Effect of Enamel Matrix Derivative on Collagen Guided Tissue Regeneration-Based Root Coverage Procedure

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Background: Enamel matrix derivative (EMD) has been shown to promote periodontal wound healing and/or regeneration when applied to tooth root surfaces in soft tissue dehiscence models. In addition, guided tissue regeneration (GTR)-based root coverage using collagen membrane (GTRC) has shown promising results. However, limited information is available regarding how EMD may influence GTRC outcome.

Methods: Twenty-six patients with Miller's Class I or II gingival recession defects of 2.5 mm were recruited for the study. Subjects were randomly assigned to receive either EMD + collagen (EMDC; test group) or collagen membrane (GTRC; control group). Clinical parameters, including plaque index (PI), gingival index (GI), relative clinical attachment levels (RCAL) to the stent, recession depth (RD), recession width (RW), probing depth (PD), gingival tissue thickness (GTT), and width of keratinized gingiva (KG) were assessed at baseline, and 3 and 6 months after surgery. A repeated measure of analysis of variance (ANOVA) was used to determine differences between treatment groups and time effect.

Results: Both treatments (GTRC and EMDC) resulted in a statistically significant decrease in RD and RW between baseline and 6 months ($P < 0.05$). However, no difference was noted between treatment groups. The percent of root coverage after 6 months was 75% for GTRC and 63% for EMDC. Complete 100% root coverage was achieved in five patients in the GTRC group, compared to only one patient in the EMDC group. There was a statistically significant gain ($P < 0.05$) in the clinical attachment level (CAL) between baseline and 6 months in both groups, as reflected on the RCAL data. No other significant differences were noted on other clinical parameters (PD, GTT, KG, GI, and PI).

Conclusions: GTR-based root coverage utilizing collagen membrane, with or without enamel matrix derivative, can be successfully used in obtaining gingival recession coverage. The application of EMD during GTRC procedures did not add additional benefit to the final clinical outcome. *J Periodontol 2004;75:1446-1457.*

KEY WORDS

Collagen/therapeutic use; enamel matrix derivative; gingival recession/therapy; guided tissue regeneration; membranes, barrier; membranes, bioabsorbable; outcome assessment; surgical flaps; tooth root; wound healing.

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Gingival recession is fairly common and its prevalence increases with age.[1,2] Recently, it was reported that more than 50% of the American population has 1 mm gingival recession in one or more sites.[3] Gingival recession, localized or generalized, may be associated with one or more surfaces, resulting in root exposures which lead to clinical problems such as root surface hypersensitivity, root caries, cervical root abrasions, difficult plaque control, and diminished esthetic/cosmetic appeal.

Buccal tooth recessions have numerous causes and are most frequently associated with overzealous tooth brushing, combined with localized prominent tooth malposition.[4] Other factors associated with marginal tissue recession are alveolar bone dehiscences,[5,6] inadequate gingival dimensions,[7] high muscle attachment and frenal pull,[8] and iatrogenic factors related to restorative and periodontal treatment procedures.[9-11]

Numerous procedures have been designed to provide predictable root coverage in order to solve these problems. Conventional mucogingival surgery includes pedicle graft procedures[12,13] (e.g., laterally sliding flap, double papilla flap, oblique rotated flap[14-16]), advanced flap procedures (e.g., coronally repositioned flap or semilunar coronally repositioned flap),[17-19] free soft tissue graft,[20-32] and free connective tissue graft.[33-37] The subepithelial connective tissue graft[38-41] is currently seen as the most predictable technique available to achieve root coverage, while maintaining a high degree of

esthetics. The disadvantages of these conventional procedures include the need for the donor-site surgical procedure as well as technical difficulty. In addition, these procedures often heal mainly with long junctional epithelium with limited connective tissue attachment.[42-44]

However, histological studies following the use of guided tissue regeneration (GTR)-based root coverage procedures showed significant formation of bone, cementum, and connective tissue attachment.[45-48] This approach was found to be predictable when non-absorbable barriers[49-56] or absorbable barriers were used. The bioabsorbable barriers offer several advantages when compared to non-resorbable membranes; these include higher patient comfort, a single surgical procedure, and an uninterrupted healing process.[48,50,54,57-67]

Numerous investigators have examined and reported the effects of collagen in periodontal wound healing, which include: chemotactic function for fibroblasts; hemostasis; ability to aggregate platelets; a low immunogenicity; and acting as a scaffold for migrating cells.[68-72] Successful root coverage was reported when bioabsorbable collagen membranes were used as a barrier device in GTR-based root coverage procedures.[73-76]

Enamel matrix derivative (EMD) has been shown to promote cementogenesis and bone formation as well as new attachment.[77-79] In addition, EMD has also demonstrated promising results in periodontal defects treatment.[78,80-98] It has been shown that EMD possesses the potential to stimulate the formation of new connective tissue, new bone, new periodontal ligament, and cementum, possibly with inserting collagen fibers.[83-85,99,100]

Cumulative evidence indicates EMD's ability to increase proliferation, migration, adhesion, and differentiation of cells responsible for tissue healing in vivo.[101] Several studies have shown that EMD may not only enhance periodontal regeneration, but also influence soft tissue healing.[82,83,102,103] Furthermore, studies of an in vitro wound-healing model, showed EMD not only had an effect on the migration of periodontal ligament cells, but also on gingival fibroblast stimulation.[104,105] Gestrelius et al.[106] reported that EMD applied to instrumented root surfaces may remain active at the location for a period of 1 to 2 weeks, which suggests EMD may influence the early healing of a soft tissue wound in the dento-gingival region. It can be speculated that EMD is present at the site during which most of the important events of gingival wound healing occur for the first 2 weeks. The efficacy of using EMD in root coverage procedures has been investigated[107,108] and shown to produce successful root coverage with healthy, thick keratinized tissue. Nevertheless, some studies[109,110] questioned the benefit of using EMD with the coronally advanced flap (CAF), since no difference was found between EMD-treated and non–EMD-treated sites. Limited information is available regarding how EMD may influence GTR-based root coverage procedures when combined with collagen membranes. Therefore, the purpose of this study was to investigate the adjunctive effect of EMD on collagen membrane GTR-based root coverage.

MATERIALS AND METHODS

Patient Population

Twenty-six systemically healthy patients (14 females and 12 males; aged 20 to 65 years; mean age 39.5) were selected from the patient pool of the Graduate Periodontic Clinic and Undergraduate Clinic at The University of Michigan School of Dentistry. Patient selection criteria for this study included: 1) non-smokers; 2) ≥18 years old; 3) buccal recession defects (≥2.5 mm) classified as either Miller's Class I or II on either lower or upper anterior teeth or premolars; 4) radiographic evidence of sufficient interdental bone (the distance between the crestal bone and cemento-enamel junction ≤2 mm); 5) clinical indication and/or patient request for recession coverage; 6) ≥0.5 mm gingival thickness at a point located 3 mm below the free gingival margin; 7) ≥2 mm keratinized gingiva; and 8) good oral hygiene. Patients with any of the following conditions were excluded from the study: 1) known allergy to bovine products; 2) pregnancy; 3) use of any tobacco products within the last 30 days; 4) presence of any unstable systemic diseases such as renal, hepatic, cardiac, endocrine, hematologic, and autoimmune diseases; 5) inability to provide informed consent; and 6) participation in another clinical trial using an investigational new drug or device within 30 days of entrance into the study.

Subject randomization to treatment groups was determined randomly by the flip of a coin. Patients were assigned to receive either the EMD‡ + collagen membrane§ + coronally advanced flap (CAF) (EMDC-test group) or the collagen membrane + CAF (GTRC-control group). All measurements were recorded by a calibrated examiner (DW).

Clinical Measurements

The following measurements were recorded for each study subject at baseline, and 3 and 6 months: the probing depth (PD), measured using a UNC probe to the nearest 0.5 mm on six aspects (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual) and recorded from the free gingival margin to the apical end of the sulcus; the relative clinical attachment level (RCAL), recorded from a reference point on a reference stent to the apical end of the sulcus as measured by a UNC probe; the width of keratinized gingiva (KG), measured from the mucogingival

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§ BioMend, Zimmer Dental, Carlsbad, CA.

junction (MGJ) to the free gingival margin using a UNC probe; the recession depth (RD), measured from the cemento-enamel junction (CEJ) to the free gingival margin at the deepest site along the tooth axis by using a UNC probe; the recession width (RW), determined by the horizontal dimension of the gingival defect at the level of the CEJ; and gingival tissue thickness (GTT), recorded using a UNC probe penetrating the gingival tissue at a mid-point on the facial aspect of the tooth, 3mm apical from the gingival margin. Plaque index (PI)[111] and gingival index (GI)[112] were measured at baseline, 1 week, 2 weeks, 4 weeks, and 3 and 6 months post-surgery.

Surgical Protocol

All surgical procedures were performed by one surgeon (MT). Under local anesthesia, each subject received an intra-sulcular incision, followed by two vertical releasing incisions (one mesial and one distal) extending beyond the MGJ. These incisions were made on the buccal aspect of the involved tooth. The vertical incisions were made at least 0.5 mm away from the adjacent tooth surface, thus leaving the adjacent marginal gingiva undisturbed. A full mucoperiosteal flap was elevated to the level of the mucogingival junction, and a partial-thickness incision was made in the area apical to the MGJ. In the adjacent interdental papillae, gingivoplasty was performed to remove the epithelium and to provide a connective tissue bed for the future coronally positioned flap. The exposed root surfaces were thoroughly scaled, root planed, and flattened by curets and/or rotary burs, removing plaque and its byproducts in the process. Root surfaces were conditioned with neutrally buffered EDTA‖ for 2 minutes to remove the smear layer, followed by copious irrigation of sterile saline. In the control group, areas were then carefully dried with light air spray. A barrier membrane composed of purified, cross-linked bovine Achilles Type I collagen was custom trimmed, positioned over the root coronal to the CEJ, with 2 to 3 mm extending beyond the bony margin, and secured with 5-0 sling-tag sutures.¶ The flap was coronally positioned to cover the membrane and was secured with two 5-0 sutures (Fig. 1). In the experimental group, the same surgical protocol was performed, except 10 mg of EMD was applied between the root surface and collagen membrane using a syringe and needle supplied by the manufacturer. Care was taken to ensure that the flap was free of tension (Fig. 2).

Preparation and Application of Enamel Matrix Derivative

EMD consists of freeze-dried enamel matrix protein, the major protein of which is amelogenin, and a viscous carrier, propylene glycol alginate. Vials were stored in a refrigerator at 2°C to 8°C. Before application, the vials were warmed at room temperature for 15 minutes. A syringe with a long, large bore (1.2 mm diameter) was used to draw the propylene glycol alginate solution from the vial. The solution was spread evenly over the freeze-dried EMD to begin solubilizing it. The EMD was drawn into a syringe with the same large bore needle, which then was replaced with a short, blunt needle before application to the root surface.

Postoperative Care

Oral postoperative instructions were provided to all patients. In addition, each patient received a sheet of written post-surgical care instructions. To prevent post-surgical infection, amoxicillin (500 mg, t.i.d.) for 10 days was prescribed. If the patient was allergic to amoxicillin, clindamycin (150 mg, t.i.d.) for 10 days was prescribed instead. The patient was asked to rinse with warm salt water twice daily for the first 2 weeks, then switched to 0.12% chlorohexidine# for the next 4 weeks. In general, no brushing or flossing was allowed on the surgical area for 3 weeks postoperatively. Sutures were removed 10 to 14 days after surgery. Oral hygiene instructions were given at the end of surgery and at each visit. Professional prophylaxis without prophy paste was performed at each post-treatment visit if indicated (i.e., visible supragingival plaque or calculus present).

Statistical Analyses

Power analysis showed that the available sample size would yield 80% power to detect 1 mm difference between the two groups. Statistical analysis with a statistical package** was performed using repeated measures of analysis of variance (ANOVA) to evaluate differences of clinical parameters between pre- and post-treatment. In addition, differences between treatment groups as well as the time effect were evaluated. The significance level was set at $\alpha = 0.05$. Analysis of the differences in PI and GI between treatment groups at the different time intervals was performed using a non-parametric test. The percentage of root coverage was calculated using the following equation: pretreatment recession depth – post-treatment recession depth/pre-treatment recession depth $\times$ 100%.

RESULTS

Twenty-six patients, 14 females and 12 males aged 20 to 65 with Miller Class I or II recession defects ≥2.5 mm were included in the study. Demographic information and distribution of recession sites by group are shown in Table 1. Statistics showed that the two groups, EMDC and GTRC, are balanced by gender and age. Two cases in the test group experienced early

‖ PrefGel, Straumann Biologics Division.
¶ Vicryl, Ethicon Inc., Johnson & Johnson Company, Somerville, NJ.
# CPL, Inc., St. Louis, MO.
** SPSS statistical package version 10.0, SPSS for Windows, SPSS Inc., Chicago, IL.

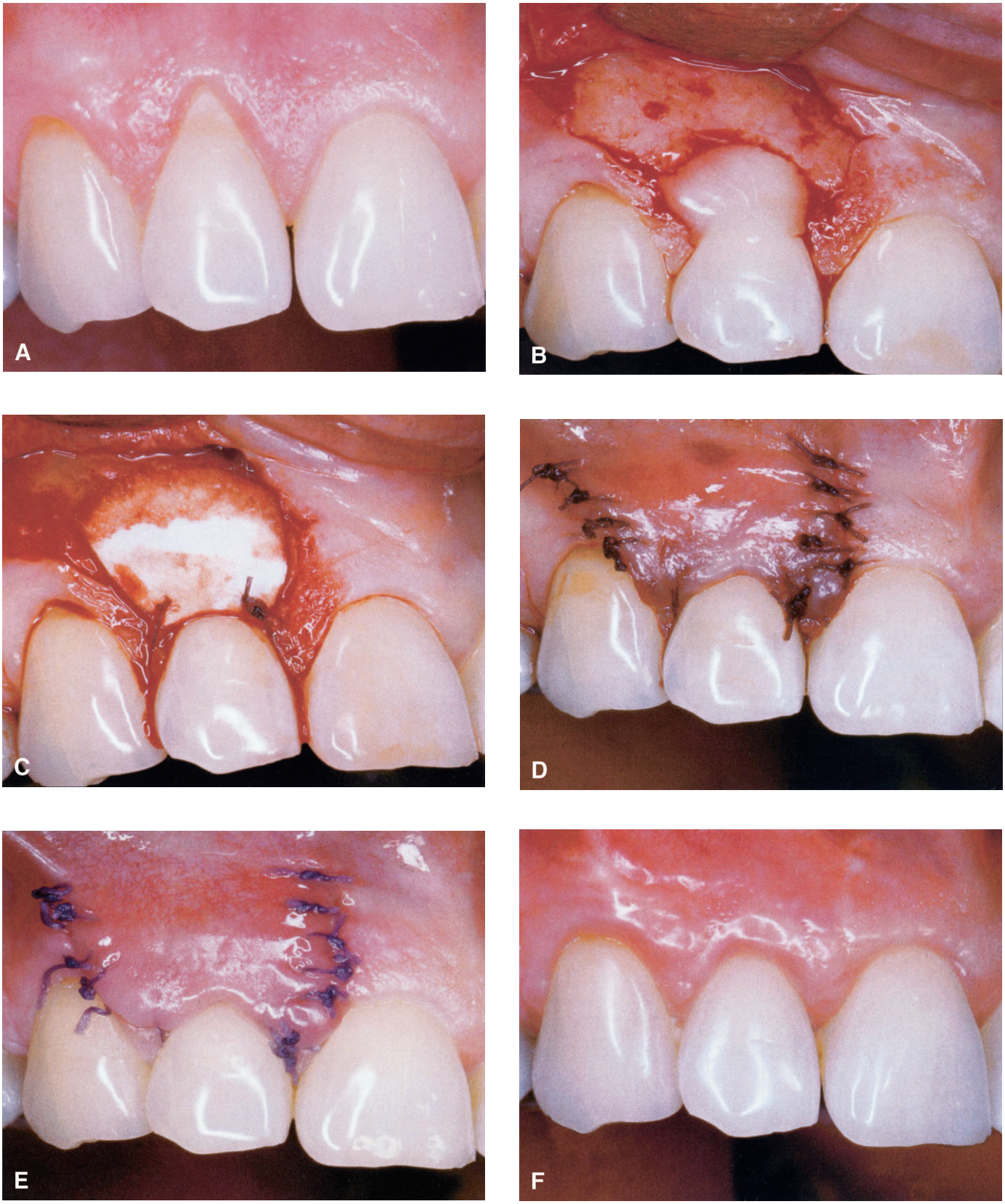

Figure 1.
A) *Preoperative view of #7 buccal recession (GTRC).* ***B)*** *EDTA application for root conditioning after root instrumentation.* ***C)*** *Collagen membrane adaptation.* ***D)*** *Flap closure.* ***E)*** *One-week healing.* ***F)*** *Six months post-treatment with GTRC.*

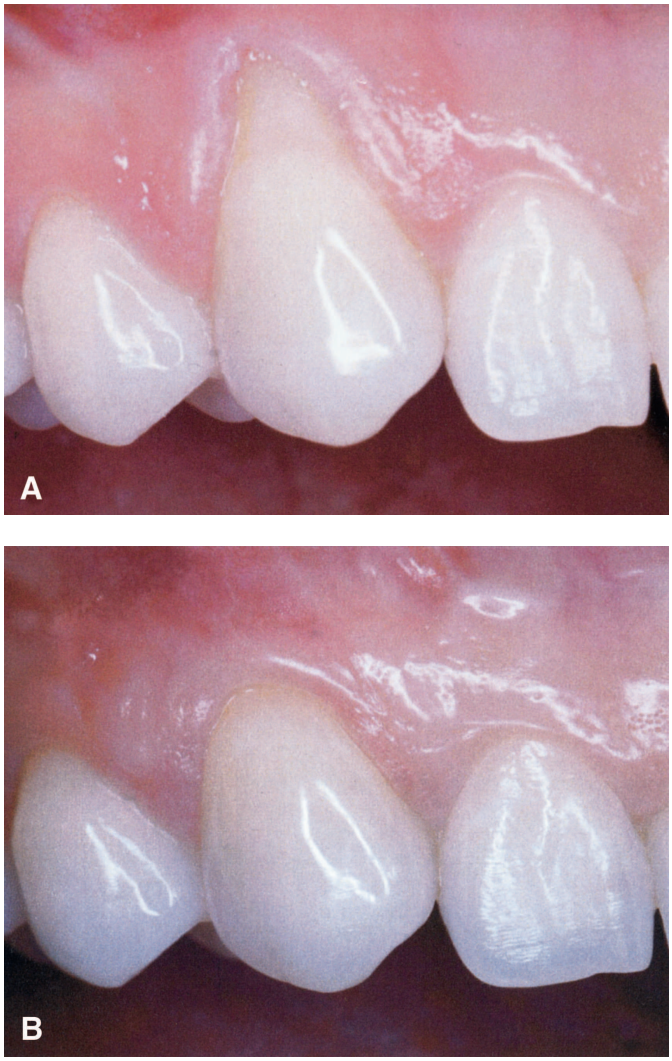

Figure 2.
A) *Pretreatment view of #6 buccal recession (EMDC).* ***B)*** *Six months post-treatment with EMDC.*

Table 1.

Patient Demographics and Recession Sites

	EMDC (test) (N = 13)	GTRC (control) (N = 13)
Age range	20-65	25-58
Gender	6 females 7 males	8 females 5 males
Recession site	Maxilla 3 canine 1 incisor 3 premolar Mandible 2 canine 4 premolar	Maxilla 6 canine 4 incisor 1 premolar Mandible 2 premolar

membrane exposure at 2 weeks post-surgery, but it disappeared uneventfully at 4 weeks postoperatively.

Measurements of each parameter were recorded at baseline and up to 6 months after surgery, as shown in Tables 2 to 4. Baseline measurements for the test and control groups showed no statistical differences in any parameters evaluated. RD for the test group decreased from 3.53 ± 0.69 mm to 1.30 ± 0.63 mm, with a difference of 2.23 ± 0.63 mm. For the control group, RD decreased from 3.23 ± 0.66 mm to 0.84 ± 0.89 mm, with a difference of 2.38 ± 0.86 mm. At 6 months, root coverage percentage was 63% ± 16.5% for the EMDC group, and 75% ± 25.6% for the GTRC group. A statistically significant difference was detected in recession reduction for both groups ($P \leq 0.01$) between baseline and 6 months; however, no statistically significant difference was noted in RD reduction between groups (Table 2).

RW measurements, as shown in Table 3, showed a change of 1.53 ± 1.05 mm (from 3.92 ± 0.49 to 2.38 ± 1.04) in the EMDC group, compared to a change of 2.30 ± 1.47 mm (from 3.76 ± 0.85 to 1.46 ± 1.71) in the GTRC group. This resulted in a statistically significant difference ($P \leq 0.01$) between baseline and 6 months in both groups, but no significant difference between the treatment groups at 6 months. A gain of the clinical attachment level (CAL) was also noted in both treatment groups, as reflected on the differences in RCAL data in Table 3; the mean of RCAL in the test group changed from 11.00 ± 1.68 mm to 9.23 ± 1.64, a difference of 1.76 ± 0.72, while the control group showed an RCAL change from 10.96 ± 1.39 mm at baseline to 9.57 ± 1.28 at 6 months, a difference of 1.38 ± 1.26. Again, the change in the relative clinical attachment level was sta-

Table 2.

Comparison of Gingival Recession Depth (means ± standard deviation, mm) at Baseline and 6 Months

	EMDC (test) (N = 13)	GTRC (control) (N = 13)	Difference (control-test)
Baseline (range)	3.53 ± 0.69 (2.5 ~ 5.0)	3.23 ± 0.66 (2.5 ~ 4.0)	–0.30 ± 0.26
6 months (range)	1.30 ± 0.63 (0 ~ 2.0)	0.84 ± 0.89 (0 ~ 3.0)	–0.46 ± 0.30
Difference	2.23 ± 0.63*	2.38 ± 0.86*	0.15 ± 0.29
% root coverage (range)	63% ± 16.5% (20% ~ 100%)	75% ± 25.6% (25% ~ 100%)	11.4% ± 8.5%

* Statistical significance within groups ($P \leq 0.01$).

Table 3.

Clinical Parameters (means ± standard deviation, mm) at Baseline and 6 Months Postoperatively

Parameter	EMDC (test) (N = 13)	GTRC (control) (N = 13)	Difference (control-test)
Recession width			
Baseline	3.92 ± 0.49	3.76 ± 0.85	–0.15 ± 0.27
6 months	2.38 ± 1.04	1.46 ± 1.71	–0.92 ± 0.55
Difference	1.53 ± 1.05*	2.30 ± 1.47*	0.76 ± 0.50
Relative clinical attachment level			
Baseline	11.00 ± 1.68	10.96 ± 1.39	–0.38 ± 0.60
6 months	9.23 ± 1.64	9.57 ± 1.28	0.34 ± 0.57
Difference	1.76 ± 0.72*	1.38 ± 1.26*	–0.38 ± 0.40
Probing depth			
Baseline	1.76 ± 0.59	1.73 ± 0.52	–0.03 ± 0.22
6 months	1.61 ± 0.65	1.30 ± 0.48	0.03 ± 0.22
Difference	0.15 ± 0.68	0.42 ± 0.70	0.26 ± 0.27
Width of keratinized gingiva			
Baseline	3.30 ± 2.04	3.61 ± 1.62	0.30 ± 0.72
6 months	3.07 ± 1.75	3.69 ± 0.85	0.61 ± 0.54
Difference	–0.23 ± 1.40	0.07 ± 1.16	0.30 ± 0.50
Gingival tissue thickness			
Baseline	0.98 ± 0.69	1.11 ± 0.21	0.13 ± 0.06
6 months	0.94 ± 0.14	1.03 ± 0.13	0.09 ± 0.05
Difference	–0.03 ± 0.13	–0.07 ± 0.18	0.03 ± 0.06

* Statistical significance within groups ($P \leq 0.01$).

Table 4.

Clinical Indices (means ± standard deviation) of Treated Sites at Different Time Points

	Gingival Index		Plaque Index	
	EMDC	GTRC	EMDC	GTRC
Baseline	0.69 ± 0.75	0.15 ± 0.55	0.69 ± 0.48	0.23 ± 0.43
1 week	0.92 ± 0.95	0.38 ± 0.65	0.76 ± 0.59	0.53 ± 0.66
2 weeks	0.61 ± 0.65	0.00 ± 0.00	0.38 ± 0.50	0.07 ± 0.27
4 weeks	0.61 ± 0.65	0.23 ± 0.43	0.69 ± 0.63	0.23 ± 0.59
3 months	0.38 ± 0.50	0.23 ± 0.34	0.84 ± 0.80	0.15 ± 0.37
6 months	0.53 ± 0.51	0.46 ± 0.51	0.38 ± 0.50	0.61 ± 0.50

tistically significant ($P \leq 0.001$) between baseline and 6 months in both groups, but there was no difference between treatment groups. As shown in Tables 3 and 4, no statistical differences were noted between groups in PD, KG, GTT, GI, and PI at any post-treatment visits (1 week, 2 weeks, 4 weeks, 3 months, and 6 months).

DISCUSSION

This study was performed to investigate the adjunctive effect of EMD on collagen membrane GTR-based root coverage. To our knowledge, the adjunctive use of EMD with a collagen membrane in the soft tissue treatment of buccal recession defects has not been reported in the literature. However, studies on the efficacy of GTR have shown predictability with use of a collagen membrane.[73-76]

In the present study, the test group (EMDC) achieved mean root coverage of 63%, while the control group (GTRC) achieved 75% at 6 months, with no significant difference between the groups for root coverage. These results are similar to those reported by numerous other GTR-based root coverage studies.[41,50,52,53,59,60,62,66,67,72,73,113-126] These authors reported a range of 50% to 76% root coverage and in most of these studies, expanded polytetrafluoroethylene (ePTFE) or bioabsorbable membranes were used in combination with the CAF.

On the other hand, the results from the GTRC group in this study (75% ± 25.6%) did not reach the root coverage levels reported by others, ranging from 81% to 93% mean root coverage.[61,63-65,74,76,115,127-130] The difference may be partially attributed to the learning curve of the operator in the present study. All surgeries in our study were performed by a graduate student, whereas experienced clinicians performed the surgical procedures in the previous studies. Another reason for the variations in results may be due to an initial difference in clinical parameters. A number of the published reports on recession treatment emphasized the size of the presurgical defect and its effect on clinical outcomes; i.e., the deeper and narrower the defect, the greater the achieved root coverage.[15,23,59,60,64,66,113,128] One study emphasized that if recession width was higher at baseline, clinical outcomes in root coverage were less than favorable.[113] The average baseline RW was 3.92 mm for the EMDC group and 3.76 mm for the GTRC group. Also, the average baseline RD used in our study was 3.53 mm for the EMDC group and 3.23 mm for the GTRC group. The pretreatment averages of our study corresponded well with other clinical reports in which an RD of ≥2 mm was used.[118,131] Some studies limited the RD to <4 mm,[74,76,109,110] while others used 4 mm.[61,75,127,128,132] The discrepancy in clinical outcomes among these studies may well be explained by Pini Prato et al.[59] who reported more favorable results

in root coverage with the GTR procedure in sites with deep (≥5 mm) recession defects.

In our study, there were 7 maxillary and 6 mandibular teeth in the EMDC group and 11 maxillary and 2 mandibular teeth in the GTRC group. More root coverage was achieved in the maxilla than mandible in both groups (data not shown). These results correspond well with results of other studies in the literature, which showed more predictable results when treating maxillary than mandibular recession defects.[61-63,65,66,74,75,115,121,129,130,133,134] In our study, maxillary canines achieved larger amounts of coverage, which agrees with the results of Muller et al.,[66] who reported the best treatment response with maxillary canines. Explanation for this repeated observation might center on the need for proper healing stabilization of the maturing clot.[135] This criterion is difficult to achieve in the mandibular teeth due to the tensile strength of the wound, which may interfere with the tissue maturation process.[113] The clinical outcome of root coverage in our study might have been affected by skewed patient distribution between the EMDC and GTRC groups, which resulted in more mandibular teeth in the EMDC group. For the group randomization, instead of flipping a coin, it would have been more desirable if another method of randomization, such as using a random number table, were used to further rule out subjectivity. Another reason for the less favorable outcome found in the EMDC group than the GTRC group might have been attributed to the finding that the EMDC group had two early membrane exposures, which could have affected the clinical outcome since membrane exposure has been found to result in less favorable treatment outcomes.[66]

Differences in study measurements may also account for the differences in outcomes achieved in our study. For example, our data were recorded to the nearest 0.5 mm, while some other studies recorded their data to the nearest 1 mm.[61,63,74,128] This difference, though seemingly small, might have affected the results. The present study used 1 mm difference to be significant with 80% of a study power because 1 mm difference is generally recommended for describing clinical significance in clinical trials in which a periodontal probe is used for clinical measurements.[136] A minimal tissue thickness of 0.5 mm at the facial recession site was included in our study. Many studies in the literature also recommended a minimal tissue thickness of 0.5 mm in order to achieve maximum root coverage.[66,114,115] However, some recommended a minimal tissue thickness of 0.8 mm to achieve 100% root coverage.[137]

After treatment, a minor mean tissue thickness loss was noted at 6 months for both treatment groups (0.03 mm in EMDC and 0.07 mm in GTRC). We assume these differences were not statistically significant. This may be interpreted by the findings from previous studies that tissues may take more than 6 months to return to their original thickness in GTR-based root coverage procedures.[47,48,56,71] The space underneath the bioabsorbable membrane is filled with the ingrowth of granulation tissue derived from the underlying structures, thus increasing tissue thickness.[66] Clinical attachment gain in our study was significant at 6 months (1.76 mm for the EMDC group and 1.38 mm for the GTRC group). Those results match earlier reports of clinical attachment gain after GTR-based root coverage procedures,[50,62,66,72,73,113,115-119,121,123,130,133,138] which reported clinical attachment gains ranging from 0.8 to 3.1 mm. However, our attachment gains fell short of the 3.2 to 4.9 mm gains reported by several other researchers.[52,60,61,65,67,74,76,115,124,128,129,132] It is important to remember that those studies included patients with deeper recession defects than those treated in our study, and, according to Jepsen et al.,[127] deeper recessions (≥4 mm) had greater attachment level gains than shallow (<4 mm) recessions. As noted above, our test (EMDC) group had greater attachment gain, but not at a statistically significant level. This may be due to the presence of true new attachment since EMD has been shown to stimulate regeneration in both animal and human models.[82,83,102] In many other reports, EMD was found to reduce PD and to increase CAL gain.[68,89,90-96,103,139,140] In addition, Hoang et al.[104] showed that EMD enhanced migration of periodontal ligament (PDL) cells and gingival fibroblasts to the root surface. It can be reasonably suggested, therefore, that the extra amount of attachment gain could be due to true attachment. However, it is premature to draw this conclusion from the results of the present study because of the lack of histological analysis.

Our study is based on the concept that EMD deposition on the root surface precedes the formation of acellular cementum, which leads to regaining of PDL and alveolar bone.[82,83] Several studies have recently been conducted using this concept to see whether EMD could be beneficial in treating gingival recession defects when combined with a connective tissue graft or a CAF; these researchers achieved greater attachment gain in the EMD group, which were statistically significant compared to controls.[107-110] Longer follow-up is needed to verify whether long-term stability of the CAL gain is observed. Even though successful root coverage with healthy, thick keratinized tissue has been reported,[107,108] some studies[109,110] questioned the benefit of using EMD with CAF procedures since no difference was found between EMD-treated and non–EMD-treated sites.

Another purpose of using EMD in the present study was to explore whether EMD could act as a space maintenance device between the membrane and the root or bone surfaces. Space maintenance is considered very important for the success of GTR-based tissue regeneration due to the concept that it allows the pleuripotential cells to repopulate the area.[50,134,141-147] However, results obtained from this study did not support that hypothesis.

The amount of keratinized gingiva in the present study did not show a significant difference between baseline and 3 or 6 months. The lack of significance in KG gain is similar to findings in previously reported studies, which showed no change in the amounts of KG between pre- and post-surgery.[56,61,66,130] Numerous other researchers, however, have reported a significant gain of KG after a longer follow-up period.[50,59,60,65,73,74,116-118,121,123,128,129,132,141] These studies reported a range of KG gain between 0.4 and 2.9 mm with a follow-up period of 12 months.

One limitation of our study lies in the fact that the results were analyzed only up to 6 months. A longer follow-up is needed to make the results more meaningful. Also, the study included no histological demonstration of the type of attachment outcomes present in either the test or control groups. Attachment outcome results with GTR reported in the literature showed consistently significant formation of bone, cementum, and connective tissue attachment.[45-47,148] Studies reporting the use of EMD with GTR found that EMD possesses a potential to stimulate the formation of new connective tissue attachment, new bone, new periodontal ligament fibers, and new cementum, possibly with inserting collagen fibers.[83-85,87,99,100,149] Another limitation of our study was that it did not evaluate the effect of smoking on the clinical treatment outcomes, since we excluded patients who were smokers. Several studies reported in the literature indicated a negative effect of smoking on GTR treatment outcomes,[61,119,128,129,132,150] as well as on using EMD as a treatment approach.[95,98,139]

Within the limitations of the present study, it can be concluded that GTR-based root coverage utilizing collagen membrane, with or without EMD, can be used successfully in obtaining gingival recession coverage. The application of EMD during collagen membrane GTR-based root coverage procedures did not add additional benefits to the final clinical outcome.

ACKNOWLEDGMENTS

This study was partially supported by the University of Michigan Periodontal Graduate Student Research Fund. The authors thank Drs. Stephen Meraw and Paul Krebsbach, Department of Periodontics/Prevention/Geriatrics, and Dr. Stephen Soehren, Department of Oral and Maxillofacial Surgery/Hospital Dentistry, University of Michigan, for their critical input and support. In addition, we would like to thank Biora, Inc., Chicago, Illinois (now Straumann Biologics Division) and Zimmer Dental (formerly Centerpulse Dental Inc.), Carlsbad, California, for donating materials for the study.

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Accepted for publication February 13, 2004.