

EFFECT OF AN ENAMEL MATRIX DERIVATIVE ON WOUND HEALING FOLLOWING GINGIVAL RECESSION COVERAGE: A RANDOMIZED, CONTROLLED, CLINICAL STUDY

Effetto dell'amelogenina sulla guarigione dei tessuti molli dopo copertura di recessioni gengivali: lavoro pilota randomizzato e controllato

Jean-Claude Imber*, Alexandra Stähli*, Elena Raptis, Giovanni E. Salvi, Sigrun Eick, Anton Sculean

med. dent. Jean-Claude Imber, Department of Periodontology, University of Bern, Switzerland

Dr. med. dent. Alexandra Stähli, Department of Oral Biology, Medical University of Vienna, Austria

med. dent. Elena Raptis, Department of Periodontology, University of Bern, Switzerland

Prof. Dr. med. dent. Giovanni E. Salvi, Professor and Vice Chairman, Department of Periodontology, University of Bern, Switzerland

Prof. Dr. med. dent. Sigrun Eick, Laboratory of Oral Microbiology, Department of Periodontology, University of Bern, Switzerland

Prof. Dr. med. dent. Anton Sculean, Professor and Chair, Department of Periodontology, University of Bern, Switzerland

**these authors contributed equally*

Running head: Gingival Recession Coverage and Emdogain

Key words: gingival recession, enamel matrix derivative, mucogingival surgery

Abstract

Aim: The aim of this randomized, controlled, single blinded clinical study was to investigate clinically and immunologically the potential effects of Emdogain on early wound healing and clinical results following treatment of gingival recessions.

Materials and Methods: A total of 40 healthy patients with Miller class I to III single or multiple gingival recessions were treated with the modified coronally advanced tunnel technique (MCAT) + subepithelial connective tissue (CTG) with or without Emdogain. Patients were consecutively enrolled and randomly assigned to Emdogain or control. Inflammatory markers and were measured at baseline, 2 days, and 1 week postoperatively. Early wound healing was expressed by a newly described Recession Healing Index (RHI). Clinical parameters were assessed at baseline, 2 weeks and 6 months postoperatively. Patient-reported outcomes were analyzed with a visual analogue scale.

Results: No statistically significant differences were detected between the 2 groups in terms of RHI, inflammatory markers and patient-reported outcomes during early wound healing. At six months following treatment, mean root coverage amounted to 78% for the test and 77% for the control group, respectively, without any statistically significant differences.

Conclusion: Within their limits, the present data have failed to show an influence of EMD on clinical and immunological parameters related to wound healing following recession coverage surgery using MCAT and CTG.

Riassunto

Scopo: Lo scopo di questo lavoro randomizzato e controllato fu di verificare se l'uso di amelogenina (Emdogain, EMD) in aggiunta alla tecnica di tunnel modificata (TM) con innesto di connettivo (TC) abbia degli effetti sulla guarigione dopo copertura di recessioni gengivali.

Materiale e metodi: In 40 pazienti con recessioni gengivali di classe Miller I-III, la tecnica di TM con innesto di TC fu utilizzata con o senza l'aggiunta di EMD. I pazienti furono arruolati e randomizzati nel gruppo test (EMD) oppure controllo (senza EMD). Marcatori infiammatori furono prelevati dal solco gengivale al giorno 0 (chirurgia) e dopo 2 e 7 giorni. I parametri clinici furono analizzati 2 settimane e 6 mesi dopo l'intervento. Un nuovo indice di guarigione dopo copertura di recessioni gengivali fu introdotto (Recession Healing Index, RHI). I parametri riportati dai pazienti furono analizzati tramite scala visuale analoga fino a 6 mesi.

Risultati: Dopo 6 mesi, la copertura media delle recessioni gengivali ammontava a 78% nel gruppo test e a 77% nel gruppo controllo ($p>0.05$). Nessuna differenza significativa ($p>0.05$) fu trovata nei marcatori infiammatori durante la guarigione tra gruppo test e gruppo controllo. L'analisi del RHI non rivelò nessuna differenza significativa tra i due gruppi.

Conclusione: I risultati di questo lavoro pilota indicano che l'aggiunta di EMD non influenza positivamente i parametri clinici ed infiammatori durante la guarigione dopo copertura di recessioni gengivali.

Introduction

Gingival recession is the exposure of the root surface due to displacement of the gingival margin apical to the cemento-enamel junction and can affect the labial, lingual and/or interproximal areas (Wennstrom, 1996). This root exposure is

frequently associated with "wedge-shaped" defects at the crevicular area (Sangnes and Gjermo, 1976), aesthetic impairment, predilection to root caries, root hypersensitivity, and difficulties to achieve optimal plaque control (Susin et al., 2004), (Daprile et al., 2007), (Serino et al., 1994), (Lovegrove and Leichter, 2004), (Allen and Miller, 1989).

Single and multiple gingival recessions can be successfully treated by means of coronally advanced flap (CAF) or the modified coronally advanced tunnel (MCAT) in combination with subepithelial connective tissue grafts (CTG) (Aroca et al., 2013, Cairo et al., 2014, Graziani et al. 2014, Sculean et al., 2014, 2016)) while recent evidence indicates that both techniques can lead to comparable outcomes (Azaripour et al., 2016).

An enamel matrix derivative (EMD) has been shown to promote periodontal regeneration by mimicking the embryonic development of the periodontal tissues (Hammarstrom, 1997), (Gestrelus et al., 2000), (Bosshardt, 2008). Clinically, EMD is used for periodontal regeneration at teeth affected by periodontitis (i.e., 2- or 3-wall intrabony defects, Class II

furcation defect), root coverage procedures, and tooth replantation (Miron et al., 2016). Histologically, the additional use of EMD with CTG in root coverage procedures results primarily in connective tissue adhesion to the root surface and a short junctional epithelium (Miron et al., 2016). Interestingly, a plethora of clinical observations and data from experimental studies have indicated that the application of EMD in conjunction with flap surgery may result in an accelerated wound healing and less inflammation compared to placebo treated sites thus pointing to its clinical relevance in modulating early wound healing (Okuda et al., 2001, Miron et al., 2016).

However, according to the best of our knowledge, until now, no randomized controlled study has evaluated the potential effects of EMD following recession coverage with CTG focusing on clinical and immunological parameters related to wound healing.

The aim of this prospective, randomized, controlled, clinical study was therefore, to characterize clinically and immunologically the early wound healing events and clinical outcomes following treatment of Miller Class I, II or III recessions by means of MCAT with and without application of EMD.

Materials and Methods

Study population

Forty patients with single or multiple Miller Class I, II or III (Miller 1985) gingival recessions were enrolled in this study. All the included patient signed an informed consent. This study protocol was in accordance with the moral, ethical and scientific principles governing clinical research as set out in the current version of the Declaration of Helsinki. Exclusion criteria comprised age < 16 years, plaque score over 25% (O'Leary 1972), history of chronic infectious or inflammatory diseases (i.e. rheumatoid arthritis, systemic lupus erythematoses, Crohn's disease, or HIV-, HCV- infection etc.), any clinical signs of an acute infection, renal failure (GFR < 30ml/min), current smoking (> 5 cigarettes per day).

Study Design

This prospective, randomized, single blinded clinical trial (Trial registration number: NCT02230787) was conducted and included a total of 40 patients undergoing elective root coverage. Upon approval of the local ethics committee (KEK-186-13- PRR-2015079) patients were randomly assigned to test (EMD + CTG) or control (CTG). The individuals who were included in the study were de-personalized for evaluation of all data, the analysis of crevicular samples and assessment of clinical data were performed in a blinded fashion.

Clinical data were recorded at baseline, at 2 days, 7 days, 14 days and 6 months after surgery, gingival crevicular fluid (GCF) was obtained at baseline, 2 and 7 days.

Surgical procedure

All surgeries were performed by one experienced periodontist (A.S.). All controls and samplings were performed by two investigators (A.St + J.-C. I.) who were blinded to the test or control group. There were examinations right before surgery, at 2 days, 7 days, 14 days and 6 months. The patients were treated with (MCAT) in combination with CTG (Fig. 1). In the test group the CTG, donor site and graft site were covered with EMD (Emdogain, Straumann AG, Switzerland), Before graft insertion into the tunnel, the roots of the test

group were treated with a pH neutral, 24% EDTA root surface conditioner (Straumann[®] PrefGel, Straumann AG, Switzerland) for 2 minutes. In the control group, no EMD was used. Follow-up visits will be performed as described below. The flow chart of the study is summarized in Figure 2.

Effectiveness parameters: measuring and times

The baseline examination included measuring of the periodontal probing depth (PPD) at the involved tooth, periodontal screening record (PSR) (AAP, ADA 1992), recession from the CEJ (mm), recording of fillings at the involved tooth). Furthermore, we assessed whether remnants of EMD were still detectable in the wound fluid at two days after surgery.

Wound healing after root coverage procedure was assessed (2 days, 7 days, 14 days) using the following, newly proposed early wound healing index (Recession Healing Index – RHI) (Fig. 3).

Wound healing was quantified and graded into 4 stages: 0: complete wound closure, no fibrin, no suppuration

1: complete wound closure, thin fibrin-layer on the wound, no suppuration

2: incomplete wound closure, thick fibrin-layer on the wound, slight suppuration 3: incomplete wound closure, suppuration or abscess formation

At the 6 months control, the examiners measured the distance from the Gingiva to the CEJ and the PPD at the involved tooth and calculated the percentage of root coverage (Fig. 4). Additionally, the post-operative healing process was judged by a patient questionnaire.

GCF samples

GCF was sampled by using the extracrevicular method to avoid traumatization. Paper strips (Periopaper, Oraflow Inc., Smithtown, NY, USA) were overlaid placed at the gingival crevice region and left in place for 30 s. Immediately after collection, samples were stored at -80°C until analyzed.

Before analyzing, GCF samples were eluted at 4°C overnight into 750 µl phosphate-buffered saline containing proteinase inhibitors (Sigma-Aldrich, Buchs, Switzerland). From the eluates, the levels of interleukin (IL)-1β, IL-8, IL10, matrixmetalloprotease (MMP)-8 and TGF-β1 were determined by using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Europe Ltd., Abingdon, UK) according to the manufacturer's instructions. Both active and total TGF-β1 were measured. For determination of total TGF-β1, samples were preheated at 99°C for 1 min. The detection levels of the kits were 1 pg/site for IL-1β, IL-8, IL-10, TGF-β1 and 100 pg/site for MMP-8.

Statistical methods

The primary outcome was the progress of wound healing assessed by the "Recession Healing Index (RHI) at day 2, 7 and 14 after surgery.

The GCF levels of the biomarkers IL-8, IL-10, IL-1beta, MMP-8 and TGF beta-1 at

2 days and 1 week after the surgery. Moreover, the traceability of EMD in the operation field was investigated two days after surgery. The patients' post-operative comfort assessed after 2 days, 1 and 2 weeks and 6 months postoperatively (documented by questionnaire) was analyzed. The root coverage in mm assessed 6 months after surgery completed the secondary outcomes.

Normality of the distribution was evaluated assessing skewness and kurtosis and applying the Kolmogorov-Smirnov test. All continuous variables were presented as means \pm SD when normally distributed and as medians and interquartile ranges when not normally distributed. Categorical variables were given as frequencies and percentages. Continuous variables were tested for differences with the Wilcoxon signed-rank test. Categorical variables were tested by the Pearson χ^2 test or the Fisher's exact test as appropriate. The differences between patients in the treatment groups were determined at each time point using the Mann-Whitney U test.

Surgical results were correlated to complications and inflammatory markers using Spearman's rank correlation, if appropriate. All statistical analyses were performed with the use of JMP (SAS Institute Inc., Cary, NC). For all tests, a two-sided $p < 0.05$ was considered statistically significant.

Results

Study participants

Patient recruitment started in September 2014 and ended in June 2016. A baseline screening was performed in totally 42 patients of whom 40 (29 females, 11 males) were entered in the study all fulfilling the inclusion criteria. The eligible patients were randomized equally into test (14 females, 6 males) and control groups (15 females, 5 males). One patient in the control group was lost during the follow up and only 2 weeks follow up data were available for this individual. Thus, only 39 patients completed the 6 months follow-up. All included surgeries were performed by the same experienced clinician (A.S.) using MCAT and CTG either with or without the adjunctive use of EMD. No systemic side effects were recorded. A total of 6 patients (3 in each treatment group) received systemic antibiotic (Amoxicillin) therapy in the first 2 weeks following surgery. The reason for the prescription of antibiotics was suppuration or abscess formation during the first postoperative week.

Baseline characteristics

Description of patient demographics and baseline data for all patients in both study groups are depicted in table 1. There were no significant differences between groups regarding age and ethnicity. There were no statistically significant differences in the periodontal screening index (PSI), recession dimensions and Miller classes. Mean PSI values for test and control group were 1.35 (\pm 0.75) and 0.99 (\pm 0.75). Mean recession length before surgery was 4.07 mm (\pm 1.28) for the test and 4.47 (\pm 2.08) for the control group. Ten patients showed gingival defects in the maxilla and 30 patients had defects in the mandible. Of the involved sites 36 were located at anterior teeth and 4 at bicuspids (3 in the mandible, 1 in the maxilla).

Recession healing index

The recession healing index (RHI) was taken at the recession site as well as at the donor site of the CTG in the palate. At 2 days the average RHI at the recession site for test and control group was 2.10 (SD 0.72) and 1.85 (SD 0.67) respectively. At 7 and 14 days the values had decreased to 1.20 (SD 0.89) and 0.40 (SD 0.50) for the test, respectively to 1.30 (SD 0.80) and 0.55 (SD 0.61) for the control group with no statistically significant difference among the groups. For the palate the respective values at 2, 7 and 14 days were 1.42 (SD 0.51), 1.00 (SD 0.86), and 0.40 (SD 0.68) for the test group. The corresponding values for the control group were 1.70 (SD 0.83), 1.45 (SD 0.83), and 0.35 (SD 0.59) (Table 2). Data analysis by the Mann-Whitney-U test revealed no statistically significant variations among the groups (2 days recession: $p=0.259$, palate $p=0.083$; 7 days recession: $p=0.676$, palate: $p=0.078$; 14 days recession: $p=0.456$ and palate: $p=0.920$).

Biomarkers

Gingival crevicular samples were harvested at baseline, 2 and 7 days after surgery and assessed for IL 8, IL-1 β , IL 10, and MMP 8. In both groups IL 8 and IL-1 β levels increased statistically significantly at 2 days when compared to baseline values. They decreased at 7 days being still statistically significantly higher than at baseline. In the test group, the IL10 levels decreased slightly ($p=0.047$) at day 7 when compared with day 2, in the control group they were lower at day 2 than at baseline ($p=0.046$). In both groups MMP8 levels were increased at day 2 compared to baseline (test group: $p<0.001$, control group: $p=0.015$). In the test group MMP8 levels were decreased at day 7 when compared to day 2 ($p=0.044$), but still higher than at baseline ($p=0.023$). There was never a statistically significant difference between test and control group at any time and for any biomarker. Results are presented in Figure 4.

GCF samples were further analyzed for active and total TGF- β 1 levels. Positive results were assessed only at day 2, total TGF β 1 was detectable in five samples (among them four in the test group) and active TGF- β 1 in one sample of the test group.

Soft tissue parameters

The values of the soft tissue parameters at baseline and the 6 months follow-up are shown in table 3. Both groups demonstrated statistically significant improvement in the coverage of the recession compared to baseline values with no significant intergroup differences. The mean recession length at baseline was 4.475 mm for the control group and 4.075 mm for the test group, the recession width 2.85 mm and 3.4 mm. Recession length after recession coverage shrunk to 1.00 and 0.944 mm respectively. The respective values expressed as percentage of root coverage at 6 months revealed an overall root coverage rate of 78.0% (SD 22.6) with 78.7% (SD 26.8) for the test and 77.4% (SD 18.1) for the control group, whereby complete root coverage was obtained in 8 test and 3 control subjects. Mean width of keratinized tissue at baseline was 1.125 mm for the control and 1.075 mm for the test group. The changes between BL and 6 month follow up showed a mean increase of 0.822 and 0.814 mm. No statistically significant differences were found between the test and the control group for the changes between baseline and the follow up period.

Patient-reported outcomes

VAS scores were evaluated at 2, 7 and 14 days after surgery. Mean VAS scores at the palate for day 2 were at 2.7 for the test and 4.0 for the control group. Hereby no statistical significant difference was demonstrated. Values further declined to the 7 and 14 day follow ups for both groups (0.7 and 0.6 for the test and 1.4 and 1.1 for the control site). Mean VAS scores at the tooth site were 2.9, 2.9 and 1.1 for the test and 5.1, 3.5 and 1.6 for the control site (Fig.6).

Discussion

The present randomized controlled clinical trial has failed to show any additional effect of using EMD as an adjunct to MCAT/CTG on wound healing as assessed through clinical and inflammatory parameters. In some cases (e.g. 3 patients in each group) suppuration and/or abscesses occurred during the first two postoperative weeks but disappeared immediately following the systemic administration of antibiotics.

Periodontal wound healing/regeneration requires adequate infection control, undisturbed early wound healing and implies adhesion, migration and proliferation of inflammatory cells in order to establish a sufficient blood supply to support the healing process. In this respect it was hypothesized that the use of EMD may enhance early wound healing and, in the same time, decrease post-operative complication rates. To assess early wound healing, a novel clinical index, the RHI, was proposed. The RHI includes parameters such as wound closure, fibrin formation, suppuration, and abscess formation and was assessed at 2, 7, and 14 days postoperatively representing a modification of the early wound healing index described by Wachtel et al. (2003) to describe early wound healing features following regenerative surgery in intrabony defects (Wachtel et al., 2003). The aim of the newly proposed index was to more accurately characterize early wound healing than simply by evaluating primary and secondary wound closure.

Numerous in vitro studies have extensively investigated in vitro cell responses to enamel matrix derivative (EMD) and have demonstrated a plethora of beneficial effects on periodontal wound healing and regeneration. EMD has been demonstrated to influence wound healing favouring the wound fill rates in vitro (Bosshardt, 2008, Miron et al. 2016), stimulating cell growth and metabolism as well as proliferation and migration of periodontal ligament cells (Haase and Bartold, 2001), (Gibson, 2008). Furthermore, EMD has been shown to increase the attachment rate of periodontal ligament cells by interfering with specific integrins (Hoang et al., 2000), (Suzuki et al., 2001), (Rincon et al., 2005) and to promote angiogenesis by enhancing mesenchymal and microvascular cell differentiation (Miron et al., 2016). In an oral mucosa wound model in the rat, the injection of EMD led to increased formation of blood vessels and collagen production thus improving early wound healing (Maymon- Gil et al., 2016). Although in vitro studies have provided evidence for a beneficial effect of EMD on wound healing and regeneration, it has been difficult to corroborate these findings in clinical studies. Wennström and Lindhe 2002 evaluated the application of EMD versus a carrier in a split mouth RCT in a group of patients receiving scaling and root planing. Patient-reported outcomes of up to 3 weeks favoured the application of EMD (Wennstrom and Lindhe, 2002). Tonetti et al. 2004 showed earlier gains in soft tissue densities after EMD application as well as high patient comfort. Here, soft tissue healing and patient morbidity at deep intrabony defects were evaluated (Tonetti et al., 2004). On the other hand, other studies could not show any

effects on wound healing following the use of EMD (Hagenaars et al., 2004).

Gingival crevicular fluid was sampled and analyzed for IL8, IL10, MMP8, IL1 β and TGF- β 1. Frequency of detection and interleukin levels were compared both between the different time-points and the two groups. For IL8 and IL1 β a statistically significant postoperative increase was noted in both groups, with a peak at day 2. Postoperative MMP8 levels were significantly increased in the EMD treated group, but remained unchanged in controls. Interleukin 10 was not increased at any time- point. The changes of inflammatory markers showed similar tendencies for both groups and can be interpreted as response to the surgical trauma with no clear tendencies among the two groups. TGF- β 1 was statistically significantly increased after the procedure in only 3 samples (e.g. one test and two controls). Consistent with these findings, previous studies have shown an increase of TGF- β 1 levels after EMD application. Maymon-Gil et al 2016 showed in the rat wound healing model an increase of TGF- β 1 and β 2, vascular endothelial growth factor, IL-1 β , matrix- metalloproteinase-1, versican, and fibronectin.

Recently, microarray analyses have been performed shedding light onto cellular responses to EMD. These studies appear to support the assumption that EMD effects are partly mediated through TGF- β activity (Brett et al., 2002); (Parkar and Tonetti, 2004); (Kapferer et al., 2011); (Stahli et al., 2014).

The evaluation of soft tissue parameters 6 months after surgery revealed a similar gain of keratinized tissue for both groups while the mean coverage rates of about 80% compare well with those obtained in other studies (Cairo et al. 2014, Graziani et al. 2014).

The use of EMD related to mean root coverage and gain of keratinized tissue has also been investigated in several clinical studies.

Some of the available data appear to indicate improved clinical results in terms of root coverage following the combination of EMD + CTG (Hagewald et al., 2002), (Cueva et al., 2004). It has been shown that EMD resulted in increased gain of keratinized tissue and improved long-term stability when compared to CAF alone (Castellanos et al., 2006). Other studies failed to show superiority of Emdogain when used with CTG and CAF after one year (Hagewald et al., 2002), (Chambrone et al., 2008). However, when looking at the 2 year- results the EMD group reached 53% of root coverage compared to 23% in the control group (Spahr et al., 2005). Other investigations based on Miller's class III gingival recession defects suggested that, the use of EMD is beneficial in augmenting the effects of the CTG in terms of root coverage, gain in clinical attachment, probing depth reduction (Sato et al., 2006), (Henriques et al., 2010). Aroca et al 2010 in a split mouth study failed to show superiority of EMD when treating multiple type III recession defects (Aroca et al., 2010). However, a recent systematic review has provided evidence that the adjunctive application of EMD onto denuded root surfaces in combination with CAF may result more often in complete root coverage (CRC) and increased keratinized tissue gain (Cairo et al., 2014).

When interpreting the present findings, it should be kept in mind that the used surgical approach (e.g. MCAT) does not completely detach the flap from the underlying bone and tooth surfaces, which in turn may additionally stabilize the blood clot. On the other hand, it cannot be ruled out that a blood contamination of the root surfaces can more easily occur when this surgical approach is adopted thus making the precipitation and persistence of EMD on the root surfaces and in the wound area unpredictable. It has been previously demonstrated that plasma proteins from blood, may alter the ability of EMD to

adsorb to root surfaces thus negatively affecting cell attachment, differentiation and proliferation (Miron et al. 2012).

In conclusion, within their limits, the present study has failed to demonstrate an influence of EMD on clinical and immunological parameters related to wound healing following recession coverage surgery using MCAT and CTG.

Conflict of interest

The authors report no conflict of interest regarding the study or products used for this trial. The study was funded by the Department of Periodontology, University of Bern, and partly by Straumann, Basel, Switzerland.

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Tables

Table 1 Patient characteristics

	Control group	Test group
Gender		
n (female)	15	14
n (male)	5	6
Age		
Mean	30.8	32.8
SD	9.9	11.1
Ethnicity		
Caucasian	19	19
Afro-American	1	1
Miller class		
I	2	4
II	10	10
III	8	6
Smoking	0	3
PSI		
Mean	0.9	1.3
SD	0.7	0.9

PSI, periodontal screening index; SD, standard deviation

Table 2 Recession healing index (RHI) and graft exposure

	recession			palate			GE 2 d (mm)	GE 7 d (mm)
	RHI 2 d	RHI 7 d	RHI 14 d	RHI 2 d	RHI 7 d	RHI 14 d		
control	1.8±0.6	1.3±0.8	0.5±0.6	1.7±0.4	1.4±0.8	0.3±0.5	0.3±0.6	0.8±1.4
test	2.1±0.7	1.2±0.8	0.4±0.5	1.4±0.5	1.0±0.8	0.4±0.6	0.1±0.4	0.2±0.9
<i>p</i> -value	0.485	0.838	0.538	0.079	0.244	0.801		

RHI, recession healing index; d, days; GE, graft exposure

Table 3 Change of parameters at recession sites between baseline and 6 months po

	Test Mean \pm SD	Control Mean \pm SD
Recession length		
Baseline	4.0 \pm 1.2	4.4 \pm 2.0
After surgery	0.9 \pm 1.3	1.0 \pm 1.0
<i>p</i> -value	< 0.001	< 0.001
Recession width		
Baseline	3.4 \pm 1.0	2.8 \pm 0.7
Width of KT		
Baseline	1.0 \pm 0.9	1.1 \pm 0.8
After surgery	1.8 \pm 1.2	1.9 \pm 1.0
<i>p</i> -value	0.001	0.001

po, postoperatively; SD, standard deviation; KT, keratinized tissue

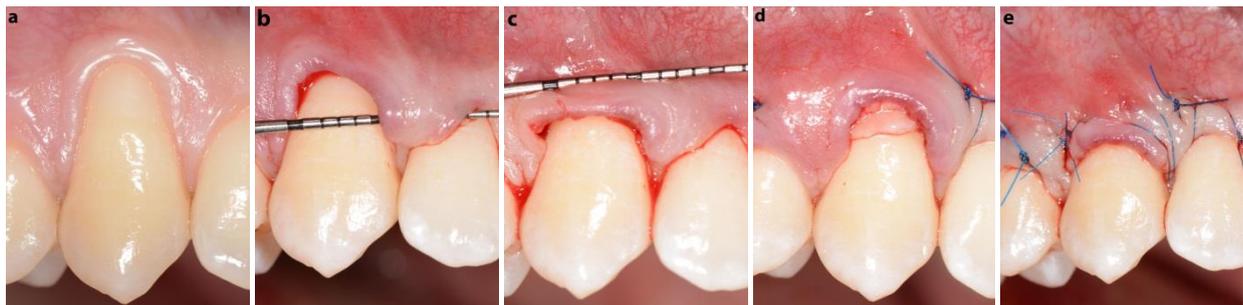


Fig. 1. Surgical technique.

a: baseline, b: tunneled flap, c: mobilisation , d: connective tissue graft, e: coronally sutured tunnel

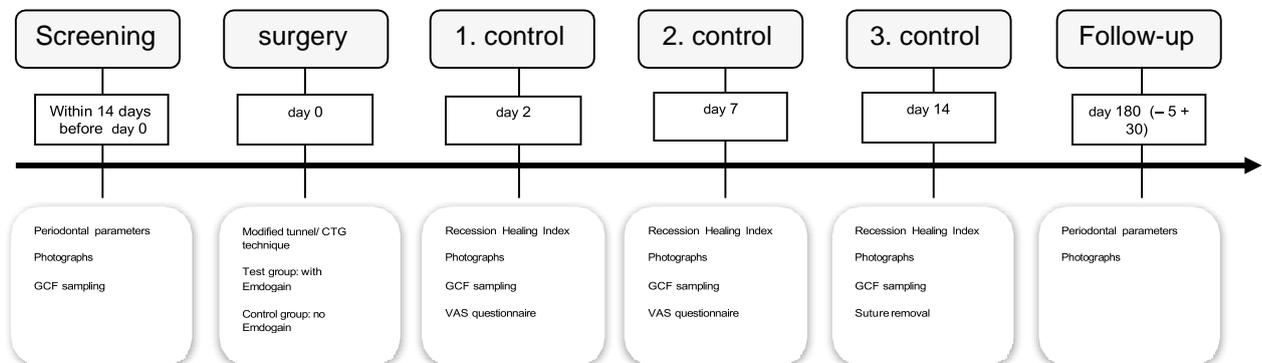


Fig. 2. Flow chart

Flow chart showing the timeline of visits with assessed parameters. GCF, gingival crevicular fluid; CTG, connective tissue graft.

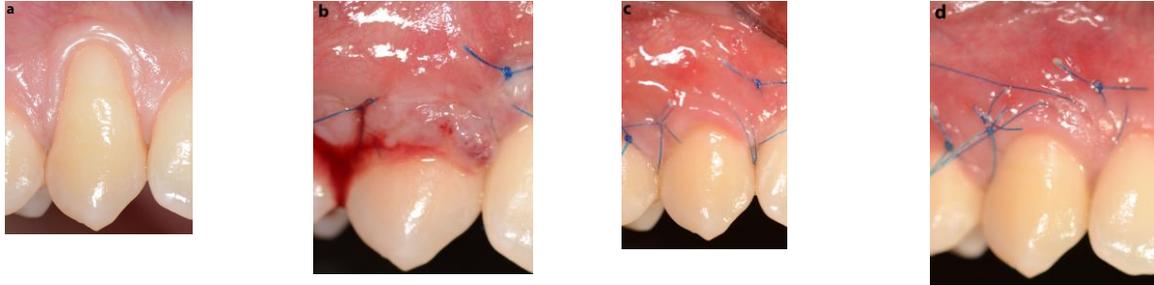


Fig. 3. *Early wound healing (example)*

a: baseline, b: 2 days post operation, c: 7 days post operation, d: 14 d post operation

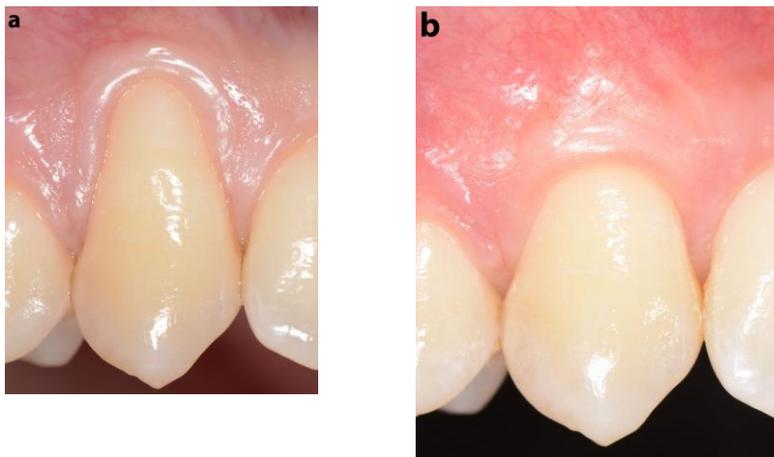


Fig. 4. *Case example*

a: baseline, b: 6 month control

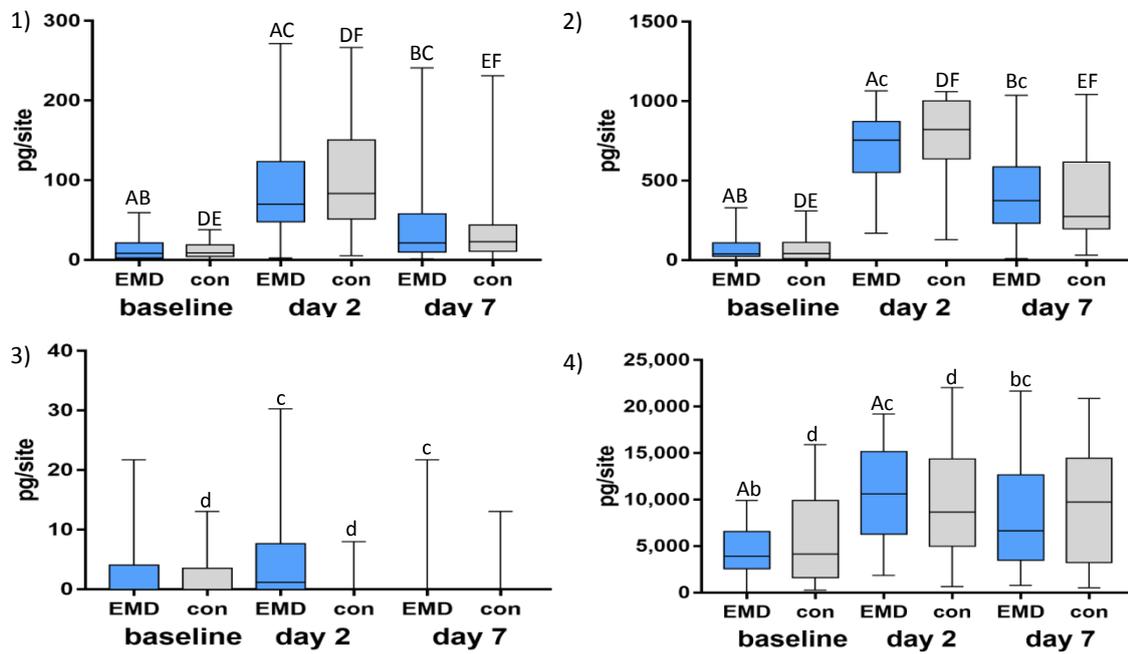


Fig. 5. Change in inflammatory markers

1) values for IL-8; 2) IL-10; 3) MMP-8; 4) IL-17

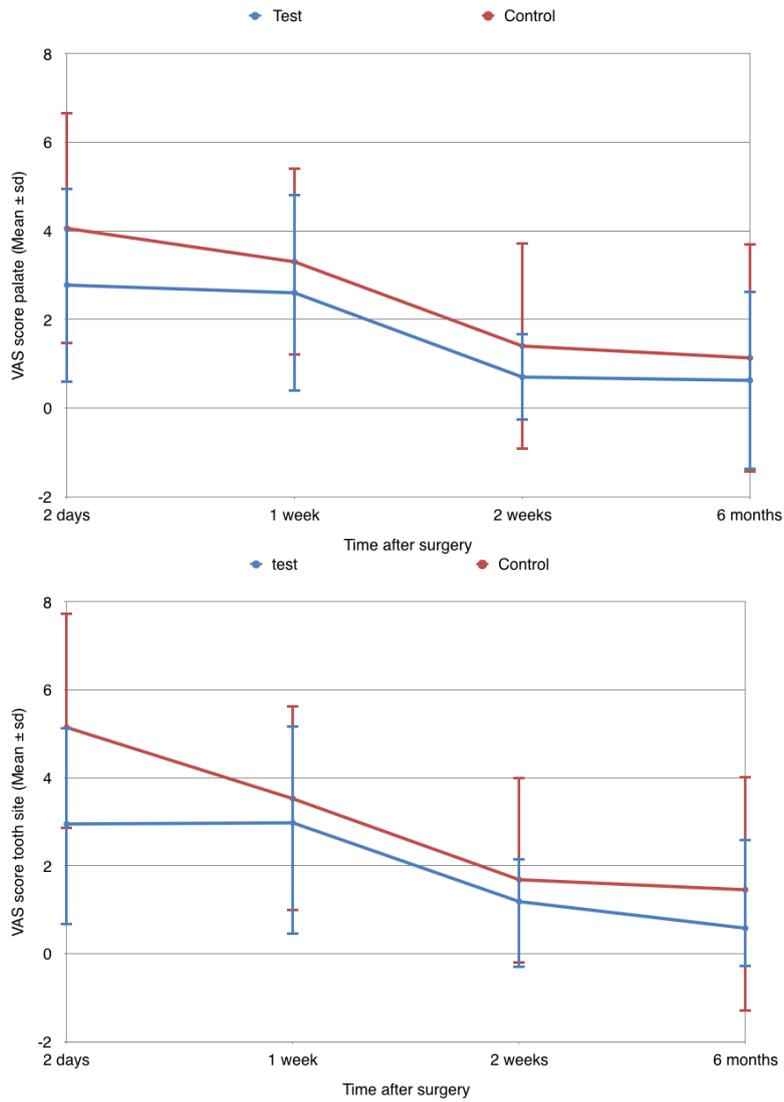


Fig. 6. VAS scores

VAS scores for pain as reported by the patients for different timepoints depicted as mean and standard deviations.