistically significant gain of attachment and decrease in Pds. However, the only statistically significant difference between the two groups was the change in CAL at 6 months (Table 3).

The PRF positioning at the CEJ may also favour initial root exposure, which has been reported in 53% of single recessions treated with a bilaminar surgical technique (Zuchelli et al. 2003). The lack of benefit of PRF in the test site of the present study do not rule out the interest of PRF in mucogingival surgery for denuded root surfaces. Surgical variables which may affect the final result are PRF consistency, relationship with a CEJ positioning, platelet concentration (Marx et al. 1998).

The present study design allows an evaluation of results with a patient centered outcome. Percentages of root coverage may not reflect patient satisfaction and our questionnaire was not sufficient to discriminate patient aesthetic outcome. In the present study only 52.3% patient showed at six months 100% root coverage in the control group and 19% in the test group. It may be more relevant at a patient level to express patient satisfaction by the percentage of patients with recessions ≤ 0.5 mm and not by the percentage of patients with 100% root coverage. This distance is the discriminating value in our probing measurements and can be considered as our minimal error of observance. With this approach the percentage of patient with satisfactory surgeries will be 38% and 71,4% respectively, for test and control group.

5.2 Treatment of multiple Miller's class III gingival recession type defetcs

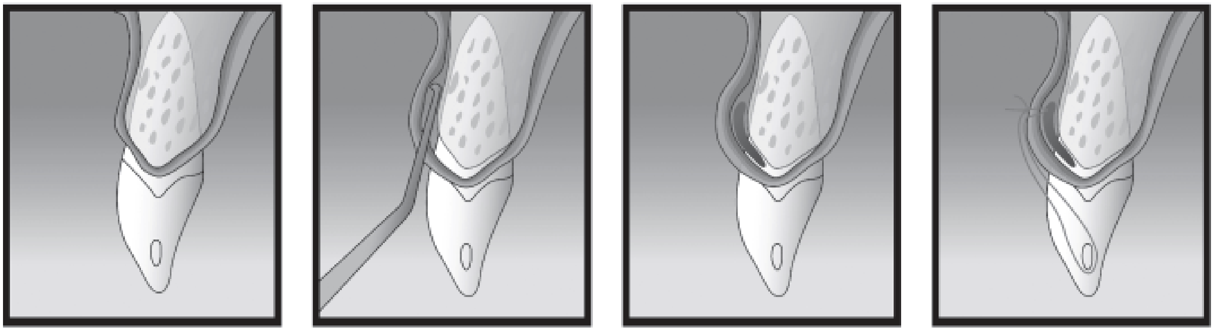
This RCT study is challenging for two clinical reasons. To our knowledge, no RCT study has been published previously that involves Miller's class III recession defects exclusively. Also, the fact that we are dealing with multiple bilateral recessions increases the surgical difficulty and the risk of failure when compared with monolateral and single recessions (Clauser et al. 2003).

Altogether 139 recessions were treated, 69 in the test group and 70 in the control group. We chose a patient-level analysis, instead of selecting the deepest recession from a surgical site. This approach may be more clinically relevant, because it will allow an appreciation of clinical outcome by surgery.

When needed, bicuspids and molars have been included in the surgical field. These posterior sites may be of concern for patients because of root sensitivity or, in the case of patients with a high-lip smile line, because of aesthetics. There were two sites with molar involvement in the test group and three in the control group. For bicuspids, the numbers were 17 and 12, respectively. The small number of molars in our study is likely to have had little effect on the overall data, but the inclusion of bicuspids will include sites with wider bucco-lingual inter-proximal bone morphology, than is the case with the cuspid-to-cuspid sites. In our study, the patient mean percentage of root coverage for the two groups is at least of 82%. This compares favourably with the 85.7% (at a patient level), obtained in the SET original paper for multiple Miller's class I and II recession defects (Allen 1994). This figure has been extrapolated from his data on seven patients who had recessions at two to five sites. Individual data were also obtained in a case series that compared results following treatment by three surgeons of various clinical experience. After 6 months and each surgeon using the same technique, root coverage was 80%, 85% and 89% (Georges et al. 2009).

The CAMT technique is quite predictable for root coverage of class III recession defects and, in many cases, provides full-root coverage. The argument that success may be obtained only occasionally (Miller 1983, Wennström 1996),can be challenged, because a similar predictability to the treatment of class I and II recessions by MARTD has been obtained. This result may be due to a number of factors. Firstly, in our technique (Fig. 6), the muco-periosteal tunnelized envelope flap was carefully released beyond the muco-gingival line and the collagen bundles, preventing the flap from being moved coronally, were separated by curettes to obtain an effect similar to that of a horizontal releasing incision in the advanced flap technique.

Figure 6. Diagram of the critical surgical steps **:**



a b c d

a) Initial gingival recession - mucogingival line (arrow). b. Gingival pouch and tunnel is dissected beyond the muco-gingival line and the collagen bundles are separated by curettes beneath the elevated flap. Papilla are then released. c. The connective tissue graft is placed slightly beneath the cemento-enamel junction and its cervical position is determined by the sutures at each end of the graft. d.The flap submerge completely the connective tissue graft and is maintained in a coronal position by sutures around the contact point. These sutures may or may not go through the graft, depending with the need for a coronal or interproximal displacement of the connective tissue graft.

Secondly, in order to promote revascularization of the graft, the CTG was completely submerged (Guiha et al. 2001). Stabilization of the CTG beneath the CEJ was provided, when needed, by mattress sutures suspended around the contact point. Thirdly, the papillae were carefully released from the underlying bone. Fourthly, the use of suspended sutures around the contact point provides good coronal stabilization of the flap during the first 2 weeks of wound healing. The beneficial effect of these sutures has also been found for class I and II recessions after the coronally positioned envelope flap procedure (Aroca et al. 2009) and an increased complete root coverage was found when advancing the flap over the CEJ of LGR (Pini Prato et al. 2005).

Chemical root conditioning was used in our study on the test sites before tunnel preparation mainly to avoid the presence of chemical beneath the papilla and the flap margin of the CAMT. In our protocol, only the exposed root surfaces of the test teeth are conditioned, whereas the root surfaces beneath the gingival margins and papillae are rinsed with saline solution only, as for the control sites. The stated clinical benefit of the EDTA protocol, to remove the smear layer to expose the collagen fibers of the cementum and to allow precipitation of EMD on a root surface free of organic elements (Blomlof et al. 1997), has been challenged by two systematic reviews, which conclude that the clinical outcome does not depend on the use of root conditioning (Mariotti 2003, Cheng et al. 2007). However, in a systematic review on the use of CAF for localized recessions, CAF alone and CAF with chemical root conditioning (EDTA) were considered unpredictable for root coverage when compared to CAF + EMD + EDTA (Cheng et al. 2007).

The two groups show a statistical mean DCP gain when compared with baseline. However, there is a discrepancy between the mean root coverage and papillary gain. The significance of anatomical features of the papillae, such as soft tissue thickness, alveolar bone height and their relationship with root coverage could not be determined for class I and II recessions (Berlucchi et al. 2005) and further studies are needed for cases that involve inter-proximal soft tissue and/or bone loss.

This failure to obtain a papillary gain similar to the amount of root coverage may be due to lack of inter-dental support with a CTG flat strip and/or regeneration. With localized papillary defects, a dense subepithelial CTG with a pyramidal shape from the tuberosity (Azzi et al. 1999, Nordland et al. 2008) will help to support the papilla, but with MARTD and a palatal donor site, we cannot customize the graft for each inter-proximal space. The subepithelial CTG is beneficial for gingival margin stabilization, but inter-proximally, after an initial swelling of the soft tissue, which sometimes fills the inter-dental space, there is a shrinkage of soft tissue during wound healing. This effect is more pronounced for class IV recession defects that have a wider distance between the crestal inter-proximal bone and the contact point. It has been reported that with a localized and wide inter-proximal space, the use of a bone graft provided some papillary gain, but further papilla enhancement was made possible only by reduction of the inter-dental tri-dimensional space with veneers (Azzi et al. 2001).

There is a nearly threefold reduction of RW for both the test and control groups (Table 4). This result is obtained in spite of a lack of inter-proximal bone support, and may be due to the support of the gingival margin and papilla provided by the subepithelial CTG, which may be slightly stretched in the inter-proximal spaces by the suspended sutures.

Interestingly, the combined use of CTG with EMD is not associated with any improved gain in root coverage or reduction of DCP. This lack of beneficial effect of EMD with a CAMT technique on root coverage after 1 year may be linked to the high efficacy of the subepithelial CTG (Chambrone et al. 2008) when compared with EMD efficacy. It is also possible that in some specific clinical or anatomical situations, we lack discriminating tools for the evaluation of early signs of regeneration. The need for a longer period of observation has been claimed for EMD treatment on LGR (Pilloni et al. 2006). When LGR were treated with CAF– EMD, versus CAF alone, there were no significant differences between the groups (Hagewald et al. 2002). But after 2 years, complete root coverage was maintained in 53% of the test sites, compared with 23% in the control group (Spahr et al. 2005). This improved stability with EMD treatment over time is not apparent within the time frame of our study. When complete root coverage was considered to be the primary outcome variable in a systematic review on CAF, single combination and LGR, definitive conclusion cannot be drawn on the advantage of EMD versus CTG and there is a need for RCTs with high power (Cairo et al. 2008). Where complete root coverage was obtained in the test group, this had been achieved by 28 days. In the control group, one case showed a creeping attachment. Where the percentage root coverage was in the range 99–75%, the control group surgeries remain stable, while in the test group, two surgeries showed a decrease in root coverage over time.

Clinically, the limited PD (Table 4) observed in the two groups may reflect the beneficial effect of the subepithelial CTG adhesion, which has been described previously (Bruno & Bowers 2000). The subepithelial CTG–EMD combination may not yield such predictable results in promoting periodontal regeneration, or its potential to inhibit the development of a long junctional epithelium (Carnio et al. 2002) cannot be evaluated clinically.

There was no significant KGW increase between the two groups or when compared with baseline. An increase of 1.52 +/- 1.05 mm was reported in a systematic review on LGR with subepithelial CTG (Oates et al. 2003), with a greater increase when the graft was left uncovered (Ouhayoun et al. 1988, Bouchard et al. 1994, Han et al. 2008). This change in KGW may be technique dependent (Cairo et al. 2008). A smaller KGW increase was found with a CAF when compared with an envelope flap (Cordioli et al. 2001). With MARTD and a tunnel technique leaving the CT graft exposed, a KGW increased was found after 3 years (Ribeiro et al. 2008, case report).

For LGR Miller's class I and II, a KGW increase was statistically significant at 18 months for a CAF–EMD group (Pilloni et al. 2006). It was more pronounced in a CAF–EMD group when the subepithelial CTG was left exposed. In our study, the mean KGW at baseline was 2.5 and 2.6 mm for the test and control group, respectively, and the mean values at patient level were comparable during the observation period. This lack of effect may be due to the spatial configuration of the subepithelial CTG with the CAMT technique and its suspended sutures during a 2-week period, which may change the inductive signals from the CTG on epithelial differentiation during the early period of wound healing (Karring et al. 1975, Ouhayoun et al. 1988).

A 1-year period of evaluation may be sufficient to evaluate the efficacy of a CAMT technique for the surgical treatment of class III gingival recessions, but a longer period of evaluation is probably necessary to assess whether these initial positive results are modified with time. Traumatic toothbrushing habits may cause interference on an immature or thin gingival margin, and the time frame may be too short to evaluate the biologic potential of the subepithelial CTG and EMD for inter-proximal hard and soft tissue regeneration or the quality of attachment.

6. CONCLUSION

These studies have shown that root coverage is predictable as well for class I, II and III Miller's recession defects. For class I and II a modified CAF was used and we can guess after our results with Class III, that a MCAT will give at least similar results.
The RCT of CAF with or without PRF has shown at six months a significant difference in gingival thickness. This increase may not be clinically significant, but this group of patient is still followed in the department of Periodontology of Semmelweis University Faculty of Dentistry and long-term evaluation at 2 years may show complementary informations. The publication of this paper rise some controversies with the promoters of the techniques, due probably to an evolution of the technique of sampling, conservation and clinical management. The relative importance of these factors need however to be tested. Surprisingly with the addition of suspended sutures to the original CAF design, we have no increased in KGW. A similar effect was observed with MCAT, at one year in spite of a recude amount of mean attached gingiva at baseline (1.1 mm), we had no significant creeping attachment when compared to the data of the literature for CAF.

The study on Class III recession defects, has shown a similar predictability than for the treatment of Class I and II recessions. This new information shows also that with these defects, the CTG combination has a similar positive effect than the EMD-CTG combination, at one year. Again the length of the observation period may be an important parameter to evaluate in particular for the effect on periodontal ligament and or connective tissue of gingival papilla.

MARTD is a good model to evaluate surgical protocols aiming at regeneration with stimulatory wound healing agents, growth factors or tissue engineering. The complexity remains however, since it may be necessary to adapt our surgical techniques to these modulating agents, which have various physical and chemical configurations.

7. SUMMARY

1. Complete root coverage of Miller's class I and II multiple recessions with a CAF can be predictable.
2. Combination with PRF of an advanced coronally flap is associated with a limited increased thickness of the gingival margin at 6 months.
3. Adjunction of PRF shows some change in gingival biotype, but the clinical relevance need to be further investigated.
4. Coverage of class III multiple recession type defects is predictable with a coronally advanced modified tunnel and CT graft at 1 year.
5. Combination with EMD in class III recession type defects is not associated with a clinical improvement at 1 year.
6. Complete root coverage is also frequently obtained with class III… but papilla gain is limited.

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