Diploma Thesis

Treatment of Localized Gingival Recession with an Enamel Matrix Protein Coated Collagen Matrix – A Randomized Controlled Trial

Short Name: EmdoDerm (Emdogain®+Mucoderm®)

submitted by

Dr. med. univ.

Marcus RIEDER

for the Academic Degree of

Doctor of Dental Medicine

(Dr. med. dent.)

at the

Medical University Graz

Department of Dental Medicine and Oral Health

Division of Restorative Dentistry, Periodontology and Prosthodontics/Oral Surgery and Orthodontics

under the Supervision of

Univ. ZA Dr. med dent. Dr. scient. med. Behrouz Arefnia, MSc

Assoc.-Prof. Priv.-Doz. Dr. med. univ. Dr. med. dent. et scient. med. Michael Payer

Graz, 12. July 2023

Declaration of Academic Integrity

I hereby confirm that the present diploma thesis is the result of my own independent scholarly work. I also confirm that in all cases, where material from the work of others (in books, articles, essays, dissertations, and on the internet) is acknowledged, quotations and paraphrases are clearly indicated. No material other than that cited in the reference list has been used. I have read and understood the Medical University's regulations and procedures concerning plagiarism.

Graz, 12/07/2023 *Marcus Rieder m.p.*

Danksagungen

Zunächst danke ich Hr. Assoz.-Prof. DDr. Michael Payer und Hr. DDr. Behrouz Arefnia, MSc für die Möglichkeit an der Universitätsklinik für Zahnmedizin und Mundgesundheit meine Diplomarbeit abzufassen. Ich bedanke mich bei Hr. DDr. Behrouz Arefnia, MSc für seine durchgehende Betreuung, klinische Durchführung der Studie, Unterstützung der Arbeit, und vormalig für seine Freundschaft und Förderung.

Meiner Familie, meiner Freundin und meinen Freunden danke ich für ihre Unterstützung und Zuspruch sowie dafür, dass sie mir während meines gesamten Studiums zur Seite standen.

Danke an Bernhard und Jan für unsere Teamarbeit in den letzten zwei Jahren und eure Analysen und Rat zu jeder Arbeit.

Zusammenfassung

Hintergrund: In der Parodontalchirurgie gibt es eine Vielzahl von etablierten Möglichkeiten, um mit unterschiedlichen chirurgischen Methoden und Materialien Rezessionen zu decken. Um die Entnahmemorbidität autologer Schleimhauttransplantate zu reduzieren, ist die Verwendung von xenogenen Kollagenmatrizen (CM) eine Standardbehandlungsoption. Die zusätzliche Anwendung von Schmelzmatrixderivaten (EMD) kann die parodontale Regeneration fördern und könnte dadurch zu einer besseren Deckung freiliegender Wurzelareale führen. Ziel dieser Studie war es, den Einfluss des additiven Einsatzes von EMD bei Rezessionsdeckungen mit einem koronalem Verschiebelappens (CAF) und einer CM (CAF + CM ± EMD) anhand digitaler und klinischer Bewertungsmethoden zu evaluieren.

Materialien und Methoden: In dieser prospektiven, randomisierten, kontrollierten Studie wurden Rezessionshöhe und -fläche, Breite und Dicke der keratinisierten Gingiva, Volumenänderung, Taschensondierungstiefe sowie das klinisches Attachmentniveau zu Beginn der Studie gemessen und über ein Jahr nachverfolgt. 15 Patient*innen ($n = 15$) mit 24 Rezessionsdefekten (Rezessionstyp 1 nach Cairo/Miller Klasse I oder II) wurden über einen Zeitraum von vier Jahren (2016-2021) nach randomizierter Zuteilung zu einer der beiden Behandlungsgruppen therapiert. Die digitale Beurteilung erfolgte 3 Monate nach dem Eingriff anhand intraoraler Scans.

Ergebnisse: Beide Behandlungsmodalitäten führten zu einer signifikanten klinischen Verbesserung der freiliegenden Wurzelareale ($p < 0,001$). Im Durchschnitt war die digital vermessene Wurzeldeckung der Gruppe A (CAF + CM) im Vergleich zur Gruppe B (CAF + CM + EMD) mit 72 ± 28 % bzw. 31 ± 32 % statistisch nicht signifikant besser ($p = 0.094$). Die klinischen Ergebnisse zeigten ein Jahr nach dem Eingriff stabile Resultate. Eine vergrößerte Breite der keratinisierten Gingiva (WKT) wurde in beiden Gruppen ein Jahr postoperativ nachgewiesen (Gruppe A/CAF + CM: 1,61 mm und Gruppe B/CAF + CM + EMD: 1,46 mm; $p = 0.690$), wobei die Gruppe A hinsichtlich der Dicke der keratinisierten Gingiva überlegen war ($p = 0.044$).

Schlussfolgerungen: Die Studie zeigt, dass es keine statistisch signifikanten Unterschiede bei der Wurzeldeckung in der CAF + CM + EMD-Gruppe im Vergleich zur CAF + CM-Gruppe gab. Die zusätzliche Verwendung von EMD zu einem koronalem Verschiebelappen und einer Kollagenmatrix bei der Behandlung von Rezessionsdefekten scheint das klinische Ergebnis nicht zu verbessern. Weiters zeigt die Studie, dass digitale Beurteilungsmethoden – wie die Verwendung intraoraler Scans – zuverlässig sind, ohne dass die Mukosa durchdrungen, Abdrücke genommen oder ein Messschieber verwendet werden muss.

Abstract

Background: In periodontal plastic surgery, there are several options to cover exposed root surfaces using various proven surgical methods and materials. To reduce the morbidity caused by autologous gingiva harvesting, the utilization of xenogeneic collagen matrices (CM; Mucoderm®, Botiss, Zossen, Germany) is a standard treatment option. The additional application of enamel matrix derivatives (EMD; Emdogain®, Straumann, Basel, Switzerland) could promote regeneration and thereby superior root coverage. This study aims to evaluate the influence of the additionally use of EMD in the treatment of gingival recession defects using a coronally advanced flap (CAF) and a CM (CAF + CM ± EMD) by means of digital and clinical assessment methods.

Materials and Methods: In this prospective, randomized, controlled study, recession height and area, width and thickness of keratinized gingiva, volume change, pocket probing depth and the clinical attachment level were measured at the baseline and followed up for one year. 15 patients ($n = 15$) with 24 gingival recession defects (Recession Type 1 after Cairo/Miller Class I or II) were treated over a four-year time period (2016-2021) after randomly being assigned into two treatment groups. The digital assessment was carried out 3 months after surgery using intraoral scans.

Results: Both treatment modalities resulted in a significant clinical improvement ($p < 0{,}001$). On average, the digitally assessed root coverage of Group A (CAF + CM) was not statistically superior compared to group B (CAF + CM + EMD) with 72 ± 28 % and 31 ± 32 %, respectively ($p = 0.094$). Clinically, outcomes showed stable results one year after surgery. Additional width of keratinized tissue (WKT) was demonstrated in both groups one year after surgery (group A/CAF + CM: 1.61 mm and group B/CAF + CM + EMD: 1.46 mm; $p = 0.690$) but with superior results in group A regarding the thickness of keratinized gingiva ($p = 0.044$).

Conclusions: The present study showed that there were no statistically significant differences in the root coverage results in the CAF + CM + EMD group compared to the CAF + CM group. The adjunctive use of EMD to a coronally advanced flap and collagen matrix in the treatment of gingival recession defects does not appear to have any clinical benefit. The digital assessment methods of this study demonstrate reliable techniques using intraoral scans, without the need to penetrate the gingiva, take impressions, or use a digital calliper tool.

Table of Contents

List of Abbreviations

RT1	recession type 1 after Cairo et al.
RT2	recession type 2 after Cairo et al.
RT3	recession type 3 after Cairo et al.
Nd-YAG	neodymium-doped yttrium aluminium garnet
FMPS	full mouth plaque score
CAF	coronally advanced flap
PPD	pocket probing depth
FMBS	full mouth bleeding score
WKT	width of keratinized gingiva
RECH	recession height
CM	collagen matrix
EMD	enamel matrix derivate
SCTG	subepithelial connective tissue graft

List of Figures

List of Tables

1 Introduction

1.1 Epidemiology and Aetiology of Gingival Recession

Gingival recession is the exposure of the root surface, or the dental implant platform caused by an apical shift of the gingival margin [1, 2]. The resulting root surface deteriorates smile aesthetics and can further lead to dentin hypersensitivity, root caries, and impaired plaque control [3]. Gingival recession can either occur generalised or localised with a prevalence ranging between 39 % and 100 %. This remarkable range is caused by varying analysis methods, age groups, and populations of epidemiological studies [3-7]. Currently, the true aetiology of gingival recession remains unclear. However, several predisposing factors have been suggested in the literature [8-10].

An advanced age and male gender are associated with extent and frequency of gingival recession defects [8, 9]. Important factors are thought to be a direct physical influence on gingival tissues and indirect plaque induced periodontal inflammation [3, 10].

Regarding mechanical influence, chronic prolonged trauma can be caused by vigorous toothbrushing (high pressure/long duration of toothbrushing), or by other variables such as the type of bristles or toothpaste. The fact that buccal surfaces of the left side of the mouth are more frequently affected supports the theory of mechanical cause of gingival recession since most people are right-handed and tend to brush their left side of the mouth more extensively [3, 11-13]. However, recent systematic reviews stated that present data are inconclusive [14-16].

Also oral piercings can cause physical damage to tooth and periodontal structures. A higher incidence of local periodontal destruction, including lingual gingival recession, was observed for teeth near lingual piercings, particularly in the mandibular incisor region, compared to the rest of the teeth [17-20]. Lower lip piercings are often associated with significantly higher plaque accumulation on adjacent teeth and frequently show buccal recession [21]. Therefore, oral piercings combine both direct physical and indirect plaque induced predisposing risk factors in the development of gingival recession.

Another cause of gingival recession could be restorative treatments, particularly those involving subgingival margins, as they can lead to plaque traps with subsequent inflammation and represent a permanent mechanical irritant to the gingiva. Studies showed that restorative treatments involving subgingival margins of restorations, in association with minimal or absent attached gingiva, are more prone to gingival recession and inflammation [22, 23]. Bone loss and apical migration of gingival tissue may occur to restore the supracrestal tissue attachment [8]. Furthermore, a thin periodontal biotype has been reported to be a predisposing factor for the development of gingival recession, especially in cases with reduced bone thickness or alveolar dehiscence resulting from a prominent tooth position out of the dental arch alignment [3].

Gingival recession can also occur or progress due to orthodontic therapy with a prevalence of 5–12 % at the end of treatment [24]. The bucco-lingual thickness of the gingiva and the direction of the tooth movement may play an important role in soft tissue alteration during orthodontic treatment. Lingual tooth movement leads to increased bucco-lingual tissue thickness in the facial region of the tooth, resulting in coronal migration of the gingival margin. In contrast, vestibular tooth movement leads to a decrease in bucco-lingual tissue thickness, resulting in a decrease in free gingival height and an increase in clinical crown height [25]. The probability of developing gingival recession defects associated with orthodontic tooth movement is higher in areas with less than two millimetres attached gingiva [22].

Inflammatory periodontal disease and localised inflammatory processes contribute to the development of gingival recession. The persistent inflammation leads to a loss of periodontal supporting tissue resulting in an apical shift of the soft tissue margin. In patients with periodontal disease, the gingival recession defects are located on all surfaces of the teeth [8]. Several other conditions have been reported to affect the presence of gingival recession, but the available literature is inconclusive. Smokeless tobacco use, traumatic occlusal forces and aberrant frenulum positions are factors that need further investigation to assess the impact on the origin of gingival recession defects [3, 26-30].

Lastly, non-carious cervical lesions are also often associated with soft tissue recession [31, 32]. The presence of these lesions lowers the chances for successful surgical root coverage [33, 34].

1.2 Classification Systems of Gingival Recession

Various classifications have been established to facilitate the diagnosis and therapy of gingival recession. The ideal classification should be treatment-oriented to assess the prognosis of surgical or non-surgical intervention and thus set the framework for the treatment plan [35].

In 1968, Sullivan and Atkins divided mucogingival defects into four classes: deep narrow, deep wide, shallow wide and shallow narrow. Better root coverage results were reported for narrow and shallow defects after a free autogenous gingival graft procedure [36]. Although the application of this classification is simple, the reproducibility of this system is low due to possible different interpretation of the extent of a mucogingival condition.

Mlinek et al. attempted to overcome these limitations by defining mucogingival recession under 3mm as shallow and narrow defects and above 3mm as deep and wide defects [37]. Unfortunately, there was no specific vertical landmark for horizontal measurement which in turn led to a large examiner dependent variability of the horizontal defect extension.

In 1985, Miller established a new classification and described the gingival recession in terms of the vertical level of the gingival margin in relation to the mucogingival junction (extent of recession) and the extent of interdental hard and soft tissue loss [38]. By dividing the gingival recession into four classes, this classification also considers the prognosis of periodontal plastic surgery procedures. Class I is described as a gingival recession not extending to the mucogingival junction without interdental hard or soft tissue loss. A mucogingival defect, which extends to or beyond the mucogingival junction and shows no interdental bone or soft tissue loss, belongs to Class II. Periodontal plastic surgery can predictably achieve complete root coverage in Class I and II gingival recession. Class III gingival recession was originally defined as a mucogingival condition with loss of interdental soft or hard tissue, or abnormal dental position. With the advent of new surgical techniques and materials, complete root coverage might be achievable by treating mild Class III recession, but the outcome is unpredictable. Class IV gingival recession is described as a severe interdental bone and soft tissue loss with absent papillae and/or severe malposition of the tooth. Periodontal plastic surgery procedures cannot obtain complete root coverage in Class IV recession [39, 40]. Although the Miller classification is widely used, it is associated with several drawbacks.

The classification has been criticised for the difficulty in distinguishing between Miller Class I and Class II since a tooth with gingival recession always has a certain amount of keratinized tissue and thus a gingival recession cannot extend to the mucogingival junction or beyond. Hence, Class I and Class II would constitute a single category. Moreover, they cannot be distinguished from a prognostic factor. There are no clear examination methods for assessing interproximal tissue loss, which makes it difficult to discern between Class III and IV. Furthermore, the degree of tooth malposition (Class III and Class IV) is not described in detail, which makes it hard to assign a gingival recession to a specific class [41].

In 2010, Mahajan amended Miller's existing classification by precisely describing classes III and IV. Class III is characterised by the fact that up to one third of the root surface is affected cervically. The tissue loss in Class IV already exceeds one third of the root surface. Both classes can be associated with mild or severe dental malocclusions [42, 43].

Radiographic examination is required in every patient and only facial mucogingival conditions can be classified using the Mahajan's adapted classification [35].

By assessing interdental clinical attachment level, Cairo et al. proposed a modern treatment-oriented classification to predict the potential for complete root coverage. Recession type 1 (RT1) is defined as a gingival recession without interproximal attachment loss. The cementoenamel junction is clinically not identifiable at both mesial and distal aspects of the tooth. Gingival recession defects with interproximal attachment loss that is less than or equal to the buccal attachment loss belong to type 2 (RT2). In recession type 3 (RT3), the extend of interproximal attachment loss is greater than the buccal attachment loss [44]. In RT1 complete root coverage can be expected. With periodontal plastic surgery procedures complete root coverage can be achieved in RT2, but the outcome is unpredictable. The success of surgical procedures is limited to incomplete root coverage in RT3 defects [44, 45]. The gingival recession classification after Cairo et al. is shown in **Figure 1** [44].

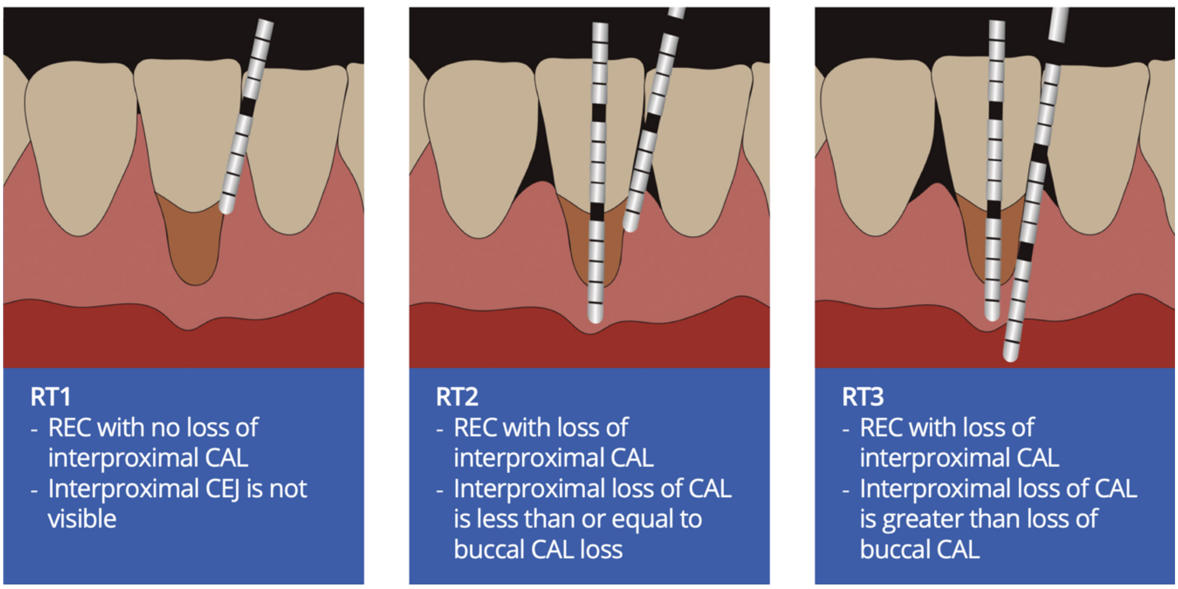

Figure 1. Classification of gingival recession defects after Cairo et alii. RT = recession type, REC = recession, CAL = clinical attachment level, CEJ = cementoenamel junction.

Not only interproximal clinical attachment level but also recession depth, periodontal biotype and characteristics of the exposed root surface are crucial clinical elements to determine the possibility of complete root coverage [23, 44, 46-48]. Therefore, an assessment of mucogingival conditions (recession depth, gingiva thickness, width of keratinized gingiva) and characteristics of the exposed root surface (undetectable cementoenamel junction, presence of a cervical step) must be performed to complete the clinical examination of gingival recession defects (**Figure 2**) [31].

Classification of mucogingival conditions (gingival phenotype) and gingival recessions					
	Gingival site			Tooth site	
	Recession depth	Gingiva thickness	Width of keratinised gingiva	CEJ (A/B)	Step (+/-)
No recession					
RT1					
RT2					
RT3					

Figure 2. Combined clinical evaluation form including gingival phenotype, gingival recession, and characteristics of the exposed root surface. CEJ = cementoenamel junction (A = detectable CEJ/B = undetectable CEJ); Step = root-surface concavity (+ = presence of a cervical step > 0.5 mm/absence of a cervical step > 0.5 mm)

1.3 Management of Gingival Recession

1.3.1 Non-surgical Treatment

There are several treatment options available to address the individual concerns of patients in terms of dentin hypersensitivity and aesthetics due to gingival recession defects. In patients with minimal gingival recession but without hypersensitivity, root caries, or a high smile line no treatment is required. However, probable causes of the gingival recession defect should be identified and eliminated. In addition, good oral hygiene and regular dental check-ups are recommended [10]. Between 10 % and 30 % of the population is affected by dentin hypersensitivity, mostly due to exposure of dentin caused by gingival recession [49]. If there are no aesthetic complaints, a non-surgical treatment plan should be attempted first before considering a surgical option [50]. There are several toothpastes that alleviate hypersensitivity by either inhibiting nerve conduction or occluding dentinal tubules. Another non-invasive option in the treatment of tooth hypersensitivity is local administration of dentin desensitizers. These are products such as hydroxyethyl methacrylate and glutaraldehyde, oxalate, potassium nitrate, and fluoride used by dentists that close dentin tubules to decrease sensitivity. Even better long- and short-term results could be achieved with a combined approach of desensitising agents and lasers. Lasers have been shown to be an effective treatment by occluding the dentinal tubules. Among the different lasers types, the Nd:YAG laser can best help alleviate these dental irritations [51]. If interventions with various desensitising products do not achieve satisfactory pain relief, restorative dentistry offers further treatment alternatives with bonding agents or cervical composite restorations [50]. Moreover, composite restorations can also be used to cover exposed root surfaces associated with non-carious cervical lesions resulting in enhanced aesthetics. In advanced cases where tooth-coloured restorations would lead to unacceptable clinical crown heights, a gingiva-coloured composite, ceramics, or removable veneers can be utilised to restore gingival aesthetics in patients where surgery is not an option [10]. Despite all these treatment options, endodontic treatment may be required as last step managing dentin hypersensitivity [1]. Gingival recession caused by tooth position outside the envelope of alveolar bone can be treated by means of orthodontic tooth movement. Any surgical intervention should be performed after completed orthodontic treatment. However, in patients who are not willing to start or resume orthodontic treatment, good results can be achieved with periodontal plastic surgery [3, 10].

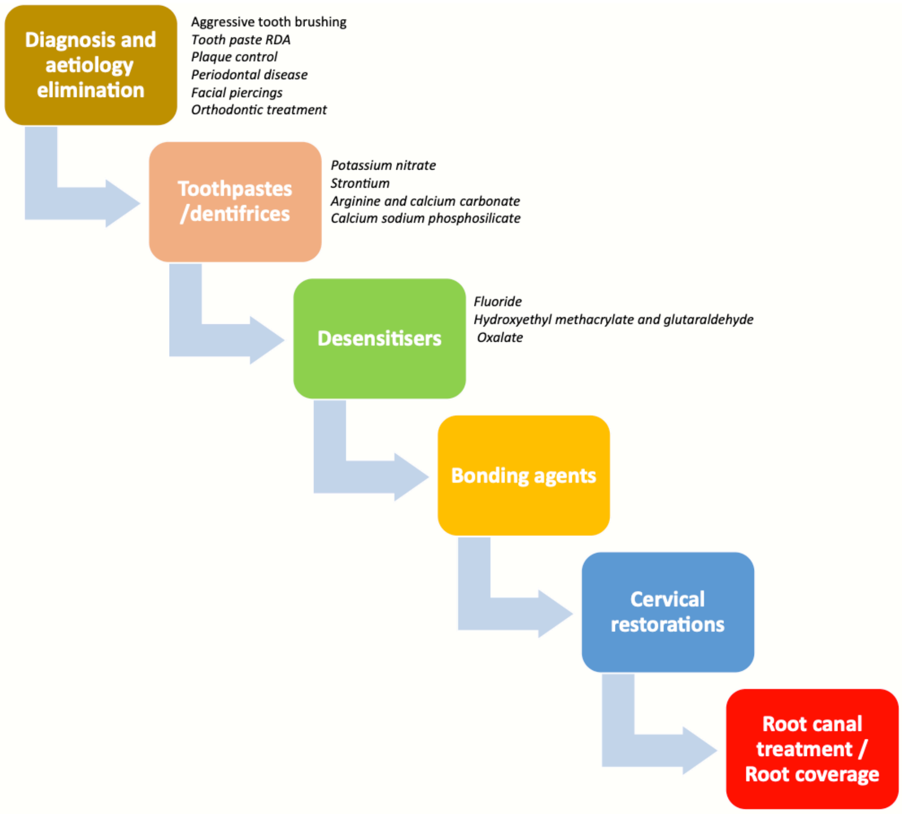

Figure 3. Illustration of non-surgical treatment options for dentin hypersensitivity.

1.3.2 Surgical Treatment

The main goal in the surgical treatment of gingival recession is a complete defect coverage in conjunction with good gingival aesthetics and minimal probing depth after wound healing [1, 52]. Although root coverage procedures were first described in the early 20th century, scientific interest did not awaken until the 1950s with several proposals for different surgical procedures to cover gingival defects [46, 53]. To date, root coverage periodontal plastic surgery techniques can be categorised as pedicle flaps, free subepithelial connective tissue flaps, matrix grafts, free gingival grafts, guided tissue regeneration and other techniques using varying biomaterials [46]. Pedicle flaps can be further divided into coronally advanced flaps and laterally repositioned flaps [1]. The decision to select a particular surgical technique depends on several factors. While some are related to the defect morphology (recession classification, clinical attachment level, width of keratinized gingiva, periodontal biotype, presence of frenula or muscle traction and depth of the vestibule), others are related to the patient (patient's expectation, compliance, systemic and local conditions, such as smoking, tooth brushing, plaque control, or pregnancy) [54, 55]. In 2019, Stein et al. presented a decision tree to help clinicians select the appropriate periodontal plastic surgery technique after careful analysis of patient-related, defect-oriented and technique-based factors (**Figure 4**) [56].

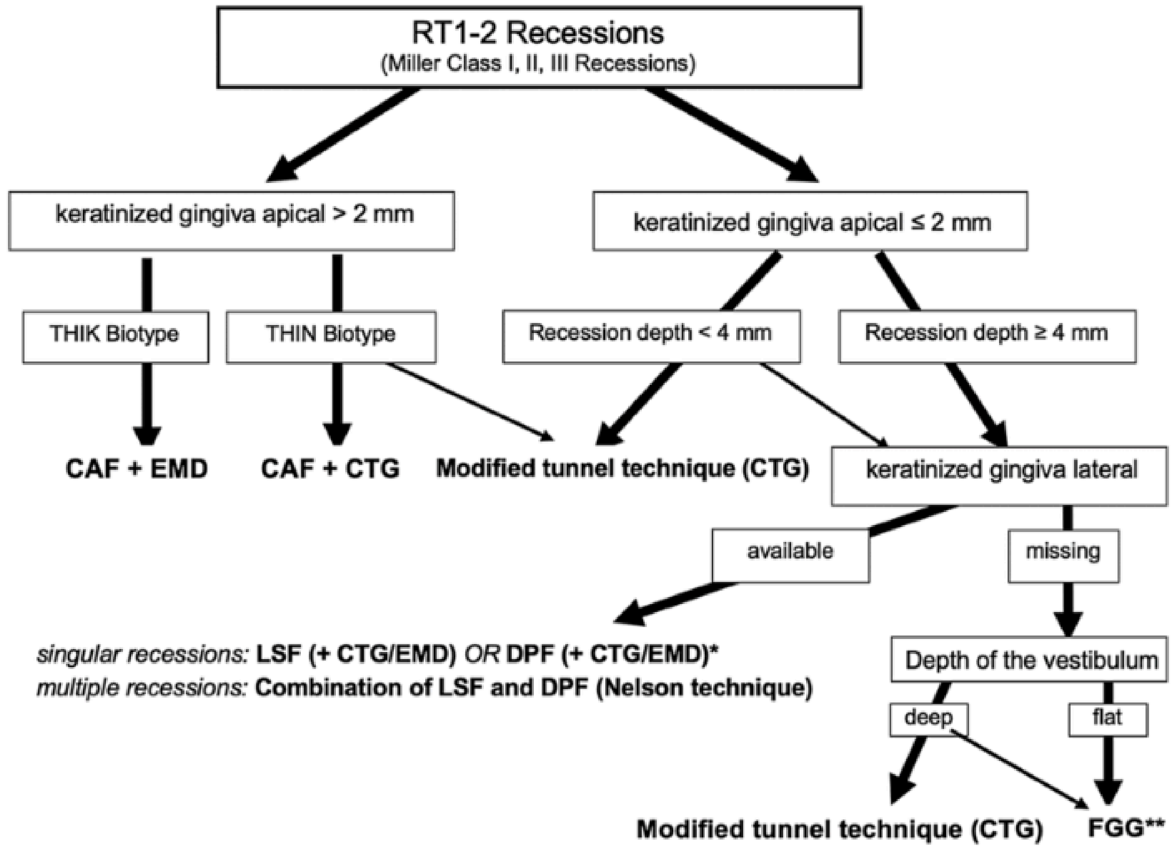

Figure 4. Stein's decision tree for the selection of the treatment strategy. CAF = coronally advanced flap, EMD = enamel matrix derivate, CTG = connective tissue graft = subepithelial connective tissue craft, LSF = lateral sliding flap = laterally repositioned flap, DPF = double papillae flap, FGG = free gingival graft,
** CAF can also be performed at a later point of time [56].

Although all periodontal plastic surgery techniques can lead to a significant reduction of gingival recession depth, it is unclear which intervention is the most effective. However, subepithelial connective tissue grafts combined with coronally advanced flaps alone or with a biomaterial/guided tissue regeneration should be preferably utilised in the treatment of gingival recession [52]. Combining coronally advanced flaps with enamel matrix derivate/matrix grafts/subepithelial connective tissue grafts can achieve better root coverage results than coronally advanced flaps alone [57].

Since the invention of the coronally advanced flap in 1926 by Norberg, this pedicle flap experienced a vast number of modifications [1]. Recent variations are coronally advanced tunnel techniques usually combined with subepithelial connective tissue grafts. It is uncertain whether this treatment technique results in similar clinical outcome (root coverage) compared to the coronally advanced flap with a subepithelial connective tissue graft. Therefore, the coronally advanced flap is still considered gold standard. However, most patients reported less pain after periodontal plastic surgery with the tunnel technique [58-60]. The double papillae flap and the laterally repositioned flap should be utilised in cases with enough keratinized gingiva available lateral to the gingival recession defect [56].

1.3.3 The Role of Enamel Matrix Derivate (EMD) in Periodontal Plastic Surgery

The adjunctive use of enamel matrix derivate in conjunction with the coronally advanced flap can improve clinical outcome and enhance wound healing [55]. In 1997, Hammarström et al. reported about the ability of enamel matrix derivate to promote periodontal regeneration [61]. Enamel matrix derivate consists largely of amelogenins along with other components such as enamelin, ameloblastin, amelotin, apin, and various proteinases which also play an important role in different aspects of periodontal regeneration [62, 63]. The behaviour of various cell types including epithelial cells, periodontal ligament fibroblasts, osteo- and cementoblasts, gingival fibroblasts, and bacteria are influenced by the enamel matrix derivate. These changes are conducted by mediating cell attachment, differentiation, proliferation, spreading, and survival as well as by the expression of transcription factors such as cytokines, growth factors, and molecules involved in the bone metabolism [64]. In addition, the enamel matrix derivate enhances angiogenic activity and periodontal soft tissue regeneration [65]. Due to these beneficial features the enamel matrix derivate is not only used in treating gingival recession defects, but also for a variety of other clinical applications in periodontal surgery such as in the treatment of furcation and intrabony defects (**Figure 5**) [66].

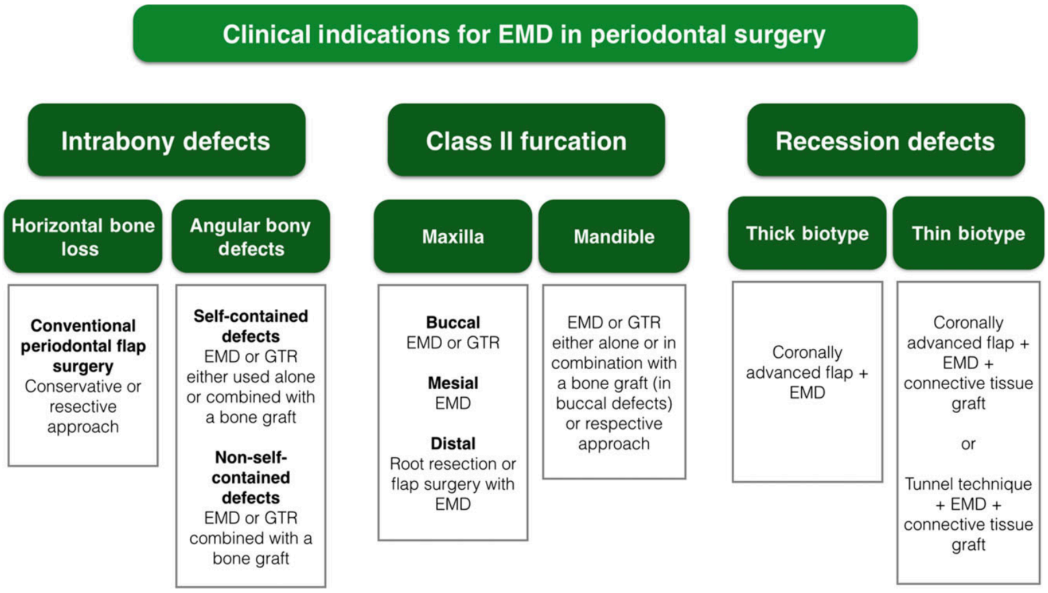

Figure 5. Clinical indications for enamel matrix derivate in periodontal surgery. EMD = enamel matrix derivate, GTR = guided tissue regeneration.

1.4 The Role of Collagen Matrix (CM) in Periodontal Plastic Surgery

Different types of collagens, predominately type I and II, have been established as a reliable and practicable biomaterial for several regenerative procedures in the field of dentistry. Available collagen materials differ by means of biodegradation and resorption and are designed for different areas of application [67]. Collagen matrices are usually manufactured from collagen-rich porcine, bovine, or human tissues. Skin typically serves as the tissue of origin, but pericardium, tendons, or intestinal mucosa are also used. Regardless of the origin tissue, almost all collagen matrices are enzymatically decellularized. After this process only the extracellular matrix remains which consists mainly of collagen and elastin. Decellularization is expected to remove all immunogenic MHC complexes to minimize rejection reactions in the recipient [68].

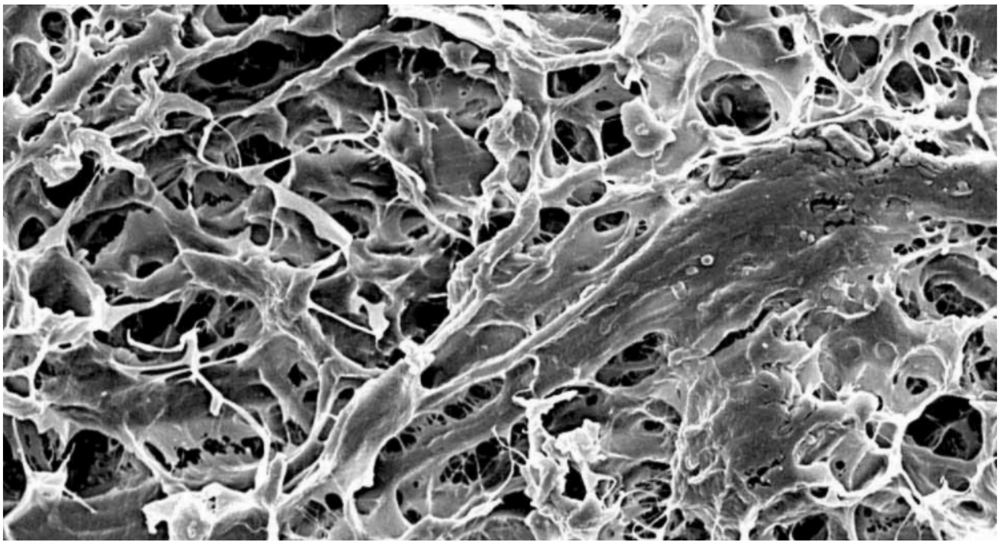

Figure 6. Microscopic image of the xenogeneic collagen matrix of Mucoderm® by biotiss [69].

In terms of soft tissue augmentation, harvesting of autologous free grafts is a standard procedure in modern periodontal plastic surgery [70]. However, these procedures are associated with pain and complications (e.g., haemorrhage, necrosis) at the donor site [71]. Hence, collagen matrix has been proposed as substitute to reduce the patients' donor site morbidity with good aesthetic and functional outcome [72]. Regarding the treatment of gingival recession defects, the use of collagen matrix improves the width of keratinized gingiva and gingival thickness compared to a coronally advanced flap alone. However, there is evidence that long-term root coverage cannot be significantly enhanced [73]. Comparing the collagen matrix with the connective tissue graft, the root coverage procedure with the autologous material had a higher percentage of long-term complete root coverage [74].

1.5 Aim of the Study

This prospective, randomized, controlled, monocentric study aimed to evaluate the periodontal plastic procedure using a coronally advanced flap with collagen matrix (Mucoderm®/CM) and enamel matrix derivate (Emdogain®/EMD) in the treatment of gingival recession defects. To the best of the authors' knowledge, this one of the first clinical investigations of this combination of medical products which should pave the way for an EMD-loaded collagen matrix as standard treatment. This should spare patients the additional harvesting procedure of autologous tissue and the associated donor site morbidity.

1.6 *Hypotheses*

1.6.1 Null Hypothesis (H0)

The use of an EMD-loaded (Emdogain®) collagen matrix (Mucoderm®) in combination with a coronally advanced flap (CAF) do not significantly differ in terms of effective root coverage of gingival recession from the periodontal plastic procedure using the coronally advance flap with an unloaded collagen matrix (Mucoderm®).

1.6.2 Alternative Hypothesis (H1)

The use of an EMD-loaded (Emdogain®) collagen matrix (Mucoderm®) in combination with a coronally advanced flap (CAF) do significantly differ in terms of effective root coverage of gingival recession from the periodontal plastic procedure using the coronally advance flap with an unloaded collagen matrix (Mucoderm®).

1.6.3 Secondary Null Hypothesis (H0)

The use of an EMD-loaded (Emdogain®) collagen matrix (Mucoderm®) in combination with a coronally advanced flap (CAF) do not significantly differ from the periodontal plastic procedure using the coronally advance flap with an unloaded collagen matrix (Mucoderm®) in terms of keratinized gingiva width in the treatment of gingival recession defects.

1.6.4 Secondary Alternative Hypothesis (H1)

The use of an EMD-loaded (Emdogain®) collagen matrix (Mucoderm®) in combination with a coronally advanced flap (CAF) do significantly differ from the periodontal plastic procedure using the coronally advance flap with an unloaded collagen matrix (Mucoderm®) in terms of keratinized gingiva width in the treatment of gingival recession defects.

2 Material und Methods

This prospective randomized, controlled study was conducted at the tertiary clinical centre of the Medical University of Graz, Austria and registered in the European Union Clinical Trials Register (NCT05799859). This clinical trial was carried out following all local legal requirements and the Declaration of Helsinki (1975) and included the approval of the Ethics Committee of the University (IRB00002556, No.: 28-123 ex 15/16). Prior to the beginning of the study, informed consent was obtained from all subjects before treatment. All surgical procedures and complete follow-ups were conducted at the University Clinic of Dentistry and Oral Health, Medical University Graz.

2.1 Study Population

The study cohort included 15 patients (Group A: n=7, Group B: n=8) from the University Clinic of Dentistry and Oral Health, Medical University Graz. The recruitment period was from October 2019 to September 2021. Participation in the study lasted 13 months per subject.

2.1.1 Inclusion Criteria

- Signed informed consent
- Age > 18 and < 80 years
- Male, female or unknown sex
- Normal overall medical condition (systemically and periodontally healthy)
- Patients with plaque control (full mouth plaque score < 20 %)
- Patients with no prior experience of root coverage procedures
- Patients with at least one gingival recession defect categorised as Miller class 1 or 2/Cairo class RT1

2.1.2 Exclusion Criteria

- General contraindications to dental surgery under local anaesthesia (e.g., severe systemic diseases, tumours, severe cardiovascular diseases, uncontrolled diabetes mellitus)
- Ongoing or previous chemo-, radio-, or bisphosphonate therapy
- Diseases of the hematopoietic system (e.g., acute, and chronic leukaemia, malignant lymphoma, multiple myeloma)
- Patients with impaired compliance
- Heavy smokers (> 10 cigarettes/day)
- Pregnancy (female patients were asked to perform a pregnancy test at visit 1, 2, 4–8 (HCG Sensitive Urine Testing))
- Disorders or treatments that impair wound healing
- Long-term treatment with high-dose steroids or anticoagulants
- Bone metabolism disorders
- (Acute) infections or vascular disorders in the region to be treated
- Known hypersensitivity to porcine collagen
- Patients with severe peripheral artery disease or autoimmune diseases
- Nursing mothers
- Extruded or malpositioned teeth

2.2 Study Design

This study was conducted as a prospective, randomised, controlled, monocentric study.

2.2.1 Treatment Overview

Prior to surgery, all patients enrolled in the study received a full periodontal examination (i.e., probing pocket depth (PPD), full mouth bleeding score (FMBS), full mouth plaque score (FMPS), width of keratinized tissue (WKT) and recession height (RECH)), followed by periodontal therapy consisting of oral hygiene instructions and professional dental prophylaxis. If necessary, deep scaling and root planing was performed. PPD (i.e., distance from gingival margin to bottom of gingival sulcus), RECH (i.e., distance from cementoenamel junction to gingival margin) and WKT (i.e., distance between gingival margin and muco-gingival junction) were measured using a calibrated periodontal probe. Four weeks after initial therapy, all patients underwent a second periodontal examination. PPD and RECH were used to determine the clinical attachment level (CAL). An intraoral scan was taken in all patients prior to surgery.

After patient selection, group allocation (1 or 2) was performed using a digital randomisation software (Randomizer® for clinical trials: www.randomizer.at):

Group A was treated with a standard porcine collagen matrix (Mucoderm®, Botiss GmbH, Berlin, Germany/ CM) + coronally advanced flap (CAF).

Group B was treated with a standard collagen matrix (Mucoderm®, Botiss GmbH, Berlin, Germany/CM) + enamel matrix protein (Emdogain®, Straumann, Basel, Switzerland/EMD) + CAF.

2.2.2 Surgical Procedure

All surgical procedures were performed by experienced periodontal surgeons at the University Clinic of Dentistry and Oral Health, Medical University Graz. Prior to surgery local anaesthesia (e.g., Articaine Ultracain® dental forte, Sanovi-Aventis, Vienna, Austria) was injected adjacent to the recession in all patients.

Surgery:

A #15 blade was used to prepare a mucosal split flap. An intra-sulcular incision and vertical relief incisions mesial and distal to the recession were made. The adjacent papillae were not integrated into the flap design and thus not split. The vertical reliefs were performed starting at the cementoenamel junction at the mesial and distal line angle of the tooth obliquely divergent in the attached gingiva and a few millimetres into the oral vestibule.

A complementary horizontal incision (periosteal slit) was made at the apical base of the flap to mobilise and release the periosteum and muscle fibres. This allowed elongation and tension-free positioning of the flap.

Root preparation was performed with hand, rotary, or ultrasonic instruments. A fine-grained diamond bur (e.g., Perio-Set®, Intensiv SA, Grancia, Switzerland) was used to remove convexities, indentations, and sharp edges on the root surface. Subsequently, the root surface was rinsed to clean and remove mobile fragments.

Thereafter, the further procedure depended on the group assignment:

Treatment group A: After measuring the gingival recession defect by means of a periodontal probe, a rehydrated collagen matrix (Mucoderm®) of appropriate size was cut. The collagen matrix was soaked in sterile saline solution at the beginning of the surgery.

Treatment group B: All previously described steps were also performed in group B, but after root planing, a synthetically produced 24 % EDTA liquid (PrefGel®, Straumann) was applied for 2 minutes and then completely rinsed off with a physiological saline solution. Additionally, collagen matrix (Mucoderm®, Botis GmbH, Berlin, Germany) and root surface were loaded with enamel matrix derivate (Emdogain®, Straumann).

Regarding both groups, fixation of the matrix was done with 6.0 absorbable single button sutures. The coronally advanced flap (CAF) was fixed at least 1mm coronal to the cementoenamel junction with 6.0 monofilament non-absorbable single button sutures.

Postoperatively, patients were instructed to abstain from brushing the treated area and rinse their mouths twice daily with a 0.12 % chlorhexidine solution for 2 weeks. Anti-inflammatory therapy was administered for at minimum of 2 days (e.g., ibuprofen 400 mg) and was extended if required. Patients were asked to document the amount and duration of analgesics taken. Suture removal and photo documentation was completed 10 days after the procedure.

2.2.3 Clinical Visits

Visit 1 (1 month before surgery ± 3 days):

The patient was informed about the clinical trial and asked for signing the informed consent form. A review of the inclusion and exclusion criteria was done. A pregnancy test was performed for women of childbearing age. Furthermore, the patient's medical history and medication were taken. Also the patient received a precise and detailed explanation of the surgical procedure. Afterwards, a periodontal examination and treatment, an intraoral scan and, if no radiological images were available or if they were older than 1 month, a radiological examination was performed. Additionally, the patient was given oral hygiene instructions.

Visit 2 (surgery):

After checking for any changes in medication, a pregnancy test was performed if applicable. After randomisation, the surgical procedure was performed according to the assigned group. Periodontal examination and photo documentation were also carried out.

Visit 3 (1 day after surgery):

Oral hygiene instructions were given to the patient and the course of healing was monitored.

Visit 4 (10 days after surgery):

Wound assessment and suture removal.

Visit 5, 7 and 8 (1, 6, 12 months after surgery ± 3 days, respectively):

A periodontal examination and photo documentation were performed. In addition, a pregnancy test was carried out for women of childbearing age. Furthermore, a radiological examination was made at visit 8.

Visit 6 (3 months after surgery ± 3 days):

An optical impression by means of an intraoral scanner was taken. A periodontal examination and photo documentation were carried out. In addition, a pregnancy test was performed for women of childbearing age.

At each visit, an assessment was made to determine whether a serious adverse event or a change in medication had occurred.

2.3 Analysed Parameters

The study's analysed primary outcome parameters were RECH and WKT after gingival recession defect coverage. PPD, FMPS, FMBS and CAL were monitored to evaluate periodontal health within the patient collective. The optical impressions in visits 1 and 6 were digitally used to analyse distance, volume change, exposed root surface, as well as gained soft tissue thickness after root coverage. The 3D-Slicer® (Available at: https://www.slicer.org, [75]) and the OraCheck® software (Dentsply Sirona. Released 2019. OraCheck for Windows, Version 5. Charlotte, NC, USA. Available at: https://www.dentsplysirona.com/en-us/discover/discover-by-brand/oracheck.html) were used to analyse the intraoral scans. Demographic data were collected concerning the following parameters: gender, age, height, weight, BMI, and medication.

2.4 Statistical Analysis

All data were anonymized before usage and stored in a protected Microsoft Excel™ database (Microsoft Released 2023. Microsoft® Excel for Mac, Version 16.70. Redmont, WA, USA). Descriptive and analytical statistics were employed to analyse the parameters of this study. Analytical statistics included T-tests, mixed model and the general linear model with repeated measures which were used when appropriate. For all calculations, a p-value of $< 0{,}05$ was considered statistically significant. All statistical analyses were performed using the statistical software package SPSS software (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY, USA). To additionally demonstrate the statistical distribution, the study's data is presented using tables and figures.

2.5 Data Management

The recorded data were collected in a case report form. The collection, transfer, storage, and analysis of personal data within this study was carried out in accordance with legal regulations. Ensuing completion of the study, test forms and investigator site files will be stored in the archives of the University Clinic of Dentistry and Oral Health, Medical University of Graz for 15 years.

3 Results

A total of 15 patients (*n = 15*) were enrolled in the main trial with 7 patients (*n = 7*; 47 %) randomized for treatment group A and 8 patients (*n = 8*; 53 %) for treatment group B. Of these 15 patients, 24 teeth (*n = 24*) were treated with periodontal plastic surgery (treatment group A/CAF + CM: *n = 11*; treatment group B/CAF + CM + EMD: *n = 13*). Patient characteristics did not differ between group A and the group B, with a similar mean age of 30.4 ± 4.6 and 31.2 ± 8.2 years ($p = 0.823$) and a similar mean BMI (group A: 20.2 ± 2.2; group B: 20.7 ± 1.6; $p = 0.583$) and sex ($p = 0.182$). All patients had a full-mouth bleeding and plaque score of < 20 % at the time of the surgery. No adverse event during the procedure or healing period was observed. The demographic case characteristics are presented in **Table 1**.

Table 1. Detailed description of patient characteristics. SD = standard deviation

	GROUP A	GROUP B	P-VALUE
AGE			*p = 0.823*
MIN	26.0	19.9	
MAX	38.3	40.7	
MEAN	30.4	31.2	
SD	4.6	8.2	
SEX			*p = 0.182*
MALE	1 (14.3 %)	3 (37.5 %)	
FEMALE	6 (85.7 %)	5 (62.5 %)	
TOTAL	7	8	
BMI			
MIN	17.4	18.9	*p = 0.583*
MAX	24.0	23.1	
MEAN	20.2	20.7	
SD	2.2	1.6	

3.1 Clinically Obtained Parameters

Mean pocket probing depth (PPD) in group A was 1.00 ± 0.00 mm and 1.60 ± 0.77 mm in group B before treatment. Following the periodontal plastic surgery procedure (visit 2), the PPD did not change in group A and decreased to 1 mm in group B. Mean PPD values over time are presented in **Figure 7.**

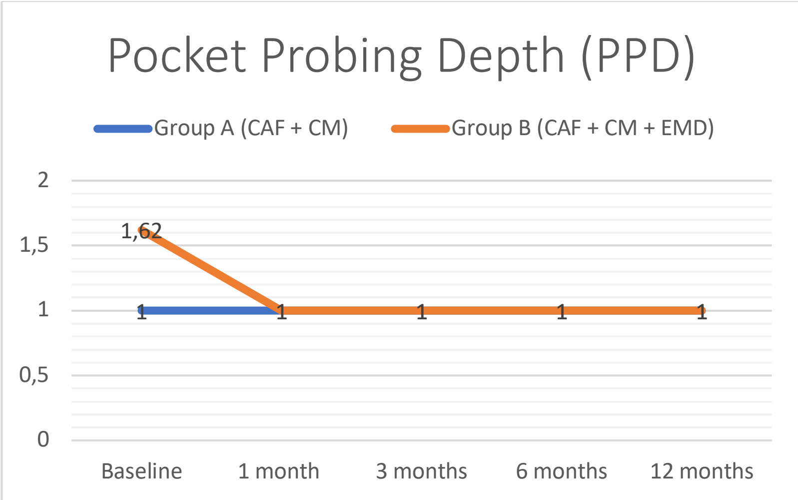

Figure 7. Mean pocket probing depth over time measured in mm. Surgery was performed at visit 2.

The mean clinically measured preoperative recession height (RECH) was similar between group A and B with 3.91 ± 1.22 and 3.77 ± 1.01, respectively. The general linear model with repeated measures showed a statistically significant change of RECH over time and slope for both groups (group A: $p < 0.001$; group B: $p < 0.001$). With reference to the distance of the two courses independent of the slope, there was no statistically significant difference identified ($p = 0.071$). The mean RECH values over time are illustrated in **Figure 8.**

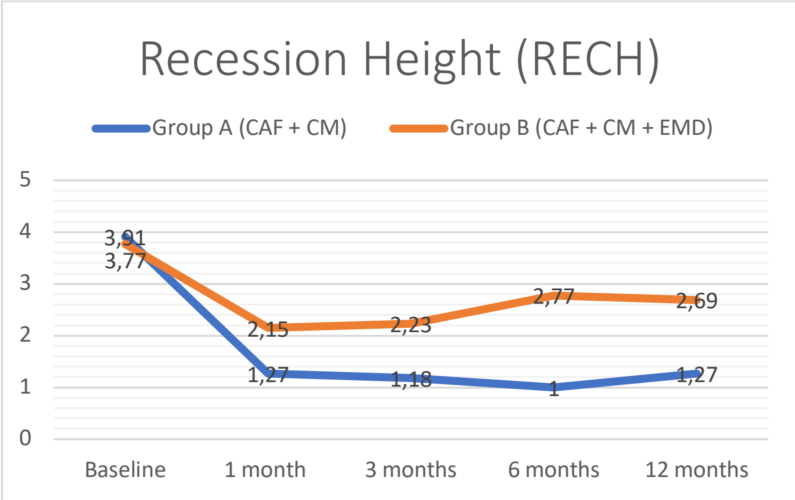

Figure 8. Mean recession height over all performed visits measured in mm.

The mean clinical attachment loss (CAL) was initially equivalent with 5.46 ± 1.51 mm in group A and 5.36 ± 1.63 mm in group B. Concerning the distance of the two courses independent of the slope, the general linear model with repeated measures showed no statistically significant difference ($p = 0.083$). Nevertheless, both groups showed a significant decrease of CAL over time (group A: $p < 0.001$; group B: $p < 0.001$) and over slope (group A: $p < 0.002$; group B: $p < 0.002$). The mean CAL values of both groups over time are depicted in **Figure 9.**

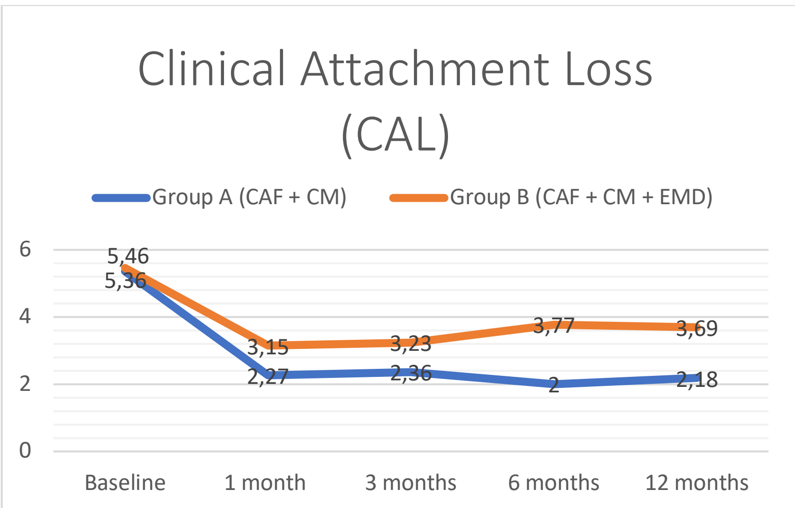

Figure 9. Mean clinical attachment level over time measured in mm.

The mean width of keratinized tissue (WKT) apical to the gingival recession defect was 1.27 ± 0.65 mm in group A and 1.31 ± 0.95 mm in group B. After periodontal plastic surgery the WKT increased in both groups significantly over time (group A: $p < 0.001$; group B: $p < 0.001$). There was no significant alteration regarding the slope over time (group A: $p = 0.074$; group B: $p = 0.074$) or difference between the groups (group A: $p = 0.690$; group B: $p = 0.690$). **Figure 10** shows clinically measured mean WKT over time.

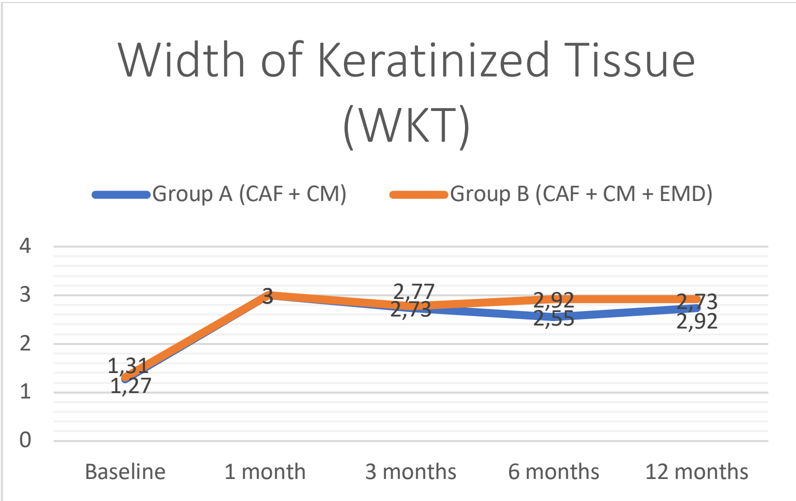

Figure 10. Mean width of keratinized gingiva over all visits measured in mm.

3.2 Digitally Obtained Parameters

Digitally measured recession defects assessed from the intraoral scans at visit 1 showed a mean RECH of 3.66 ± 1.35 mm in group A and 3.60 ± 1.09 mm in group B. Three months after surgery (visit 6), the RECH were reassessed and showed an improvement to 1.18 ± 1.07 mm and 2.47 ± 1.47 mm, respectively. This corresponds to a root coverage of 69 ± 28 % in group A and 36 ± 32 % in group A. There is no statistically significant difference between the groups with regard to root coverage; both measured as the difference in RECH between baseline and three months postoperatively ($p = 0.054$) and the percentual coverage ($p = 0.094$) (**Figure 11**).

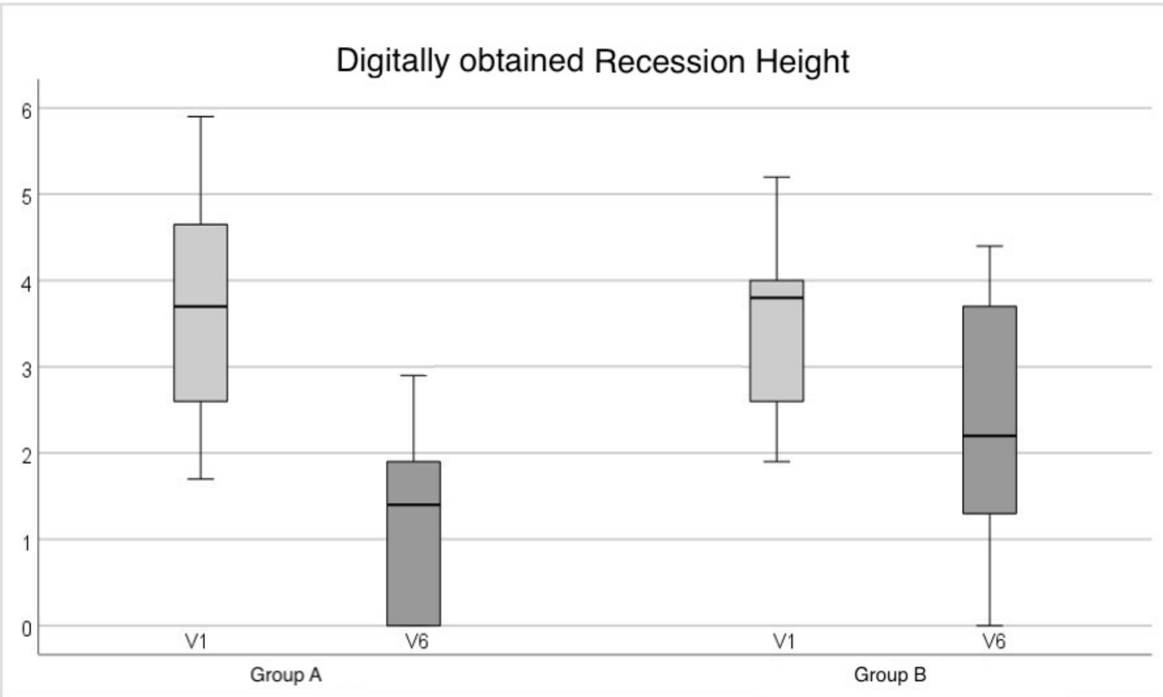

Figure 11. Boxplots illustrating the changes in recession height before and 3 months after periodontal plastic surgery with Mucoderm (group A) or Mucoderm with Emdogain (group B). RECH were digitally measured in mm using intraoral scans.

Preoperatively, the mean area of recession (i.e., exposed root surface) was 11.45 ± 6.01 mm^2 and 13.95 ± 5.23 mm^2 in Groups A and B, respectively. Three months postoperatively, the mean exposed root surface area decreased to 3.02 ± 2.57 mm^2 in the CAF + CM group and to 10.59 ± 7.01 mm^2 in the CAF + CM + EMD group ($p = 0.065$). A statistically significant difference between the groups regarding the percentual root coverage was detected with 72 ± 28 % (Group A/CAF + CM) and 31 ± 32 % (Group B/CAF + CM + EMD) ($p = 0.031$). The reported range of root coverage in Group B is a result of a treated recession that worsened after the conducted periodontal plastic surgery. The digitally obtained results regarding exposed root surface are illustrated in **Figure 12.**

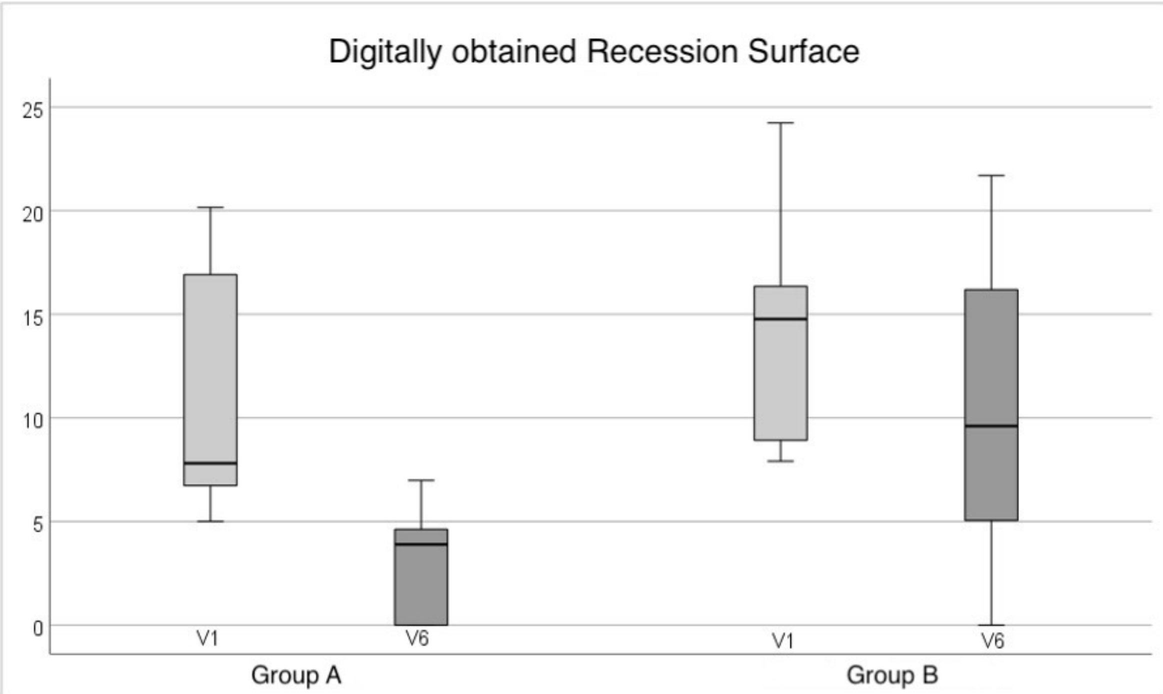

Figure 12. Boxplots showing the changes in recession surface/exposed root surface before and 3 months after periodontal plastic surgery with CAF + CM (group A) or CAF + CM + EMD (group B). Gingival recession surface was digitally measured in mm using intraoral scans.

Volume changes before and after surgery ranged from 3–33 mm^3 (mean 19 ± 10 mm^3) in group A and from minus 2–32 mm^3 (mean 10 ± 8 mm^3) in group B (**Figure 13**). There was no significantly difference between the two groups ($p = 0.066$). **Figure 14** depicts the assessment of the volume changes by means of the OraCheck® software.

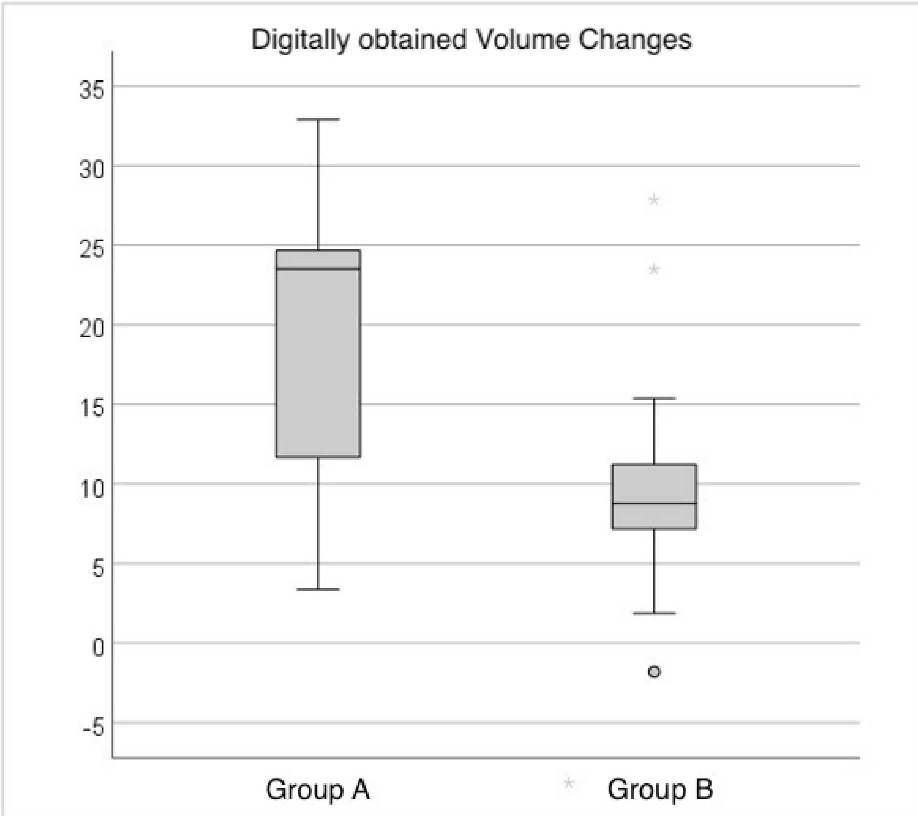

Figure 13. Boxplots representing the volume changes in mm^3 between visits 1 (preoperatively) and visit 6 (3 months after surgery).

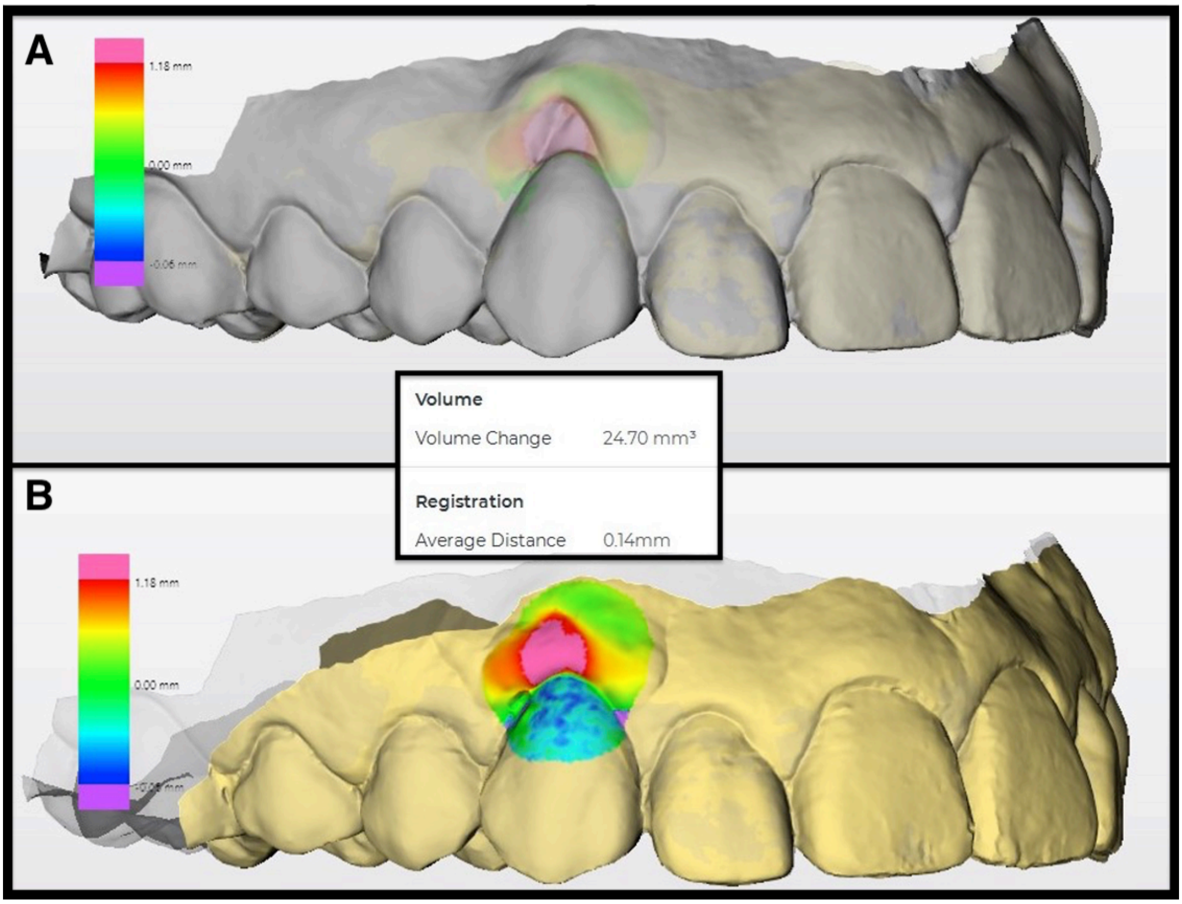

Figure 14. Digital assessment of the volume change before and 3 months after periodontal plastic surgery. A: Preoperative scan of the upper jaw. B: Final aspect after 6 months. Colors indicate the difference between the matched oral scans.

The thickness of soft tissue coverage was measured in the mid sagittal plane of the treated tooth. In group A, the mean soft tissue thickness covering the gingival recession defect measured 1.05 ± 0.44 mm and ranged from 0.5 to 2.18 mm. The soft tissue thickness in group B ranged from 0 to 1.22 mm with a mean value of 0.56 ± 0.41 mm (**Figure 15**). The mixed model test demonstrated that the difference between the CAF + CM and the CAF + CM + EMD groups is statistically significant with a p-value of 0.044.

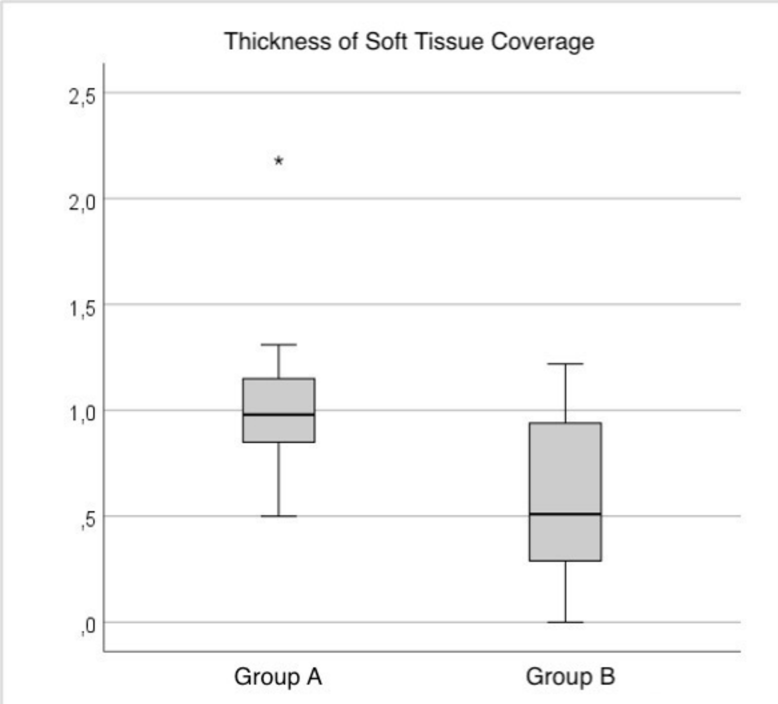

Figure 15. Boxplots illustrating the thickness of the gained soft tissue covering the previous gingival recession defect measured in mm.

4 Discussion

In periodontal plastic surgery, there are several options to cover exposed root surfaces using a variety of proven surgical methods and materials. The principal goal of all alternatives is to achieve predictable, long-term, and complete root coverage in conjunction with healthy gingival conditions and low patient morbidity. To reduce morbidity caused by autologous gingiva harvesting and to allow simultaneous treatment of multiple recession defects, the use of xenogeneic collagen matrices (CM) is a standard treatment option which offers several advantages for both the patient and surgeon. Various studies have already described benefits of biomaterials in the treatment of gingival recession compared to coronally advanced flaps (CAF) [52, 73, 76-78]. The biological intent of the collagen matrix is to act as a barrier against the proliferation of epithelial cells into the defect [79]. Additional application of enamel matrix derivatives (EMD) should improve the environment of periodontal-associated cells to promote regeneration and thereby better root coverage [80].

This randomized controlled study included RT1 defects which were treated by a CAF-technique using a xenogeneic collagen matrix (group A: Mucoderm®/CAF + CM) alone or in combination with an enamel matrix derivate (group B: Mucoderm® + Emdogain®/CAF + CM + EMD). This study used clinically measured parameters (RECH, CAL, WKT, PPD) and compared them with digitally obtained parameters using intraoral scans (RECH, recession surface, volume change, thickness of soft tissue coverage).

Both tested approaches demonstrated a statistically significant improvement regarding the root coverage in RT1 recession. Evaluating the RECH outcomes clinically with a periodontal probe, both groups showed stable results over the complete follow-up period (1 year) and there was no statistically significant difference between the two groups detectable. With respect to the digitally obtained percentage of root coverage, group A (CAF + CM) was superior to group B (CAF + CM + EMD) with 72 ± 28 % and 31 ± 32 %, respectively. This percentage was calculated based on the area of the exposed root surfaces (baseline and 3 months postoperatively, measured in mm^2) which was obtained through intraoral scans and analysed digitally. Although quite similar results were obtained when the percentages were compared based on the maximum extent of gingival recession defects (69 ± 28 % in group A and 36 ± 32 % in group B), no statistically significant difference could not be detected. This difference is due to the measurement method and raises the question as to which digital evaluation method should be considered the gold standard in periodontal plastic surgery. Measuring the actual change of the exposed root surface may be more appropriate rather than to measuring recession height. **Figure 16** shows the digital workflow using the 3D-Slicer® software.

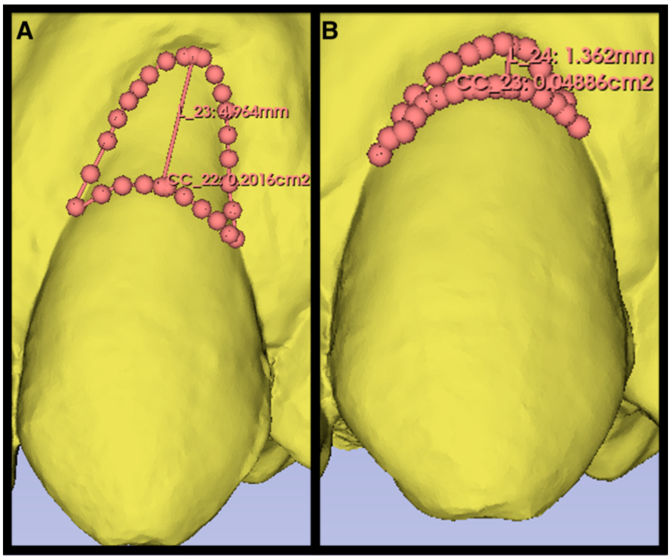

Figure 16. Digitally obtained surface of the exposed root. A: preoperative scan of a 40-year-old patient with a recession height of 4.96 mm and a recession area of 20.16 mm^2, B: 3 months postoperative scan with a recession height of 1.36 mm and a recession area of 4.88 mm^2.

The obtained root coverage results of this study are inferior when compared to the results of Sangiorgio et al. (6 months postoperatively: CAF + CM: percentage of root coverage: 87.2 ± 15.01 % and CAF + CM + EMD: percentage of root coverage: 91.59 ± 11.08 %). The varying inclusion criteria of the studies could be an explanation for these circumstances. In the present study, tooth type (incisor, canine, molar) and location (maxilla or mandible) did not play a role, however, in the study by Sangiorgio et al. only maxillary canines or premolars were treated. When comparing the two treatment groups in this study, three molars were treated in group B (CAF + CM + EMD) and no molar in group A (CAF + CM). The more surgically demanding procedure for posterior teeth, may have led to a worsening of the postoperative outcome; which would accordingly lead to inferior results in group B. In group B, 9 out of 13 teeth were in the mandible, while in group A, 9 out of 11 teeth were in the maxilla. The shallow vestibulum depth, the higher incidence of aberrant frenula with consecutive pull of the soft tissues, the inferior vascularisation compared to the maxilla, as well as the generally smaller surgical field (e.g., lower incisors) in the mandible may have led to a deterioration of root coverage. The difference between the two investigated groups demonstrates the importance of proper patient selection in periodontal plastic surgery.

EMD is successfully used in a wide range of periodontal plastic surgery procedures, including root coverage, periodontal regeneration, and implant dentistry because of its ability to regenerate lost periodontal tissue, as demonstrated in several studies, in both humans and animals. Histological evaluation showed formation of new bone, cementum, and periodontal ligament [81-84]. A histomorphometric study in minipigs revealed that the use of EMD can improve periodontal regeneration regardless of its combination with a collagen matrix [79]. Additionally, higher postoperative soft tissue thickness was observed when treated with both EMD and CM, rather than when using only one biomaterial, or CAF alone [79]. In the present study, the soft tissue thickness was also determined digitally, which is a novel reliable assessment method without having to penetrate the gingiva (**Figure 17**) [85]. The digitally obtained results showed statistically significant superior results in group A (CAF + CM) compared to group B (CAF + CM + EMD). An investigation by Chambrone and Tatakis described the positive effect of keratinized tissue thickness on the long-term prevention of gingival recession [86].

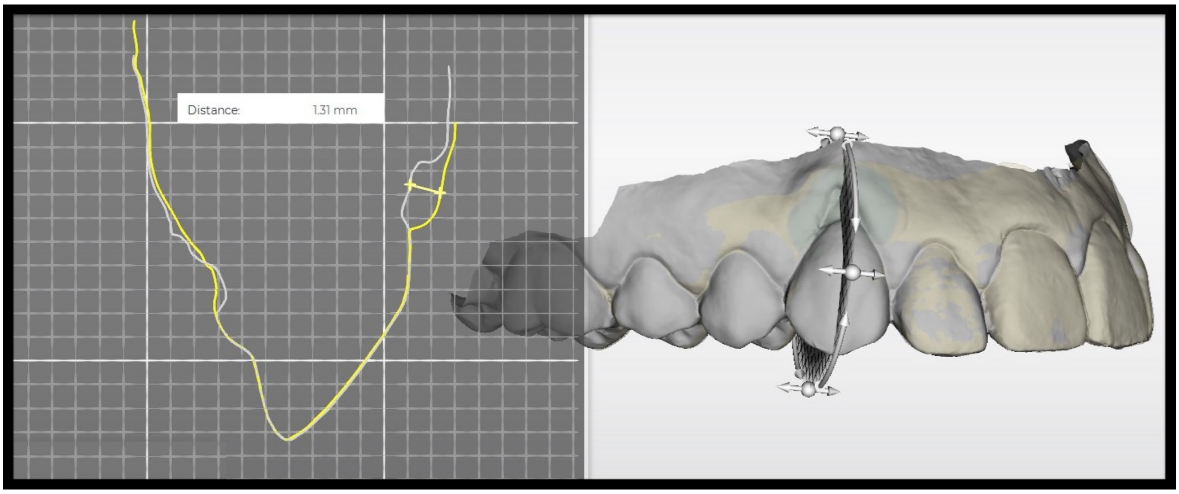

Figure 17. Digitally obtained change in soft tissue thickness using the OraCheck® software by Dentsply Sirona.

There are several studies available comparing the solely use of EMD to a combination of EMD and subcutaneous tissue graft (SCTG). One year postoperatively, Rasperini et al. reported a root coverage of 90 % in the SCTG + EMD group and 80 % in the STCG group [87]. In comparison, Roman et al. observed 82.3 % for SCTG + EMD and 89.8 % for SCTG alone [88]. In both studies, the concurrent use of EMD and SCTG did not show a significant additional benefit. While the use of EMD provides significant improvement over CAF alone, multiple combinations of biomaterials may offer similar or fewer benefits compared to periodontal plastic procedures using only one biomaterial [89]. However, a histologic examination of human buccal recession defects demonstrated that sites treated with EMD showed periodontal regeneration. In SCTG treated sites this kind of regeneration could not be found, despite similar clinical outcomes [82].

The present study demonstrates additional width of keratinized tissue (WKT) in both groups one year postoperatively (group A/CAF + CM: 1.61 mm and group B/CAF + CM + EMD: 1.46 mm). This is in accordance with the results of Cardaropoli et al. and McGuire and Scheyer, which reported a WKT gain after periodontal plastic surgery using CAF + CM of 1.23 and 1.34 mm, respectively [90, 91]. Comparing CAF + CM with CAF, Jepsen et al. reported a superior outcome of the CAF + CM group with 0.37 mm more WKT gain compared to the CAF group [73]. Evaluating the impact of EMD regarding the WKT, Cardaro et al. observed no impact using EMD (CAF: 0.31 mm and CAF + EMD: 0.28 mm), whereas Del Pizzo et al. demonstrated a WKT gain over time (CAF: 0.47 mm and CAF + EMD: 1.00 mm) [78, 92]. Despite these findings, several authors have observed no statistically significant difference when comparing various treatment alternatives [76, 93-95].

Although specific inclusion and exclusion criteria were applied in this study and established standard evaluation methods were used, some limitations must be considered when interpreting the study's results. The study's sample size with 15 patients and 24 treated sites prohibits generalizing the found results. However, the conducted prospective randomized controlled trial is one of first investigations evaluating the periodontal plastic surgery procedure using a coronally advanced flap (CAF) with a collagen matrix (CM) and an enamel matrix derivate (EMD) over a 12 month time period [76, 96]. In this study, the kind of teeth (i.e., incisor, canine, premolar, molar) were not an inclusion criterion. Therefore, the influencing effect that results from the more surgically demanding procedure for posterior and mandible teeth could not have been evaluated in this study. This bias has to be presented as it demonstrates the clinically important factor of patient and site selection for periodontal plastic surgery procedures and additionally allows reasonable outcome predictions for both the patient and surgeon.

5 Conclusion

The present trial evaluated the periodontal plastic surgery procedure using a coronally advanced flap (CAF) with a collagen matrix (CM) and an enamel matrix derivate (EMD) to cover gingival recession defects within a homogenous patient collective. This procedure was compared to the established surgical procedure using a CAF with a CM. All patients ($n = 15$) in this randomized controlled trial underwent gingival recession coverage with intraoperative allocation in one of the two treatment groups and received a follow up until one year postoperatively. Regarding the null hypothesis (H0) that the use of an EMD-loaded CM in combination with a CAF do not significantly differ in terms of effective root coverage of gingival recession from the periodontal plastic procedure using the CAF with an unloaded CM, no statistically significant differences were found. Consequently, the hypothesis (H0) can be confirmed. Regarding the second null hypothesis (H0) that these procedures do not differ in terms of keratinized gingiva width in the treatment of gingival recession defects, no statistically significant differences could be obtained. Hence, the second null hypothesis (H0) can be confirmed. Although both surgical techniques showed statistically significant improvement of the gingival recession defect, the additional use of EMD seems not to clinically improve the outcome. The present study indicated inferior root coverage results of the CAF + CM + EMD compared to the CAF + CM group. The found divergences between the treatment groups are likely associated to the small study size causing a heterogenous distribution of treated teeth. Nevertheless, it demonstrates the essential role of patient selection and offers outcome predictions in cases besides the ideal maxillary canine. Both surgical techniques showed gain regarding the keratinized tissue width (WKT). Additionally, reliable evaluation methods of periodontal plastic surgery procedures were presented without the need of penetrating the gingiva, take impressions, or use a digital caliper tool.

As a future work project further similar studies on a multicentred basis with a larger patient collective are required to analyse the EMD-loaded CM periodontal plastic surgery technique in more detail. Additionally, a split-mouth design would be needed to evaluate the gingival recession defect coverage technique's influence even better regarding the outcome prediction in all teeth.

References

1. Imber, J.C. and A. Kasaj, *Treatment of Gingival Recession: When and How?* Int Dent J, 2021. **71**(3): p. 178-187.
2. Meza Mauricio, J., et al., *Does enamel matrix derivative application provide additional clinical benefits in the treatment of maxillary Miller class I and II gingival recession? A systematic review and meta-analysis.* Clin Oral Investig, 2021. **25**(4): p. 1613-1626.
3. Kassab, M.M. and R.E. Cohen, *The etiology and prevalence of gingival recession.* J Am Dent Assoc, 2003. **134**(2): p. 220-5.
4. Vignoletti, F., et al., *Prevalence and risk indicators of gingival recessions in an Italian school of dentistry and dental hygiene: a cross-sectional study.* Clinical Oral Investigations, 2020. **24**(2): p. 991-1000.
5. Thomson, W.M., R. Hashim, and A.R. Pack, *The prevalence and intraoral distribution of periodontal attachment loss in a birth cohort of 26-year-olds.* J Periodontol, 2000. **71**(12): p. 1840-5.
6. Seong, J., et al., *Prevalence of gingival recession and study of associated related factors in young UK adults.* Journal of Dentistry, 2018. **76**: p. 58-67.
7. Albandar, J.M. and A. Kingman, *Gingival recession, gingival bleeding, and dental calculus in adults 30 years of age and older in the United States, 1988-1994.* J Periodontol, 1999. **70**(1): p. 30-43.
8. Baker, P. and C. Spedding, *The aetiology of gingival recession.* Dent Update, 2002. **29**(2): p. 59-62.
9. Gorman, W.J., *Prevalence and etiology of gingival recession.* J Periodontol, 1967. **38**(4): p. 316-22.
10. Patel, M., P.J. Nixon, and M.F. Chan, *Gingival recession: Part 1. Aetiology and non-surgical management.* Br Dent J, 2011. **211**(6): p. 251-4.
11. Gillette, W.B. and R.L. Van House, *Ill effects of improper oral hygeine procedure.* J Am Dent Assoc, 1980. **101**(3): p. 476-80.
12. Breitenmoser, J., W. Mörmann, and H.R. Mühlemann, *Damaging effects of toothbrush bristle end form on gingiva.* J Periodontol, 1979. **50**(4): p. 212-6.
13. Khocht, A., et al., *Gingival recession in relation to history of hard toothbrush use.* J Periodontol, 1993. **64**(9): p. 900-5.
14. Heasman, P.A., et al., *Evidence for the occurrence of gingival recession and non-carious cervical lesions as a consequence of traumatic toothbrushing.* J Clin Periodontol, 2015. **42 Suppl 16**: p. S237-55.
15. Rajapakse, P.S., et al., *Does tooth brushing influence the development and progression of non-inflammatory gingival recession? A systematic review.* J Clin Periodontol, 2007. **34**(12): p. 1046-61.
16. Matthews, D.C., *No good evidence to link toothbrushing trauma to gingival recession.* Evid Based Dent, 2008. **9**(2): p. 49.
17. Schmidt, J.C., et al., *On the association between oral piercings and periodontal conditions-A case series.* Int J Dent Hyg, 2019. **17**(4): p. 318-326.
18. Er, N., et al., *An unusual cause of gingival recession: oral piercing.* J Periodontol, 2000. **71**(11): p. 1767-9.
19. Giuca, M.R., et al., *Dental and periodontal complications of labial and tongue piercing.* J Biol Regul Homeost Agents, 2012. **26**(3): p. 553-60.

20. Tomaževič, T., R. Gašperšič, and R. Kosem, *Occurrence of Dental Injuries and Periodontal Complications in Tongue-piercing Jewellery Users.* Oral Health Prev Dent, 2017. **15**(3): p. 293-297.
21. Kapferer, I. and U.S. Beier, *Lateral lower lip piercing--prevalence of associated oral complications: a split-mouth cross-sectional study.* Quintessence Int, 2012. **43**(9): p. 747-52.
22. Kim, D.M. and R. Neiva, *Periodontal soft tissue non-root coverage procedures: a systematic review from the AAP Regeneration Workshop.* J Periodontol, 2015. **86**(2 Suppl): p. S56-72.
23. Cortellini, P. and N.F. Bissada, *Mucogingival conditions in the natural dentition: Narrative review, case definitions, and diagnostic considerations.* J Periodontol, 2018. **89 Suppl 1**: p. S204-s213.
24. Renkema, A.M., et al., *Gingival labial recessions in orthodontically treated and untreated individuals: a case - control study.* J Clin Periodontol, 2013. **40**(6): p. 631-7.
25. Joss-Vassalli, I., et al., *Orthodontic therapy and gingival recession: a systematic review.* Orthod Craniofac Res, 2010. **13**(3): p. 127-41.
26. Anand, P.S., et al., *Comparison of periodontal destruction patterns among patients with and without the habit of smokeless tobacco use--a retrospective study.* J Periodontal Res, 2013. **48**(5): p. 623-31.
27. Anand, P.S., et al., *Patterns of Periodontal Destruction among Smokeless Tobacco Users in a Central Indian Population.* Healthcare (Basel), 2021. **9**(6).
28. Bergström, J., et al., *Smokeless tobacco (snuff) use and periodontal bone loss.* J Clin Periodontol, 2006. **33**(8): p. 549-54.
29. Hugoson, A. and M. Rolandsson, *Periodontal disease in relation to smoking and the use of Swedish snus: epidemiological studies covering 20 years (1983-2003).* J Clin Periodontol, 2011. **38**(9): p. 809-16.
30. Jepsen, S., et al., *Periodontal manifestations of systemic diseases and developmental and acquired conditions: Consensus report of workgroup 3 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions.* J Periodontol, 2018. **89 Suppl 1**: p. S237-s248.
31. Pini-Prato, G., et al., *Classification of dental surface defects in areas of gingival recession.* J Periodontol, 2010. **81**(6): p. 885-90.
32. Bhusari, P., et al., *Classification & prevalence of dental surface defects in areas of gingival recession- a clinical study.* J Clin Diagn Res, 2014. **8**(7): p. Zf01-4.
33. Santamaria, M.P., et al., *The influence of local anatomy on the outcome of treatment of gingival recession associated with non-carious cervical lesions.* J Periodontol, 2010. **81**(7): p. 1027-34.
34. Pini-Prato, G., et al., *Influence of inter-dental tissues and root surface condition on complete root coverage following treatment of gingival recessions: a 1-year retrospective study.* J Clin Periodontol, 2015. **42**(6): p. 567-74.
35. Mahajan, A., et al., *Decision-making in classifying gingival recession defects - A systematic review.* Natl J Maxillofac Surg, 2019. **10**(2): p. 206-211.
36. Sullivan, H.C. and J.H. Atkins, *Free autogenous gingival grafts. I. Principles of successful grafting.* Periodontics, 1968. **6**(3): p. 121-9.

37. Mlinek, A., H. Smukler, and A. Buchner, *The use of free gingival grafts for the coverage of denuded roots.* J Periodontol, 1973. **44**(4): p. 248-54.
38. Miller, P.D., Jr., *A classification of marginal tissue recession.* Int J Periodontics Restorative Dent, 1985. **5**(2): p. 8-13.
39. Fernández-Jiménez, A., et al., *Complete root coverage in the treatment of Miller class III or RT2 gingival recessions: a systematic review and meta-analysis.* BMC Oral Health, 2021. **21**(1): p. 145.
40. Miller, P.D., *Miller Classification of Marginal Tissue Recession Revisited After 35 Years.* Compend Contin Educ Dent, 2018. **39**(8): p. 514-520.
41. Pini-Prato, G., *The Miller classification of gingival recession: limits and drawbacks.* J Clin Periodontol, 2011. **38**(3): p. 243-5.
42. Mahajan, A., et al., *Reliability study of Mahajan's classification of gingival recession: A pioneer clinical study.* J Indian Soc Periodontol, 2014. **18**(1): p. 38-42.
43. Mahajan, A., et al., *Comparison of miller's classification of gingival recession defects with mahajan's classification of gingival recession defect: A reliability study.* Dental Hypotheses, 2010. **1**(2): p. 45-50.
44. Cairo, F., et al., *The interproximal clinical attachment level to classify gingival recessions and predict root coverage outcomes: an explorative and reliability study.* J Clin Periodontol, 2011. **38**(7): p. 661-6.
45. Tonetti, M.S. and S. Jepsen, *Clinical efficacy of periodontal plastic surgery procedures: consensus report of Group 2 of the 10th European Workshop on Periodontology.* J Clin Periodontol, 2014. **41 Suppl 15**: p. S36-43.
46. Chambrone, L., R.C.N. de Castro Pinto, and L.A. Chambrone, *The concepts of evidence-based periodontal plastic surgery: Application of the principles of evidence-based dentistry for the treatment of recession-type defects.* Periodontol 2000, 2019. **79**(1): p. 81-106.
47. Baldi, C., et al., *Coronally advanced flap procedure for root coverage. Is flap thickness a relevant predictor to achieve root coverage? A 19-case series.* J Periodontol, 1999. **70**(9): p. 1077-84.
48. Hwang, D. and H.L. Wang, *Flap thickness as a predictor of root coverage: a systematic review.* J Periodontol, 2006. **77**(10): p. 1625-34.
49. Bartold, P.M., *Dentinal hypersensitivity: a review.* Aust Dent J, 2006. **51**(3): p. 212-8; quiz 276.
50. Clark, D. and L. Levin, *Non-surgical management of tooth hypersensitivity.* International Dental Journal, 2016. **66**(5): p. 249-256.
51. Rezazadeh, F., P. Dehghanian, and D. Jafarpour, *Laser Effects on the Prevention and Treatment of Dentinal Hypersensitivity: A Systematic Review.* J Lasers Med Sci, 2019. **10**(1): p. 1-11.
52. Chambrone, L., et al., *Root coverage procedures for treating localised and multiple recession-type defects.* Cochrane Database Syst Rev, 2018. **10**(10): p. Cd007161.
53. Baer, P.N. and S.D. Benjamin, *Gingival grafts: a historical note.* J Periodontol, 1981. **52**(4): p. 206-7.
54. Zucchelli, G. and M. De Sanctis, *Treatment of multiple recession-type defects in patients with esthetic demands.* J Periodontol, 2000. **71**(9): p. 1506-14.
55. Rasperini, G., R. Acunzo, and E. Limiroli, *Decision Making in Gingival Recession Treatment: Scientific Evidence and Clinical Experience.* Clin Adv Periodontics, 2011. **1**(1): p. 41-52.

56. Stein, J., *Management of gingival recessions: options and prognostic factors.* Clinical Dentistry Reviewed, 2019. **3**.
57. Chambrone, L., et al., *Evidence-based periodontal plastic surgery. II. An individual data meta-analysis for evaluating factors in achieving complete root coverage.* J Periodontol, 2012. **83**(4): p. 477-90.
58. Salhi, L., et al., *Coronally advanced flap versus the pouch technique combined with a connective tissue graft to treat Miller's class I gingival recession: a randomized controlled trial.* J Clin Periodontol, 2014. **41**(4): p. 387- 95.
59. Santamaria, M.P., et al., *Connective tissue graft and tunnel or trapezoidal flap for the treatment of single maxillary gingival recessions: a randomized clinical trial.* J Clin Periodontol, 2017. **44**(5): p. 540-547.
60. Azaripour, A., et al., *Root coverage with connective tissue graft associated with coronally advanced flap or tunnel technique: a randomized, double-blind, mono-centre clinical trial.* J Clin Periodontol, 2016. **43**(12): p. 1142-1150.
61. Hammarström, L., L. Heijl, and S. Gestrelius, *Periodontal regeneration in a buccal dehiscence model in monkeys after application of enamel matrix proteins.* J Clin Periodontol, 1997. **24**(9 Pt 2): p. 669-77.
62. Bartlett, J.D., et al., *3. Protein-protein interactions of the developing enamel matrix.* Curr Top Dev Biol, 2006. **74**: p. 57-115.
63. Lyngstadaas, S.P., et al., *Enamel matrix proteins; old molecules for new applications.* Orthod Craniofac Res, 2009. **12**(3): p. 243-53.
64. Bosshardt, D.D., *Biological mediators and periodontal regeneration: a review of enamel matrix proteins at the cellular and molecular levels.* J Clin Periodontol, 2008. **35**(8 Suppl): p. 87-105.
65. Miron, R.J., M. Dard, and M. Weinreb, *Enamel matrix derivative, inflammation and soft tissue wound healing.* J Periodontal Res, 2015. **50**(5): p. 555-69.
66. Miron, R.J., et al., *Twenty years of enamel matrix derivative: the past, the present and the future.* J Clin Periodontol, 2016. **43**(8): p. 668-83.
67. Rothamel, D., et al., *Biodegradation pattern and tissue integration of native and cross-linked porcine collagen soft tissue augmentation matrices - an experimental study in the rat.* Head Face Med, 2014. **10**: p. 10.
68. Kim, Y.S., et al., *Applications of decellularized extracellular matrix in bone and cartilage tissue engineering.* Bioeng Transl Med, 2019. **4**(1): p. 83-95.
69. biotiss-biomaterials;. *Mucoderm®* Available from: https://botiss-dental.com/de/products/mucoderm-de/#literature.
70. Thoma, D.S., et al., *A systematic review assessing soft tissue augmentation techniques.* Clin Oral Implants Res, 2009. **20 Suppl 4**: p. 146-65.
71. Wessel, J.R. and D.N. Tatakis, *Patient outcomes following subepithelial connective tissue graft and free gingival graft procedures.* J Periodontol, 2008. **79**(3): p. 425-30.
72. Gholami, G.A., et al., *Comparison of the clinical outcomes of connective tissue and acellular dermal matrix in combination with double papillary flap for root coverage: A 6-month trial.* Dent Res J (Isfahan), 2013. **10**(4): p. 506-13.
73. Jepsen, K., et al., *Treatment of gingival recession defects with a coronally advanced flap and a xenogeneic collagen matrix: a multicenter randomized clinical trial.* J Clin Periodontol, 2013. **40**(1): p. 82-9.

74. Atieh, M.A., et al., *Xenogeneic collagen matrix for periodontal plastic surgery procedures: a systematic review and meta-analysis.* J Periodontal Res, 2016. **51**(4): p. 438-52.
75. Fedorov, A., et al., *3D Slicer as an image computing platform for the Quantitative Imaging Network.* Magn Reson Imaging, 2012. **30**(9): p. 1323-41.
76. Sangiorgio, J.P.M., et al., *Xenogenous Collagen Matrix and/or Enamel Matrix Derivative for Treatment of Localized Gingival Recessions: A Randomized Clinical Trial. Part I: Clinical Outcomes.* J Periodontol, 2017. **88**(12): p. 1309-1318.
77. Hägewald, S., et al., *Comparative study of Emdogain and coronally advanced flap technique in the treatment of human gingival recessions. A prospective controlled clinical study.* J Clin Periodontol, 2002. **29**(1): p. 35-41.
78. Del Pizzo, M., et al., *Coronally advanced flap with or without enamel matrix derivative for root coverage: a 2-year study.* J Clin Periodontol, 2005. **32**(11): p. 1181-7.
79. Franca-Grohmann, I.L., et al., *Treatment of dehiscence-type defects with collagen matrix and/or enamel matrix derivative: Histomorphometric study in minipigs.* J Periodontol, 2020. **91**(7): p. 967-974.
80. Koop, R., J. Merheb, and M. Quirynen, *Periodontal regeneration with enamel matrix derivative in reconstructive periodontal therapy: a systematic review.* J Periodontol, 2012. **83**(6): p. 707-20.
81. Sallum, E.A., et al., *Enamel matrix derivative and guided tissue regeneration in the treatment of dehiscence-type defects: a histomorphometric study in dogs.* J Periodontol, 2004. **75**(10): p. 1357-63.
82. McGuire, M.K., E.T. Scheyer, and P. Schupbach, *A Prospective, Case-Controlled Study Evaluating the Use of Enamel Matrix Derivative on Human Buccal Recession Defects: A Human Histologic Examination.* J Periodontol, 2016. **87**(6): p. 645-53.
83. Sculean, A., et al., *Clinical and histologic evaluation of an enamel matrix derivative combined with a biphasic calcium phosphate for the treatment of human intrabony periodontal defects.* J Periodontol, 2008. **79**(10): p. 1991-9.
84. Corrêa, M.G., et al., *Histometric analysis of the effect of enamel matrix derivative on the healing of periodontal defects in rats with diabetes.* J Periodontol, 2013. **84**(9): p. 1309-18.
85. Han, Y., et al., *[Digital evaluation of supracrestal gingival thickness induced by periodontal regenerative and corticotomy surgery in patients with skeletal class Ⅲ malocclusion].* Zhonghua Kou Qiang Yi Xue Za Zhi, 2020. **55**(2): p. 73-79.
86. Chambrone, L. and D.N. Tatakis, *Periodontal soft tissue root coverage procedures: a systematic review from the AAP Regeneration Workshop.* J Periodontol, 2015. **86**(2 Suppl): p. S8-51.
87. Rasperini, G., et al., *Subepithelial connective tissue graft for treatment of gingival recessions with and without enamel matrix derivative: a multicenter, randomized controlled clinical trial.* Int J Periodontics Restorative Dent, 2011. **31**(2): p. 133-9.
88. Roman, A., et al., *Subepithelial connective tissue graft with or without enamel matrix derivative for the treatment of Miller class I and II gingival*

recessions: a controlled randomized clinical trial. J Periodontal Res, 2013. **48**(5): p. 563-72.
89. Cairo, F., M. Nieri, and U. Pagliaro, *Efficacy of periodontal plastic surgery procedures in the treatment of localized facial gingival recessions. A systematic review.* J Clin Periodontol, 2014. **41 Suppl 15**: p. S44-62.
90. McGuire, M.K. and E.T. Scheyer, *Xenogeneic collagen matrix with coronally advanced flap compared to connective tissue with coronally advanced flap for the treatment of dehiscence-type recession defects.* J Periodontol, 2010. **81**(8): p. 1108-17.
91. Cardaropoli, D., et al., *Treatment of gingival recession defects using coronally advanced flap with a porcine collagen matrix compared to coronally advanced flap with connective tissue graft: a randomized controlled clinical trial.* J Periodontol, 2012. **83**(3): p. 321-8.
92. Cordaro, L., V.M. di Torresanto, and F. Torsello, *Split-mouth comparison of a coronally advanced flap with or without enamel matrix derivative for coverage of multiple gingival recession defects: 6- and 24-month follow-up.* Int J Periodontics Restorative Dent, 2012. **32**(1): p. e10-20.
93. Aroca, S., et al., *Treatment of class III multiple gingival recessions: a randomized-clinical trial.* J Clin Periodontol, 2010. **37**(1): p. 88-97.
94. Henriques, P.S., et al., *Application of subepithelial connective tissue graft with or without enamel matrix derivative for root coverage: a split-mouth randomized study.* J Oral Sci, 2010. **52**(3): p. 463-71.
95. Moreira, A.R.O., et al., *Coronally advanced flap with or without porcine collagen matrix for root coverage: a randomized clinical trial.* Clin Oral Investig, 2016. **20**(9): p. 2539-2549.
96. Costa, P.P., et al., *Root Coverage in Smokers with Acellular Dermal Matrix Graft and Enamel Matrix Derivative: A 12-Month Randomized Clinical Trial.* Int J Periodontics Restorative Dent, 2016. **36**(4): p. 525-31.