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ORIGINAL ARTICLE

Effect of enamel matrix derivative on wound healing following gingival recession coverage using the modified coronally advanced tunnel and subepithelial connective tissue graft: a randomised, controlled, clinical study

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Abstract

Objectives The potential effect of enamel matrix derivative (EMD) on wound healing following recession coverage surgery is still controversially discussed in the literature. The aim of this randomised, controlled, single blinded clinical study was, therefore, to investigate clinically and immunologically the potential effects of EMD on early wound healing and clinical results following treatment of single and multiple gingival recessions by the modified coronally advanced tunnel technique (MCAT) and subepithelial connective tissue graft (sCTG).

Materials and methods A total of 40 systemically healthy patients with Miller class I, II or III single or multiple gingival recessions were treated with MCAT + sCTG with or without EMD. Patients were consecutively enrolled and randomly assigned to test or control treatment. Inflammatory markers (interleukin (IL)-1β, IL-8, IL-10 and matrix metalloprotease (MMP)-8) were measured at baseline, 2 days and 1 week postoperatively. The following clinical parameters were assessed at baseline and at 6 months postoperatively: Recession Depth (RD), Recession Width (RW), Width of Keratinized Tissue (KT) and Probing Depth (PD). Patient-reported outcomes were analysed by means of a visual analogue scale.

Results No statistically significant differences were detected between the 2 groups in terms of inflammatory markers and patient-reported outcomes during early wound healing. In the test group, RD was reduced from 4.0 ± 1.2 mm at baseline to 0.9 ± 1.3 mm at 6 months ($p < 0.001$), while the corresponding values in the control group were 4.5 ± 2.0 mm at baseline and 1.0 ± 1.0 mm at 6 months, respectively. At 6 months, mean root coverage measured 78 ± 26% in the test group and 77 ± 18% in the control group, respectively.

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

Clinical relevance Early wound healing following recession coverage by means of MCAT and sCTG does not seem to be influenced by the additional application of EMD.

Keywords Mucogingival surgery · Enamel matrix derivative · Wound healing · Recession coverage

Alexandra Stähli and Jean-Claude Imber contributed equally to this work.

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Introduction

Gingival recession is the exposure of the root surface due to displacement of the gingival margin apical to the cementoenamel junction and can affect the labial, lingual and/or interproximal areas [1]. This root exposure is frequently associated with "wedge-shaped" defects at the crevicular area, aesthetic impairment, predilection to root caries, root hypersensitivity and difficulties to achieve optimal plaque control [2–5].

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 (sCTG) [6–11], while recent evidence indicates that both techniques can lead to comparable outcomes [12]. The MCAT implies preparation of a full thickness flap without vertical releasing incisions and incisions in the area of the papillae to improve blood supply and wound stability [8–11]. The coronal displacement of the tunnel enables predictable coverage of the recessions and of the soft tissue graft, thus optimizing graft survival, recession coverage and tissue blending [8–11].

Enamel matrix derivative (EMD) has been shown to promote periodontal regeneration by mimicking the embryonic development of the periodontal tissues [13, 14]. 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 [15]. Findings from preclinical studies and human case reports indicate that for root coverage procedures, the use of EMD alone or in combination with sCTG enhances periodontal wound healing/regeneration as demonstrated histologically through the formation of the periodontal ligament, root cementum and alveolar bone [15, 16]. Very recently, the potential effects of EMD and sCTG in conjunction with a coronally advanced flap (CAF) for recession coverage were evaluated clinically and histologically in dogs [16]. The results have shown statistically significantly higher improvements in terms of probing depth reduction and clinical attachment gain in the defects treated with CAF + EMD + sCTG compared with the controls (i.e. CAF + sCGT). The corresponding histological analysis revealed that treatment with CAF + EMD + sCTG resulted in statistically significantly shorter epithelium length and greater complete periodontal regeneration (i.e. new cementum, new periodontal ligament and new bone) compared with the controls (i.e. CAF + sCTG) [16].

Moreover, a number of clinical and experimental studies have suggested that the application of EMD in conjunction with flap surgery may lead to accelerated wound healing and less inflammation compared to placebo-treated sites, thus pointing to its potential clinical relevance in modulating early wound healing [15, 17].

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

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

Materials and methods

Study population

Forty patients with single or multiple Miller class I, II or III gingival recessions [18] were enrolled in this randomised controlled clinical study. In cases of multiple gingival recessions, the deepest defect was selected for evaluation. All included patients signed an informed consent. This study protocol was in accordance with the moral, ethical and scientific principles governing clinical research set out in the current version of the Declaration of Helsinki. Exclusion criteria comprised age < 16 years, full mouth plaque score over 25% [19], history of chronic infectious or inflammatory diseases (i.e. uncontrolled diabetes, rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease or HIV- and HCV-infection), any clinical signs of an acute infection, renal failure (GFR < 30 ml/min) and current smoking (> 5 cigarettes per day).

Study design

This prospective, randomised, single blinded clinical trial (Trial registration number: NCT02230787) was conducted and it 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 + sCTG) or control (sCTG). The individuals who were included in the study were de-personalised for evaluation of all data. The analysis of crevicular samples and assessment of clinical data were performed in a blinded fashion. The study design is depicted in Fig. 1. 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 and at 2 days and 7 days.

Surgical procedure and postoperative protocol

All treatments were performed by the same periodontist (A.S.) with extensive experience in plastic-aesthetic periodontal surgery with the MCAT technique [10, 11]. Pre- and postoperative examinations and samplings were performed by two investigators (A.St. + J.-C. I.) who were blinded to the provided treatment. Examinations were scheduled immediately before surgery, at 2 days, 7 days, 14 days and at 6 months. Patients were treated with MCAT in combination with sCTG either with or without EMD (Fig. 2). In the test group, the sCTG, the palatal donor site and root surfaces were covered with EMD (Straumann® Emdogain, Straumann AG, Switzerland). Before graft insertion into the tunnel, the roots of the test group were conditioned for 2 min with a 24% EDTA (Straumann® PrefGel, Straumann AG, Switzerland) to remove the smear layer. In the control group, neither EDTA nor EMD was used. All patients were given analgesics (mefenamic acid 500 mg, max. 3 × day,

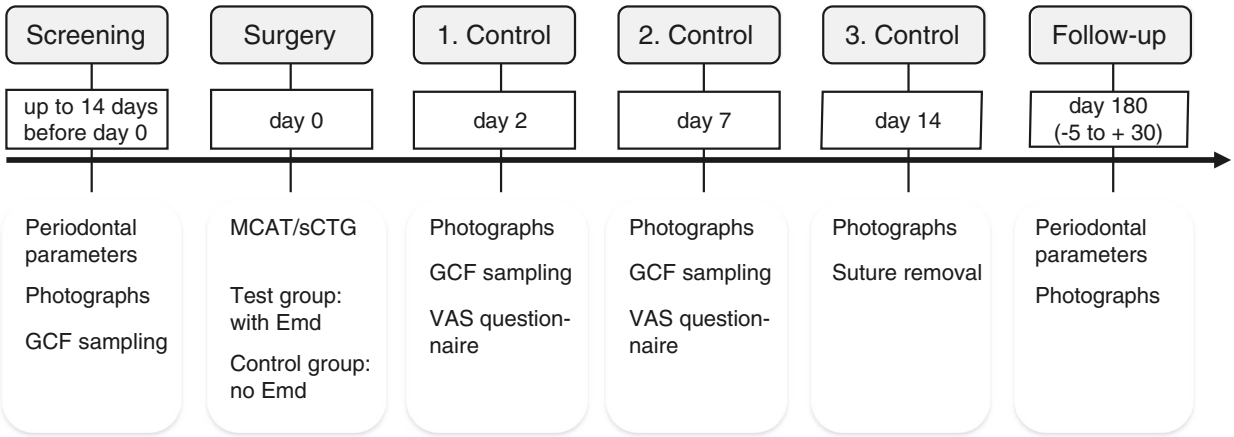

Fig. 1 Flow chart. GCF, gingival crevicular fluid; MCAT, modified coronally advanced tunnel; sCTG, subepithelial connective tissue graft

Mephadolor 500 Neo, Mepha, Switzerland) for pain release during the first 3–5 days after surgery. Furthermore, the patients were instructed not to brush their teeth in the operated area until suture removal after 2 weeks. For chemical plaque control, 0.2% chlorhexidine digluconate containing mouth rinses (Chlorhexamed forte, GSK Consumer Healthcare Schweiz AG, Switzerland) were used for 1 min twice daily during the next 2 weeks postoperatively. Mechanical plaque control was resumed by means of a soft surgical brush (Paro AG, Kilchberg, Switzerland) after suture removal at 2 weeks. Regular tooth brushing including also interdental cleaning was resumed at 4 weeks postoperatively.

Gingival crevicular fluid samples

GCF was sampled by using the extracrevicular method [20] to avoid traumatisation. 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 analysed.

Before analysis, 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, matrix metalloprotease (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 and TGF-β1 and 100 pg/site for MMP-8. Furthermore, we assessed whether remnants of EMD were still detectable in the wound fluid at 2 days after surgery.

Clinical parameters

The baseline examination included the following measurements: periodontal screening record (PSR) [21], probing depth (PD) and recession depth (RD) (i.e. distance from the CEJ or restoration margin, if the CEJ was not visible, and gingival margin and recession width (RW)). The postoperative healing was evaluated through a patient questionnaire.

At 6 months, PD and RD were again measured and the percentage of root coverage was calculated.

Statistical methods

The primary outcome was the GCF level of 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 also investigated at 2 days after surgery.

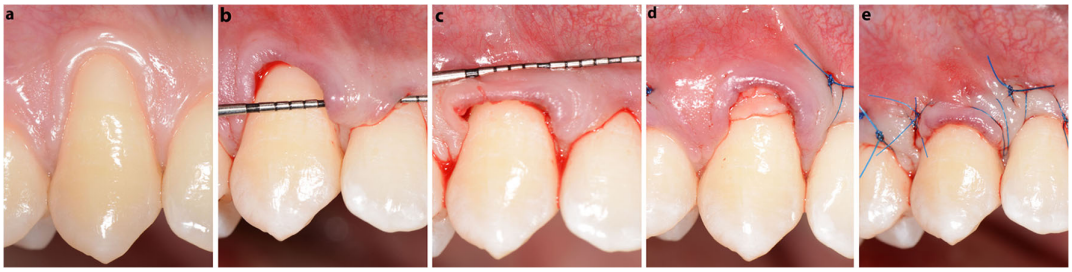

Fig. 2 Surgical technique. **a** baseline, **b** tunnelled flap, **c** mobilisation, **d** connective tissue graft, **e** coronally sutured tunnel

The patients' postoperative comfort assessed after 2 days, 1 and 2 weeks and 6 months postoperatively (documented by questionnaire) was analysed. 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 ± 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 Pearson's chi-squared test or Fisher's exact test as appropriate. The differences between patients in the treatment groups were determined at each timepoint using the Mann-Whitney U test.

The clinical outcomes in terms of root coverage were correlated with complications and inflammatory markers using Spearman's rank correlation. 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 a total of 42 patients of whom 40 (29 females, 11 males) were entered in the study all fulfilling the inclusion criteria. The eligible patients were randomised 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-week follow-up data were available for this individual. Thus, only 39 patients completed the 6-month follow-up. No systemic side effects were recorded. Three patients from the test group were smokers (i.e. less than 10 cigarettes/day), while none of the patients in the control group was a smoker. None of the patients had a history of diabetes. A total of 6 patients (3 in each treatment group) received systemic antibiotics (amoxicillin) 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

There were no statistically significant differences between the groups in terms of age and ethnicity, periodontal screening record (PSR), recession dimensions and Miller classes (Table 1). Mean PSR values for test and control group were 1.3 ± 0.9 and 0.9 ± 0.7, respectively. Mean RD before surgery measured 4.0 ± 1.3 mm in the test and 4.5 ± 2.0 mm in the control group with the corresponding values for RW of 3.4 ± 1.0 mm in the test group and 2.8 ± 0.7 mm, in the control group.

Table 1 Patient characteristics

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

PSR, periodontal screening record; *SD*, standard deviation

Out of the 40 patients, 10 displayed the gingival recessions in the maxilla, while in 30 patients, the defects were localised in the mandible. Out of the treated sites, 36 were located at anterior teeth and 4 at bicuspids (3 in the mandible, 1 in the maxilla).

Biomarkers

Gingival crevicular samples were harvested at baseline and at 2 and 7 days after surgery and assessed for IL 8, IL-1β, IL 10 and MMP 8. In both groups, IL 8 and IL-1β levels increased statistically significantly at 2 days when compared to baseline values. They decreased at 7 days, being, however, 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 no statistically significant difference between test and control group at any time and for any biomarker (Fig. 3).

GCF samples were further analysed for active and total TGF-β1 levels. Positive results were assessed only at day 2, total TGFβ1 was detectable in five samples (among them were four in the test group) and active TGF-β1 in one sample

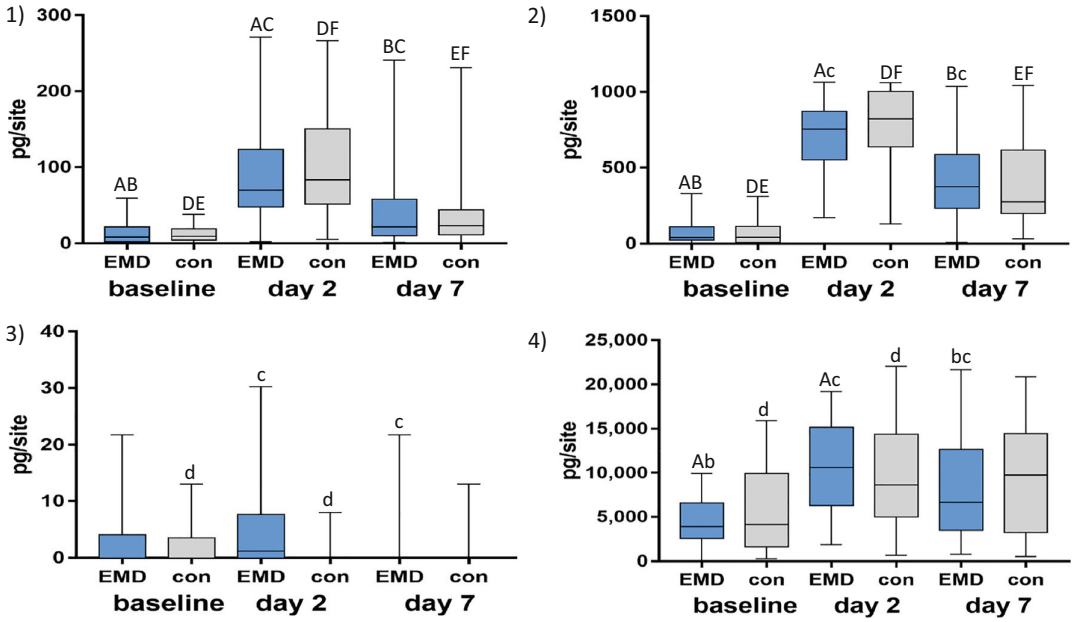

Fig. 3 Change in inflammatory markers. (1) IL-8; (2) IL-10; (3) MMP-8; (4) IL-1β; Footnotes: Statistical significances, test (EMD) group: A, B, C—$p < 0.01$; b, c—$p < 0.05$; control group: D, E, F—$p < 0.01$; d—$p < 0.05$.

of the test group. Thereby, only three samples reached statistical significance.

Soft tissue parameters

The clinical results at baseline and 6-month follow-up are shown in Table 2. At 6 months, mean RD measured 0.9 ± 1.3 mm in the test group and 1.0 ± 1.0 mm in the control group, respectively. The corresponding values in terms of percentage of mean root coverage measured were 78 ± 26% in the test and 77 ± 18% in the control group, respectively. Complete root coverage was obtained in 8 test and 3 control subjects. At baseline, the mean width of KT measured 1.0 ± 0.9 mm in the test and 1.1 ± 0.8 mm in the control group. At 6 months, KT increased statistically significantly ($p < 0.001$) in both groups and measured 1.8 ± 1.2 mm in the test and 1.9 ± 1.0 mm in the control group, respectively. No statistically significant differences were found in terms of KT between the two groups at any time-point.

Table 2 Change of clinical parameters at recession sites between baseline and 6 months po

	Test Mean ± SD	Control Mean ± SD
Recession depth (mm)		
Baseline	4.0 ± 1.2	4.5 ± 2.0
6 months	0.9 ± 1.3	1.0 ± 1.0
p value	< 0.001	< 0.001
Recession width (mm)		
Baseline	3.4 ± 1.0	2.8 ± 0.7
Width of KT (mm)		
Baseline	1.0 ± 0.9	1.1 ± 0.8
6 months	1.8 ± 1.2	1.9 ± 1.0
p value	0.001	0.001

po, postoperatively; *SD*, standard deviation; *KT*, keratinised tissue

Patient-reported outcomes

VAS scores were evaluated at 2, 7 and 14 days after surgery (Fig. 4). Mean VAS scores at the palate for day 2 were at 2.7 for the test and 4.0 for the control group. 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 without statistically significant differences.

Discussion

The present randomised controlled clinical study has failed to show an additional effect on early wound healing, as assessed by inflammatory parameters, and on the patient-reported outcomes, following the use of EMD in conjunction with recession coverage by means of sCTG and MCAT. Despite the fact that at 6 months, complete root coverage was obtained in 8 test and 3 control subjects, the corresponding values in terms of mean root coverage measured 78 ± 26% in the test and 77 ±

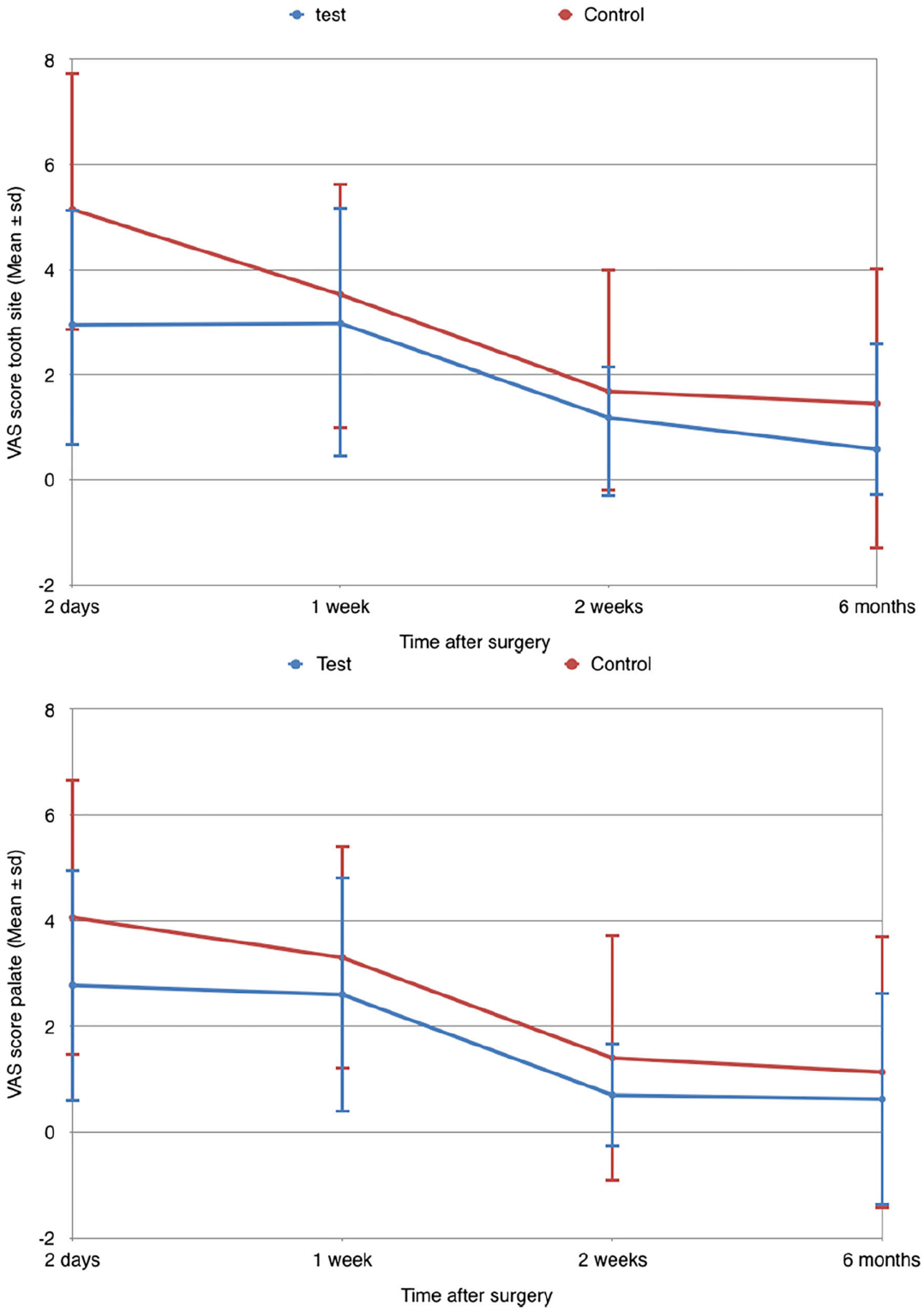

Fig. 4 Patient-reported outcomes for the palate and the recession site

18% in the control group, respectively, without statistically significant differences between the two groups.

An interesting observation was that in six patients, e.g. in three patients in each group, suppuration and/or abscesses

occurred during the first two postoperative weeks, but disappeared immediately following the systemic administration of antibiotics (e.g. amoxicillin). This observation is clinically relevant since it raises the question on the need for routine administration of systemic antibiotics following recession coverage surgery by means of MCAT to reduce postoperative infection. Obviously, this pertinent clinical question should be investigated in further randomised, controlled clinical studies.

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 hypothesised that the use of EMD may enhance early wound healing and, at the same time, decrease postoperative complication rates.

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 wound fill rates in vitro [15], stimulating cell growth and metabolism as well as proliferation and migration of periodontal ligament cells [22, 23]. Furthermore, EMD has been shown to increase the attachment rate of periodontal ligament cells by interfering with specific integrins [24–27] and to promote angiogenesis by enhancing mesenchymal and microvascular cell differentiation [15]. 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 [28]. 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 [29]. Tonetti et al. 2004 showed earlier gains in soft tissue densities after EMD application as well as high patient comfort. In that study, soft tissue healing and patient morbidity were evaluated following treatment of intrabony defects with open flap debridement (OFD) and application of EMD [30]. Other studies, however, have failed to show any differences in terms of early wound healing following flap surgery with or without EMD [31].

Gingival crevicular fluid was sampled and analysed for IL-8, IL-10, MMP-8, IL-1β and TGF-β1. Frequency of detection and interleukin levels were compared both between the different time-points and the two groups. For IL-8 and IL-1β, 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. IL-10 was not increased at any time-point. The changes of inflammatory markers showed similar tendencies for both groups and can be interpreted as a response to the surgical trauma with no clear tendencies among the two groups. TGF- β1 was statistically significantly increased after the procedure in only 3 samples. Consistent with these findings, previous studies have shown an increase of TGF- β1 levels after EMD application. Maymon-Maymon-Gil et al. 2016 showed in the rat wound healing model an increase of TGF-β1 and β2, vascular endothelial growth factor, IL-1β, MMP-1, versican and fibronectin [28].

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 [32–35].

The evaluation of soft tissue parameters 6 months after surgery revealed a similar gain of keratinised tissue for both groups while the mean coverage rates of about 80% compare well with those obtained in other studies where MCAT was used [8–11]. The additional effect of EMD for root coverage with or without sCTG has been investigated in several clinical studies. Interestingly, when EMD was used in conjunction with CAF either alone or combined with sCTG, higher improvements in terms of recession coverage were obtained compared to treatment without EMD [6, 36–40], but no differences were found when the recession coverage was performed by means of MCAT [8]. The present results compare well with those of a previous split-mouth study, which have failed to show any differences in recession coverage following treatment of multiple adjacent Miller class III recessions with MCAT and CTG with and without EMD [8]. When interpreting the findings, it should be kept in mind that during MCAT, the flap is not completely detached from the underlying bone and tooth surfaces, which may additionally stabilise the blood clot during early wound healing. Therefore, it may be anticipated that blood contamination of the root surfaces may occur more easily when MCAT is performed which, in turn, could negatively affect the precipitation and persistence of EMD on the root surfaces and in the wound area. 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 [41].

Other aspects that need to be also discussed when interpreting the present findings are the potential influence of EDTA root conditioning on the clinical outcomes and the experience of the clinician. While limited data from a very recent systematic review suggest that root conditioning with EDTA in conjunction with root coverage using CAF and sCTG, may additionally improve the clinical outcomes [42], until now, at least to the best of our knowledge, no studies have evaluated the potential influence of EDTA on the clinical outcomes when the surgery was performed with MCAT. Obviously, this issue needs to be addressed in further studies. Moreover, it has to be kept in mind that the outcomes of reconstructive periodontal surgery are largely dependent on the experience of the clinician [6, 7]. Since, in the present

study, all surgical procedures were performed by the same periodontist (A.S.) with extensive experience in plastic-aesthetic periodontal surgery using the MCAT technique, it cannot be ruled out that the results might have been different if the procedures would have been performed by less experienced clinicians such as postgraduate students in training. Last but not the least, it can be also anticipated that the obtained results in terms of recession coverage were also influenced by the strict inclusion criteria (i.e. all patients had a good level of oral hygiene and were systemically healthy, while only three patients from the test group reported smoking less than 8 cigarettes/day). It has been repeatedly demonstrated that the outcomes of plastic-aesthetic periodontal surgery are largely influenced by careful patient selection [6, 7].

Conclusion

Within its 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 sCTG.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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