A Randomized Clinical Trial Comparing Enamel Matrix Derivative and Membrane Treatment of Buccal Class II Furcation Involvement in Mandibular Molars. Part I: Study Design and Results for Primary Outcomes

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Background: The objective of this multicenter, randomized trial was to compare enamel matrix derivative (EMD; test) with barrier membranes (control) for the treatment of mandibular buccal Class II furcation defects.

Methods: Forty-five patients with 90 comparable defects on contralateral molars were included. Defects were randomly assigned to EMD or bioabsorbable barrier membrane; the contralateral defect received the alternative treatment. Assessments at baseline and 8 and 14 months included gingival margin levels, probing depths, bleeding on probing, vertical attachment levels, and vertical bone sounding from a stent at five buccal sites/tooth. Defect dimensions were recorded at surgery and during reentry at 14 months. Change of open horizontal furcation depth was the primary outcome variable. Adverse reactions and patient perceptions were also noted.

Results: Both treatment modalities led to significant clinical improvements. The median reduction of open horizontal furcation depth was 2.8 mm with the corresponding interquartile interval (1.5 mm, 3.5 mm) at test sites compared with 1.8 mm (1.0 mm, 2.8 mm) at control sites. The Hodges-Lehmann estimator of the advantage (reduction test versus control) was 0.75 mm (95% confidence interval [CI]: 0.125 mm, 1.375 mm, \(P = 0.033\), Wilcoxon). The frequency of complete furcation closure was 8/45 (test) and 3/45 (control); partial closure, 27/45 in both groups; no change, 9/45 and 11/45, respectively; and deterioration, 1/45 and 4/45, respectively. The frequency of no pain or no swelling at 1 week post-surgery was 62% and 44%, respectively, at the test sites and 12% and 6% at the control sites.

Conclusion: There was a significantly greater reduction in horizontal furcation depth and a comparatively lower incidence of postoperative pain/swelling following enamel matrix derivative compared to membrane therapy. *J Periodontol 2004;75:1150-1160.

KEY WORDS
Clinical trials, randomized; comparison studies; enamel matrix derivative; furcation/surgery; guided bone regeneration; membranes, barrier.

Furcation involvements present one of the greatest challenges in periodontal therapy because furcation-involved molar teeth respond less favorably to conventional periodontal therapy than non-involved molar or non-molar teeth.¹,² In the past, several techniques have been proposed and promoted to treat furcated molars and thereby improve their prognosis. Various regenerative procedures have been tried with the aim of reducing the furcation depth.³-⁶

Successful regeneration of periodontal furcation defects is clinically defined as the complete elimination of horizontal and vertical defect components by bone fill. Histologically, a successful furcation fill is characterized by periodontal regeneration, defined as the formation of new bone, new cementum, and a new periodontal ligament over previously plaque-exposed root surfaces. As histologic evidence for successful furcation regeneration is not a practical outcome variable for controlled clinical trials, any change in direct bone measurements (at surgery and at reentry) serves as a primary outcome variable to evaluate clin-
The prospect of using barrier membranes to achieve improvements in furcation defects has led to numerous clinical studies in the past years, and comprehensive review articles have been published on the outcomes of membrane therapy in furcation defects. A recent systematic review assessed the efficacy of membrane therapy in the treatment of periodontal furcation defects measured against standard surgical periodontal treatment; i.e., open flap debridement. For the primary outcome, reduction in horizontal furcation depth assessed at surgical reentry, the weighted mean difference between guided tissue regeneration (GTR) and the control was 1.51 mm (95% CI: 0.39 mm to 2.62 mm; <0.001) in mandibular Class II furcations.

An alternative approach may be provided by the use of a commercially available enamel matrix derivative (EMD). Enamel matrix proteins are secreted by cells of Hertwig’s epithelial root sheath during root development. They are thought to be a crucial factor in initiating the formation of acellular root cementum and stimulating development of the periodontal ligament and alveolar bone. Animal studies have demonstrated that the application of EMD facilitated periodontal regeneration. Clinical and histologic studies could demonstrate favorable outcomes with the use of EMD in vertical periodontal defects. However, at present there are no data available on the effects of EMD in the treatment of periodontal furcation defects.

The present multi-center study was conducted to assess the effects of EMD treatment in buccal Class II furcation-involved mandibular molars, and to compare the effects of this therapy with those of the standard membrane treatment. Here, we describe the study design and results for the primary outcomes.

**MATERIALS AND METHODS**

**Study Design and Randomization**

The multicenter study had an examiner-masked, split-mouth, randomized design. The investigation was performed in five centers involving a total of five clinicians and five masked examiners. The furcation defects were described and recorded, and then randomized to treatment with either EMD or a bioabsorbable barrier membrane, the active control. The first surgery was always performed on the left side. The computer-generated randomization code was kept in a sealed envelope provided by a central randomization facility, and revealed to the clinician only after the first surgical site had been prepared. The second (contralateral molar) surgery was performed within 2 to 6 weeks. No patient or defect characteristics were available to the central randomization registrar. Patient outcomes were evaluated during the healing period, while clinical outcomes were evaluated at 8 and 14 months. The study design is outlined in Figure 1.

**Investigators’ Meeting and Examiner Calibration**

An investigators’ meeting was conducted to standardize the examination and surgical procedures. A video illustrating the surgical procedures ensured that a standardized approach was performed at the different study centers. A calibration exercise was performed to obtain acceptable intraexaminer consistency in the probing assessments. The examiners measured probing depth (PD) and clinical attachment level (CAL) from a customized acrylic stent, and classified the furcation involvements at 35 to 60 sites. The sites measured were comparable to the sites to be measured in the study. Repeated measurements were performed at least 1 hour later. The results were described and recorded, and then randomized to treatment with either EMD or a bioabsorbable barrier membrane, the active control. The first surgery was always performed on the left side. The computer-generated randomization code was kept in a sealed envelope provided by a central randomization facility, and revealed to the clinician only after the first surgical site had been prepared. The second (contralateral molar) surgery was performed within 2 to 6 weeks. No patient or defect characteristics were available to the central randomization registrar. Patient outcomes were evaluated during the healing period, while clinical outcomes were evaluated at 8 and 14 months. The study design is outlined in Figure 1.
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 Patients and Defects
Patients were recruited at four university dental schools and one private periodontal practice. Patients had to present with full-mouth plaque scores and full-mouth bleeding scores of <25% at study baseline (following completion of the initial periodontal treatment phase). Patients all had buccal Class II furcation involvements (horizontal probing depth of >3 mm) in both lower first or second molars (i.e., the compared teeth were contralateral). If suitable pairs of first and second contralateral mandibular molars were present in one patient, the first molars were selected for the study. The study protocol and procedures, including the associated risks and benefits of each therapy, were explained in detail to the patients. Each patient signed a consent form. The study was performed according to the Declaration of Helsinki II, and the study protocol was reviewed and approved by the International Ethics Committee in Freiburg, Germany.

The selected teeth were required to have proximal bone levels at or above the fornix of the furcation. In addition, they had to present with a zone of keratinized tissue of at least 2 mm adjacent to the furcation defect, in order to provide coverage of the furcation entrance during surgery. If the selected teeth had been subjected to root canal treatment, they had to present with a zone of keratinized tissue, any remaining subgingival calculus, and osteal access flap (not involving the papilla) was raised (Figs. 2 and 3). In this way, the surgical area was limited to the experimental site. Granulation tissue, any remaining subgingival calculus, and enamel pearls were removed (Figs. 2B and 3B). The morphology of the furcation defect was then examined and recorded. The contralateral defects were then treated with the control membrane therapy, according to the randomization. The morphology of the furcation defect was then examined and recorded. The first furcations were treated with either EMD or the control membrane therapy, according to the randomization. The contralateral defects were then treated with the alternative therapy.

Sample Size Calculation
Efficacy of therapy was described by change of horizontal furcation depth between surgery and reentry (primary parameter). Two therapies, application of EMD and the placement of a bioabsorbable barrier membrane, were compared. A sample size of 60 patients was estimated to have 90% power to detect a difference in change of horizontal depth of 0.7 mm, assuming a standard deviation of differences of changes of 1.6 mm, using a parametric test procedure with a 0.05 two-sided significance level.

Pretreatment
All subjects went through initial treatment, including instruction in proper oral hygiene measures, scaling, and professional tooth cleaning (including the furcations). Surgical treatment of the furcation defects was not scheduled until the patient could demonstrate an adequate standard of supragingival plaque control.

Clinical Measurements
The following clinical parameters were evaluated in duplicate on the day of surgery and at 8 and 14 months: bleeding on probing, probing depth, clinical attachment level, and location of gingival margin. Full-mouth plaque scores and site plaque scores were measured after 2, 3, 6, 8, and 14 months.

Vertical soft tissue measurements were made at five buccal sites (mesial, mid-mesial root, mid-furcation, mid-distal root, distal) using an individually manufactured acrylic stent with grooves and a standard periodontal probe. All measurements were recorded to the nearest 0.5 mm.

Following local anesthesia, a vertical bone sounding was performed at the same five sites from the stent. Horizontal bone sounding was made in the furcation area. The furcation defects were classified on a four-stage scale using a Nabers probe: Class 0: no furcation involvement; Class I: initial furcation involvement (barely probeable up to 3 mm); Class II: furcation involvement (clearly probeable more than 3 mm); or Class III: through- and-through furcation involvement.

Surgical Procedures
Following local anesthesia, a full-thickness (muco-periosteal) access flap (not involving the papilla) was raised on the buccal surface of the alveolar process and relieving incisions were made (Figs. 2 and 3). In this way, the surgical area was limited to the experimental site. Granulation tissue, any remaining subgingival calculus, and enamel pearls were removed (Figs. 2B and 3B). The furcation area was cleaned by hand instrumentation, oscillating scalers, and rotating diamond burs.†† The morphology of the furcation defect was then examined and recorded. The first furcations were treated with either EMD or the control membrane therapy, according to the randomization. The contralateral defects were then treated with the alternative therapy.

In sites receiving EMD therapy, the smear layer was removed by conditioning with EDTA gel†† (sterile 24% EDTA gel, pH 6.7) for 2 minutes, followed by thorough rinsing with a sterile saline solution. Excess fluids were removed, leaving the surgical area clear. EMD was then
Figure 2.
A) Preoperative view of tooth #47 affected by a buccal Class II furcation defect. B) Debridement after full-thickness flap elevation. C) Application of enamel matrix derivative to the defect. D) Flap closure sutured in order to completely cover the coronal entrance of the furcation. E) Clinical view 14 months following surgery. F) Defect during the reentry procedure.
Figure 3.
A) Preoperative view of tooth #37 affected by a buccal Class II furcation defect. B) Debridement after full-thickness flap elevation. C) Placement of barrier membrane secured over the furcation entrance. D) Flap closure sutured in order to completely cover the coronal entrance of the furcation. E) Clinical view 14 months following surgery. F) Defect during the reentry procedure.
immediately applied, starting at the farthest end of the involved furcation, covering the entire denuded root surface (Fig. 2C). At sites treated with membrane therapy, conditioning with EDTA was not performed, and the membrane was adapted to the defect and secured by a sling suture (Fig. 3C). The surgical flap was then replaced; if necessary, it was extended coronally after periosteal fenestration, to ensure that it covered the entrance of the furcation. The flap was sutured, using non-irritating sutures§§ (4/0) to ensure tight adaptation of the flap margins and a stable wound (Figs. 2D and 3D). The sutures were removed 2 weeks postsurgery. The mean number of days between the two surgeries was 26.

**Intrasurgical Measurements**

Assessments of the furcation defects were made at baseline (during surgery), and at 14 months during a surgical reentry procedure (Figs. 2F and 3F). Duplicate measurements were always made, and the mean of the two taken.

The primary outcome measure was the horizontal depth of the furcation at its deepest point. This was measured using a periodontal probe with a rubber stopper that was adjusted to the buccal prominence of the roots. Vertical measurements of the hard tissue boundaries were made using the individually manufactured acrylic stent. These measurements were taken at five sites from the stent to the mesial and distal buccal bone crest, mid-distal and mid-mesial root, and mid-furcation. In addition, the height and width of the furcation entrance were determined (Fig. 4).

**Post-Surgical Infection Control and Maintenance Care**

Patients were advised to rinse with a 0.2% chlorhexidine solution twice a day for 2 weeks following surgery. For the following 4 weeks, they used a chlorhexidine gel applied in the area of surgery with a cotton swab. Each patient was re instructed in proper oral hygiene measures when toothbrushing was reinstated 6 weeks after surgery.

Any adverse reactions or post-surgical complications were recorded using a questionnaire at the time of suture removal (1 to 3 weeks after surgery) and by open questioning throughout the study.

During the clinical healing phase, all patients underwent a gentle supragingival professional tooth cleaning by means of a rubber cup with toothpaste at weeks 1, 2, 3, 4, and 6. All patients were maintained in a maintenance program and received full-mouth prophylaxis at 3, 6, 8, and 14 months. However, no subgingival instrumentation was performed in the furcations until the 14-month examination.

**Data Management and Statistical Analysis**

For the analysis, three populations were considered. For assessments of safety, all patients receiving at least one treatment were analyzed (safety population). In the intention-to-treat (ITT) analysis, all patients were included who belonged to the safety population and who met the inclusion and exclusion criteria (ITT population). The per-protocol (PP) analysis included all patients in the ITT population who did not violate the inclusion or exclusion criteria and who were not considered major protocol violators and received a full course of treatment (PP population).

All data were double entered into two databases by independent data typists. Quality and validity of data were checked. Analyses were carried out using a statistical software program. In order to get exact estimators for the treatment effects, another program was applied to the computed data. As all measurements were performed twice, means were calculated and used in the analysis.

The study was designed to assess both the effectiveness of EMD treatment and to compare its efficacy with bioabsorbable barrier membrane treatment. The analysis of the primary parameter, change of open horizontal furcation depth between surgery and 14 months post-surgery, was performed in a confirmatory manner. The distribution of the observed values and of the computed changes or differences was described by the number of non-missing values, median, first and third quartiles, and range. As a normal distribution could not be assumed, the non-parametric Wilcoxon two-sample test was used to test the null hypothesis of no different treatment effects between both treatments. As a non-parametric estimator of the advantage (reduction test versus control), the Hodges-Lehmann estimator and the

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§§ Ethibond Excel, Ethicon, Norderstedt, Germany.
¶¶ SAS Institute, Cary, NC.
¶¶ StatXact Version 4.0.1 for Windows, CYTEL Software Corporation, Cambridge, MA.
corresponding 95% CI were computed. Before this analysis was done, one of the first steps was to test the possible center effect and the possible interaction between the factors center and treatment.

Descriptive statistical methods were applied to the secondary parameters and descriptive statistical measures (number of non-missing values, median, first and third quartiles, and range) or frequency tables were calculated separately for sequence and/or treatment. The calculated \( P \) values of the statistical tests were also interpreted in a descriptive manner. They are a measure of how the observed data differ from that expected under the assumption of no different treatment effect. Therefore, they can be used only as a guide to the relative importance of the findings.

As mentioned above, normal distribution could not be assumed for the data obtained in this study. In the literature, very often the distribution of the observed parameters is described by the normal distribution presuming mean and standard deviation (SD). To enable the comparison of the results of this study with the literature, the mean and standard deviation were computed as well as the median and interquartile intervals.

**RESULTS**

**Patients**

A total of 51 patients (26 men, 25 women) were consecutively recruited and randomly assigned to one of the two treatment sequences. It was not possible to recruit the desired sample size of 60 patients due to difficulties in finding patients who met the inclusion criteria; that is, presenting with matching furcation defects in contralateral mandibular molars.

As a result of fulfilling an exclusion criterion, unveiling before or during the second surgery, three patients had to be excluded from the ITT population. Owing to protocol violations, a further three patients had to be excluded from the PP population (two patients did not give consent for reentry assessments to be made, and one was lost to follow-up after 6 months).

The PP population comprised 24 men and 21 women, with a mean age of 53 years (median 54 years; range 28 to 73 years), who contributed 27 pairs of first molars and 18 pairs of second molars to the study. Nine of the patients were current smokers (seven to 20 cigarettes per day) (Table 1).

**Treatment Assessments**

No treatment by center interaction was observed. Both treatment modalities led to significant improvements regarding the primary outcome: change of open horizontal furcation depth between baseline and reentry (Figs. 5 and 6).

Disregarding the sequences, the median reduction of open horizontal furcation depth was 2.8 mm, with the corresponding interquartile interval (1.5 mm, 3.5 mm) at test sites, compared to 1.8 mm, and the interquartile interval (1.0 mm, 2.8 mm) at control sites. The mean reduction was 2.6 \( \pm \) 1.8 mm at test sites compared with 1.9 \( \pm \) 1.4 mm at control sites. In order to get an un-biased estimation and taking into account the study design, the Hodges-Lehmann estimator of the advantage (reduction test versus control) was 0.75 mm (95% CI: 0.125 mm, 1.375 mm; \( P = 0.033 \), Wilcoxon). Outcomes for the individual pairs of furcation defects are displayed in Figure 6.

As judged clinically, EMD treatment reduced 35 of the 45 (78%) defects, eight (18%) of them completely. Nine (20%) of the defects did not improve and 1 (2%) deteriorated. In contrast, membrane treatment reduced 30 of the 45 (67%) defects, three (7%) of them com-

**Table 1.**

**Patient Characteristics at Baseline (n = 45*)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LE–RM</td>
</tr>
<tr>
<td>N patients</td>
<td>18</td>
</tr>
<tr>
<td>Age in years (range)</td>
<td>52.5 (33-62)</td>
</tr>
<tr>
<td>Gender (% female)</td>
<td>61</td>
</tr>
<tr>
<td>Smokers (≤20 cigarettes/day; %)</td>
<td>22</td>
</tr>
</tbody>
</table>

* Six patients were excluded from the PP analysis (three met exclusion criteria before or during the second surgery, two did not give consent for reentry to be made, and one was lost to follow-up).

LE = left EMD; RM = right membrane; RE = right EMD; LM = left membrane.
DISCUSSION
The results of the present randomized, multi-center clinical trial demonstrate that for mandibular buccal Class II furcation involvements both the application of EMD as well as GTR membrane therapy result in a significant improvement in the primary outcome parameter, i.e., horizontal furcation depth assessed during reentry. However, EMD was statistically significantly more beneficial for reducing the horizontal furcation depth than GTR, which was used as an active control in the present study.

The placement of GTR barrier membranes represents the recommended and most well-documented regenerative treatment modality for mandibular Class II furcation defects.3-6 In these review articles, the reported mean values for horizontal bone fill in Class II furcations following membrane therapy ranged from 0.5 to 2.5 mm. The type of bioabsorbable membrane used in the present study, in particular, had previously shown promising results.24,25

In a recent systematic review of GTR in furcation defects,8 it was demonstrated that when GTR was compared with open flap debridement, the weighted mean difference with regard to reduction in horizontal furcation depth assessed during reentry was 1.5 mm ($P < 0.001$). In the few studies that qualified for meta-analysis, the observed mean reduction of horizontal furcation depth ranged from 0.2 to 4.4 mm. At the same time, it was concluded that a complete closure of Class II molar furcation defects following placement of barrier membranes appeared to be an unpredictable outcome that occurred in less than 10% of cases, as evaluated at reentry.26 Thus, the results following membrane therapy in the present study, where we observed a mean horizontal bone gain of 1.9 mm and clinical closure in only

Figure 6.
A) Treatment by treatment scatterplot for individual pairs of furcation defects treated by EMD and barrier membrane. Change in horizontal furcation depth (surgery to reentry). B) Treatment by treatment scatterplot for individual pairs of furcation defects treated by EMD and barrier membrane. Relative change in horizontal furcation depth (surgery to reentry, % defect fill). LE = left EMD; RM = right membrane; RE = right EMD; LM = left membrane.

Adverse Reactions and Patient Perceptions
The frequency of no pain or no swelling (1 week postoperative) was 62% and 44%, respectively, at the test sites, and 12% and 6%, respectively, at the control sites in the ITT population (Fig. 8).

Antibiotics were prescribed twice after EMD surgery and eight times after membrane surgery; this difference was statistically significant ($P = 0.034$).

Figure 7.
Distribution of furcation class at 14 months following EMD or barrier membrane treatment.
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Figure 8.
A) Postoperative pain (1 week postsurgery) following EMD or barrier membrane treatment (N = 48 patients). B) Postoperative swelling (1 week postsurgery) following EMD or barrier membrane treatment (N = 48 patients).

three out of 45 treated defects, are in agreement with previous work.

In contrast, EMD treatment showed a higher degree of predictability than GTR, as more defects were closed completely and fewer defects deteriorated. At present, there are no data available from other studies on the effects of EMD application in furcation defects. In comparative studies on intrabony defects, the results following EMD application were equivalent to those of membrane therapy.14,19,27

In the present study, change in furcation status was judged clinically before surgical reentry using a Nabers probe. These values may not necessarily resemble the ones obtained from open bone measurements, since it should be remembered that a 100% defect resolution measured by a periodontal probe with a rubber stopper, as used in the present study, would require a bone fill up to the level of the buccal root prominence. Earlier studies attempted to determine change in defect volume and defect resolution by the analysis of study models obtained from impressions taken during surgery and at reentry,28 whereas in a more recent publication probing bone level measurements with furcation probes were proposed in order to avoid the trauma from a surgical reentry procedure.29 Thus, at present there appears to be no generally accepted modality to determine bone fill of furcations during clinical regenerative trials.

At a recent consensus conference it was stated that more emphasis should be given to patient-centered outcomes and evaluation of adverse effects of regenerative therapy.26 The split-mouth design of the present study allowed for a direct comparison of the patient’s perception of both treatment modalities. Lack of pain, improved healing of the soft tissues, and limited inflammation of the operated areas have been common clinical observations following surgical periodontal therapy with EMD; however, such observations are purely clinical and difficult to explain. Data from a recent study investigating wound healing in the dento-gingival region following soft tissue curettage and EMD application support the favorable healing response we observed in the present study.30 The biological mechanisms behind these effects of EMD on the early phase of healing, however, are presently not understood. EMD was thought to be present at the instrumented site for a period of 1 to 2 weeks,31 but in a more recent report it could be demonstrated by means of immunohistochemistry that EMD is present on treated root surfaces for up to 4 weeks following periodontal surgery.32 Even though no conclusions can be drawn from these studies regarding the persistence of EMD in the soft and hard tissues surrounding the periodontal defects, it has to be assumed that EMD has an effect on the critical steps of periodontal wound healing that occur during the early healing phase. It is not likely that the enhanced healing is related to an early epithelialization of the soft tissue wound, since epithelial cells may not be influenced by EMD.31 On the other hand, it has been shown that EMD facilitates the attachment and proliferation of periodontal ligament cells and stimulates fibroblasts to release growth factors such as transforming growth factor-β, a mediator known to influence the early phase of mucosal healing.33,34 Another factor that might positively affect the early wound healing is the antimicrobial property of EMD that was studied in an ex vivo dental plaque model.35 Further studies are needed to elucidate the mechanisms behind the influence of EMD on soft tissue wound healing.
In conclusion, the present study demonstrates that the application of EMD results in a significantly greater reduction of horizontal furcation depth, the main outcome parameter, than standard membrane treatment of buccal mandibular Class II furcation defects. At the same time, there are fewer adverse events and postoperative complications during the early healing period, resulting in less discomfort for the patient undergoing regenerative furcation therapy.

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