

Treatment of Intrabony Defects With Enamel Matrix Proteins or Barrier Membranes: Results From a Multicenter Practice-Based Clinical Trial

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Background: This prospective multicenter, randomized, controlled clinical trial compared the clinical outcomes of enamel matrix proteins (EMD) versus placement of a bioabsorbable membrane in conjunction with guided tissue regeneration (GTR).

Methods: Seventy-five patients with advanced chronic periodontitis were recruited in seven centers in three countries. All patients had at least one intrabony defect of ≥ 3 mm. Heavy smokers (≥ 20 cigarettes/day) were excluded. The surgical procedures included access for root instrumentation using the simplified papilla preservation flap and either the application of EMD or the placement of a GTR membrane. At baseline and 1 year following the interventions, clinical attachment levels (CAL), probing depths (PD), recession (REC), full-mouth plaque scores, and full-mouth bleeding scores were assessed. A total of 67 patients completed the study.

Results: At 1 year, the EMD defects gained 3.1 ± 1.8 mm of CAL, versus 2.5 ± 1.9 mm for GTR defects. Probing depth reduction was 3.8 ± 1.5 mm and 3.3 ± 1.5 mm, respectively. A multivariate analysis indicated that the differences between EMD and GTR treatments were not significant while a center effect and baseline PD significantly influenced CAL gains. No significant differences in terms of frequency distribution of the outcomes were observed. All cases treated with GTR presented at least one surgical complication, mostly membrane exposure, while only 6% of EMD treated sites displayed complications ($P < 0.0001$).

Conclusions: The results of this trial failed to demonstrate superiority of one treatment modality over the other. GTR outcomes in this trial were lower than anticipated based on previous evidence. This was attributed to the high prevalence of post-surgical complications in the GTR group. *J Periodontol* 2004;75:726-733.

KEY WORDS

Clinical trials, controlled; clinical trials, randomized; comparison studies; guided tissue regeneration; membranes, bioabsorbable; multicenter studies; proteins, enamel matrix/therapeutic use.

The ultimate goal of periodontal treatment is the regeneration of tissues that have been lost to periodontal disease. Considerable histological and clinical evidence gathered over the last 2 decades indicates that the regeneration of periodontal tissues lost as a result of periodontitis can be achieved in humans. In particular, two clinical approaches have been routinely employed with considerable success: bone grafting¹ and guided tissue regeneration (GTR) with barrier membranes.²

GTR is one of the best documented regenerative approaches. Cumulative evidence from randomized clinical trials indicates that GTR is an efficacious and predictable procedure for the treatment of intraosseous periodontal defects.³⁻⁵ A review published in 2000, which summarized the clinical outcomes following application of GTR to the treatment of deep intrabony defects using weighted means, indicated that clinically significant attachment level gains of 3.7 ± 1.8 mm were obtained with GTR.² However, the weighted mean difference between GTR alone and open flap debridement was just 1.11 mm (95% confidence interval [CI] 0.63 to 1.59) when clinical trials were evaluated by a systematic review.⁶ Although GTR has been proven to promote the regrowth of the destroyed periodontium, the clinical application is often difficult and several confounding factors

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have been described as important modifiers of this regenerative outcome.^{7,8}

As a result, substantial variations in clinical predictability, degree of efficacy, and histological outcomes are found in the literature.^{1,2} These shortcomings associated with GTR and advances in developmental biology have fostered a growing interest in the possibility of modulating the periodontal wound healing process using biological mediators. Current knowledge of cellular events involved in tooth formation supports the development of a new approach to periodontal regeneration by focusing on the potential role of mediators expressed by Hertwig's root sheath, mainly enamel matrix proteins (EMD), in reconstructing the periodontal ligament.⁹ Applying proteins derived from the enamel matrix has provided histological evidence for periodontal regeneration in monkeys^{9,10} and humans.¹¹⁻¹⁴ Subsequent clinical studies provided evidence of significant clinical attachment level gains and probing depth reductions in case series.¹⁵⁻¹⁹ Clinical trials have also reported improved gains in clinical attachment levels following the application of enamel matrix derivative (EMD) in the regeneration of intrabony defects with respect to access flap alone.²⁰⁻²⁷ Similar therapeutic outcomes were reported in studies where EMD treatment was compared directly to GTR, both with bioabsorbable^{17,21,25} and non-resorbable membranes.^{23,26} In all these studies, the clinical efficiency of EMD in the treatment of human periodontal intrabony defects was established through significant CAL gains and PD reductions. However, there is minimal direct evidence evaluating the possible additional benefit expected from the application of EMD when compared with GTR and this may limit the routine use of this treatment option.

The objective of the present clinical investigation was therefore to compare, in a practice-based multicenter, randomized controlled clinical trial, the outcomes obtained following treatment of intrabony defects with papilla preservation flap surgery with application of EMD or with the application of GTR with bioabsorbable membranes.

MATERIALS AND METHODS

Experimental Design

A parallel group, randomized, multicenter, controlled clinical trial was designed to test the efficacy of two treatment modalities in intrabony periodontal defects. The test treatment consisted of access of the defect with papilla preservation flaps, surgical debridement, root conditioning, and application of EMD^{††} to the debrided root surface. The same procedure was performed in the control group except that root conditioning and EMD application were omitted and a bioabsorbable membrane^{‡‡} was placed according to the principles of GTR. A single defect was treated in each patient. Surgical site-related outcomes were evaluated during the healing period, while clinical outcomes were evaluated at 1 year. This investigation was performed at seven periodontal

practices in Spain, Portugal, and Italy constituting a practice-based research network. In each center a single clinician served as examiner and surgeon. To limit assessment bias, clinicians did not have previous patient measurements available to them and used a pressure sensitive probe. Each clinical center was connected with and supervised by a central monitoring facility at the University College London, United Kingdom.

Investigators' Meeting and Calibration

An investigator meeting was performed as previously described.²⁷ In brief, a calibration exercise was performed to obtain acceptable intra- and inter-examiner reproducibility for probing depth, recession of the gingival margin, and evaluation of defect anatomy. Intra-examiner reproducibility was evaluated as standard deviation of the difference of triplicate measurements. All investigators reached the target of a standard deviation lower than 0.4 mm for attachment levels. Inter-examiner variability was evaluated as standard deviation of the difference from the gold standard represented by author IZ. The computed value for attachment level was less than 0.5 mm for all clinicians.

Study Population

Inclusion and exclusion criteria were as previously reported.⁵ In brief, patients younger than 21 years, with uncontrolled or poorly controlled diabetes, unstable or life threatening conditions, requiring antibiotic prophylaxis, or heavy smokers (>20 cigarettes/day) were excluded. Only patients with a diagnosis of severe periodontitis previously treated by oral hygiene instructions and scaling and root planing were invited to participate. These subjects had to present with full mouth plaque scores (FMPS) and/or full mouth bleeding scores (FMBS) <25% at study baseline (following completion of the initial periodontal treatment phase).^{7,8} The patients were informed in detail about the possible risks and benefits and were asked to give their consent to the trial. The Ethical Committee of the University Complutense of Madrid, Spain approved the study protocol.

Entry criterion was the presence of a deep intrabony defect (≥ 3 mm), located in the interproximal area, in anterior and premolar teeth. Defects extending into a furcation were not included. Defect depth and absence of furcation involvement were preliminarily identified during screening, and were confirmed during surgery. Inclusion of defects involving the mesial aspect of the lower first molar was individually evaluated for access and thickness of the alveolar ridge (ability to preserve the papilla). The presence of a 2 to 3 mm band of keratinized tissue to allow surgical manipulation, flap adaptation, and suturing according to the protocol was also required.

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Pretreatment

Control of periodontal infection was achieved prior to the experimental phase by an initial treatment consisting of patient motivation, oral hygiene instructions, and scaling and root planing. When indicated, clinicians supplemented mechanical debridement with antiseptics.

Randomization and Study Power

After verifying entry criteria, 75 patients gave informed consent and were enrolled into the study. All subjects were assigned a patient number and were assigned to one of the two treatment regimens using a random number table. Clinicians were not aware of treatment allocation until after root debridement. Based on previous pilot data⁵ on variance of CAL gains, the study was designed to have sufficient power to detect a true difference of 1.0 mm of CAL gain with alpha set at 0.05 and a power of 0.8.

Clinical Measurements

Before anesthesia, the following clinical parameters were evaluated on the day of surgery and 1 year later. Full mouth plaque scores (FMPS) were recorded as the percentage of total surfaces (4 per tooth) which revealed the presence of plaque. Bleeding on probing from the bottom of the pocket was assessed dichotomously at a force of 0.3 N with a manual pressure sensitive probe.^{§§} Full mouth bleeding scores (FMBS) were then calculated. Probing depth (PD) and gingival margin recession (REC) were recorded to the nearest millimeter with a manual pressure sensitive probe by trained investigators at the deepest location of the selected interdental site. All measurements were taken with the same pressure sensitive manual periodontal probe at 0.3 N. Clinical attachment levels, calculated as the sum of PD and REC, were the primary outcome variable.

Surgical Procedures

Test and control defects were accessed using simplified papilla preservation flaps.²⁸ The exposed defects were carefully scaled and root planed to remove residual mineralized deposits, but not necessarily the root cementum. A combination of sonic, ultrasonic, and/or hand instrumentation was used. Root surfaces at test sites were conditioned with a neutral pH EDTA gel^{|||} for 2 minutes.^{29,30} In these sites, EMD gel was applied on the root surface and to overfill the defect. The flaps were then replaced and sutured employing non-

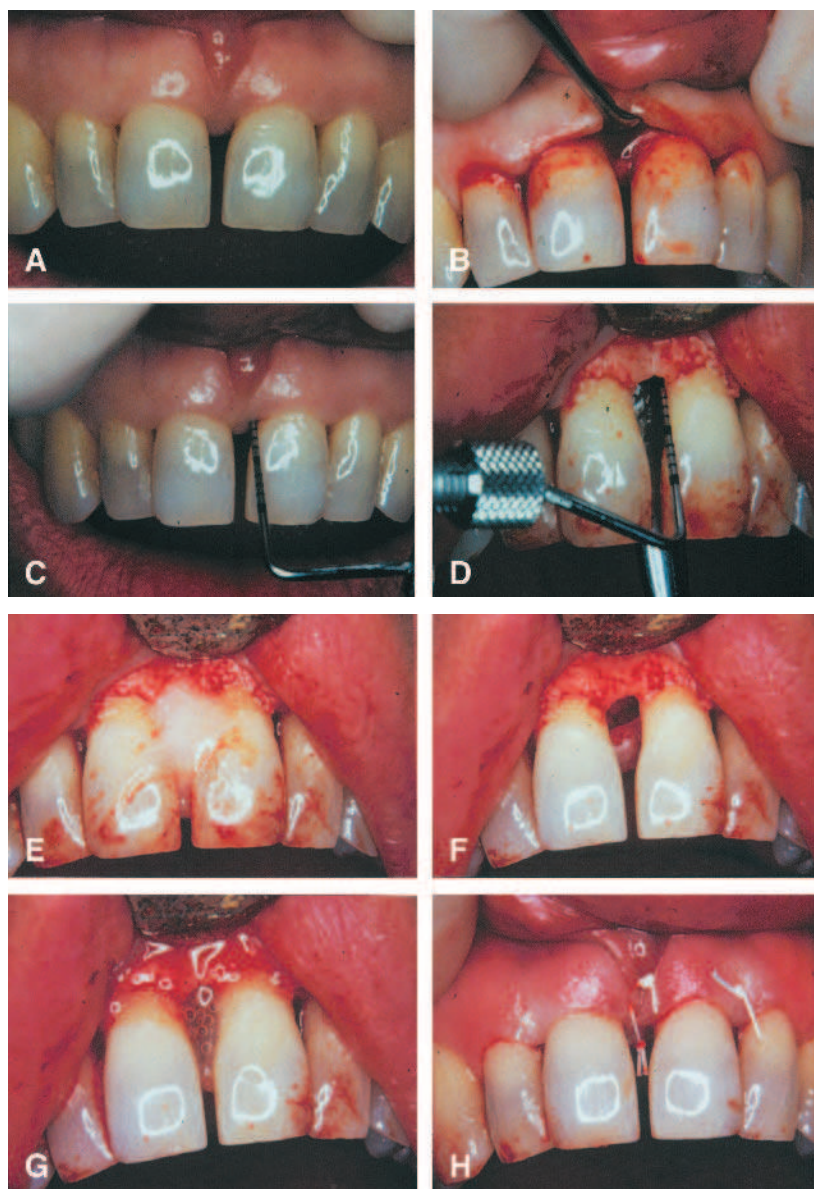


Figure 1.

Test surgical procedure (EMD) using simplified papilla preservation flaps. **A)** Before surgery; **B)** design of surgical incisions; **C)** measurement of the clinical attachment level; **D)** measurement of the intraosseous defect; **E)** EDTA application; **F)** conditioned roots and intraosseous defects; **G)** EMD application; **H)** suturing the flaps. Achieving primary closure.

resorbable expanded polytetrafluoroethylene sutures^{¶¶} as previously described² (Fig. 1). The control procedure was identical except that rather than applying EDTA and EMD, a bioabsorbable periodontal membrane was placed following the principles of guided tissue regeneration² (Fig. 2).

§§ Brodonic probe, Dentamar, The Netherlands, equipped with a PCP-UNC 15 tip, Hu-Friedy, Leimen, Germany.

||| PrepHgel, Biora AB, Huddinge, Sweden.

¶¶ Gore-Tex, W.L. Gore & Associates, Inc., Flagstaff, AZ.

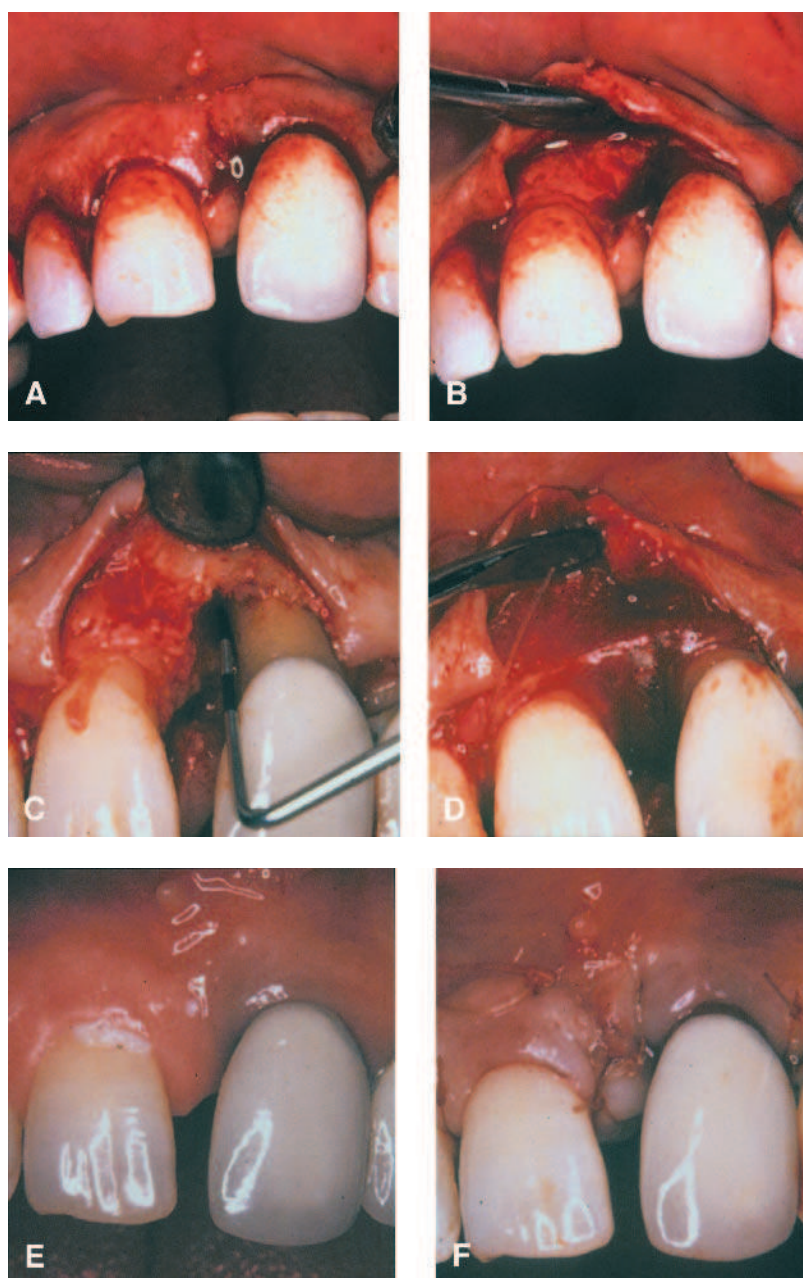


Figure 2.

Control surgical procedure (GTR) using simplified papilla preservation flaps. **A)** Design of surgical incisions; **B)** flap elevation; **C)** measurement of the intraosseous defect; **D)** placement of the bioabsorbable periodontal membrane; **E)** before surgery; **F)** suturing the flaps. Achieving primary closure.

Intrasurgical Clinical Measurements

The following defect morphology parameters were evaluated after debridement of the area: 1) distance from the cemento-enamel junction (CEJ) to the bottom of the defect (CEJ-BD); and 2) distance from the CEJ to the most coronal extension of the interdental bone crest (CEJ-BC) to the nearest millimeter. These measurements were performed at the deepest interdental point of the defect (i.e., the deepest point of the site defined

by the interdental line angles of the affected tooth). The intrabony component of the defect (INFRA) was calculated as $INFRA = (CEJ-BD) - (CEJ-BC)$. The number of bony walls of the predominant component of the defect was also recorded.

The duration of the surgical procedure was timed and the number of teeth involved in the surgical procedure was recorded.

Post-Surgical Instructions and Infection Control

Postoperative pain and edema were controlled with either 600 mg ibuprofen or 500 mg acetaminophen. Patients were instructed to rinse twice daily with 0.12% chlorhexidine and to use modified oral hygiene procedures in the treated area for the first 4 postoperative weeks. They were instructed to start gentle wiping of the operated dento-gingival area with a post-surgical toothbrush^{##} soaked in a 0.12% chlorhexidine solution from the third postoperative day. No interdental cleaning was allowed in the first four postoperative weeks. Smokers were asked to limit and possibly avoid smoking.

Post-Surgical Controls and Professional Tooth Cleaning (Weeks 1 to 6)

Sutures were removed after 1 week. Post-surgical controls and professional tooth cleaning consisting of supragingival prophylaxis with a rubber cup and 0.2% chlorhexidine gel,^{***} were performed at weeks 1, 2, 3, 4, and 6. At these time points, presence of edema, hematoma, suppuration, flap dehiscence, patient complaints, and membrane exposure (in the control group) were dichotomously recorded.

Maintenance Care (Months 3, 6, and 9)

All patients were enrolled in maintenance care programs and received full-mouth professional prophylaxis and calculus removal at 3, 6, and 9 months as previously detailed.⁵ No probing of the defect site was attempted until the final examination at 1 year.

Data Management and Statistical Analysis

Data were entered in a microcomputer and proofed for entry errors. The resulting database was locked and loaded into a statistical software program^{†††} for calculations and analyses. Data are expressed as means \pm SD. Imbalances in the test and control groups

^{##} Vitis Surgical, Dentaid SA, Barcelona, Spain.

^{***} PerioAid Gel, Dentaid SA.

^{†††} Version 8.03, SAS Institute, Cary, NC.

arising from the randomization process were evaluated using the unpaired *t* test for continuous variables and the chi-square test for categorical variables. The significance of the treatment effects on the dependent variables CAL changes and PD changes was estimated by constructing generalized linear models using the general linear modeling (GLM) procedure. The clinical center and the treatment by center interaction were incorporated as stratification factors.^{31,32} In case of a non-significant treatment-by-center interaction, the interaction term was removed from the analysis and the main effect model was applied.³² Final models were selected by elimination of non-significant factors. Model diagnostics included distribution of errors and analysis of residuals. Adjusted means were calculated using the least square means and PDIFF statements of the GLM procedure. Data were also analyzed as frequency distributions employing the Mantel-Haenszel chi-square test to compare distributions of outcomes at test and control sites. For all analyses the alpha error was set at 0.05.

RESULTS

Patient Retention and Missing Data

A total of 75 patients were entered and randomized; three withdrew informed consent before surgery. During the 1-year follow-up period, five patients were lost to follow-up, leaving complete observations available for 67 patients: 35 test and 32 control (89.3% of entered patients). All subsequent analyses were performed on this population. The patient and defect characteristics of the test and control groups yielded no significant differences between any of the patient associated variables (Table 1).

Oral Hygiene

Baseline FMPS and FMBS are displayed in Table 1. At 1 year, the FMPS was $11\% \pm 5\%$ for test and $13\% \pm 11\%$ for control treated patients ($P = 0.23$). Similarly, the FMBS was $9.5\% \pm 9\%$ for test and $11\% \pm 11.5\%$ for control subjects ($P = 0.71$).

Clinical Outcomes

For the primary outcome variable, the average gain in clinical attachment was 3.1 ± 1.8 mm after EMD application (test) and 2.5 ± 1.9 mm after GTR placement (control). One year after therapy, probing depth reductions were 3.8 ± 1.5 mm for the test group and 3.3 ± 1.5 mm for controls. Between baseline and 1 year, the gingival margin receded of 0.6 ± 0.9 mm for the test group and 0.7 ± 0.9 mm for the control group.

The significance of the treatment effect (gains in clinical attachment level) was evaluated taking into account the potential sources of variability arising from the multicenter design of the study and the previously described covariates.^{3,7,33} Since no treatment-by-

center interaction was observed, the main effect model was applied.³² The following variables were used in the model: treatment, center effect, smoking status, baseline PD, defect depth, and intrabony component (Table 2). The constructed multivariate model was statistically significant and explained 58% of the observed variability in CAL gain. A highly significant center effect was observed ($P = 0.0021$). The difference between the center that obtained the largest CAL gains and the one with the smallest was 2.6 mm. No significant differences were observed comparing the outcomes of the test and the control therapy ($P = 0.07$). Among the considered defect characteristics, the initial probing depth was a significant covariate ($P < 0.021$). However,

Table 1.

Baseline Patient and Defect Characteristics

Variable	EMD	GTR	P Value
N patients	35	32	.
Age	50.9 ± 7.7	52 ± 9.1	0.78
Gender (% females)	54.29	53.13	0.92
Smoker (% <20 cig/day)	28.57	35.48	0.55
FMPS	14 ± 7	15 ± 8	0.71
FMBS	10 ± 8	12 ± 8	0.23
PD (mm)	7.9 ± 1.8	8 ± 1.7	0.63
CAL (mm)	9.5 ± 2.6	9.7 ± 2.3	0.74
CEJ-BD (mm)	9.7 ± 2.9	9.9 ± 2.6	0.72
Intrabony component (mm)	6.2 ± 2.3	5.9 ± 2.1	0.81
Predominantly 1 wall (%)	17.24	14.81	0.84
Predominantly 2 walls (%)	20.69	29.63	0.84
Predominantly 3 walls (%)	62.07	55.56	0.84

Table 2.

Multivariate Analysis of CAL Gain

Parameter	Estimate	P Value
Treatment effect (EMD vs. GTR)	0.8 ± 0.4	0.07 (NS)
Center effect (worst vs. best)	-2.6 ± 0.8	0.0021
Smoking (yes vs. no)	-0.03 ± 0.5	0.95 (NS)
Baseline PD (mm)	0.7 ± 0.2	0.021
Defect depth CEJ-BD (mm)	0.1 ± 0.1	0.44 (NS)
Intrabony (mm)	-0.4 ± 0.2	0.07 (NS)

Significance of model $P < 0.0001$, adjusted $R^2 = 0.58$.

smoking and defect depth and intrabony components did not influence significantly the CAL gains. Adjusted means in CAL gain were 2.03 mm (95% confidence interval [CI] 0.82 to 3.24) for GTR and 2.78 mm (1.65 to 3.91) for EMD. The adjusted mean difference between test and control was -0.75 mm (-1.6 to 0.1 , $P = 0.08$, NS). In this respect, it is important to highlight that comparison of the 95% CI of the adjusted mean difference between test and control (-1.6 to 0.1 mm) with the range of clinical indifference indicated that differences of potential clinical significance were within the observed confidence interval. This indicates that, in spite of having performed a sample size calculation during the planning stages of the protocol, the study had insufficient power to detect potentially clinically relevant differences between the two treatments.

The frequency distribution of various CAL gains at test and control sites is given in Table 3. No significant differences between treatments were observed comparing the frequencies of obtaining <2 mm CAL gain, 2 to 3 mm CAL gain, and >3 mm CAL gain ($P = 0.28$, Mantel-Haenszel chi square test).

Finally, the impact of complications affecting the treatment outcomes in both groups was analyzed. A composite index was constructed defining the presence or absence of a complication as detection of any of the following at any of the postoperative visits (1, 3, 6, 9 weeks): lack of primary closure, flap dehiscence, membrane exposure (GTR only), or suppuration.

A significant difference in the incidence of complications was found between test and controls. In the GTR group, 100% of the cases had at least one complication at one or more of the postoperative visits, while in the EMD cases the incidence was 6% ($P < 0.0001$ Mantel-Haenszel chi square test). In the GTR group, membrane exposure occurred in 35% of the cases at 1 week, in 62% at 3 weeks, and in 29% at 6 weeks. When the impact of the composite index of complications on CAL gains was analyzed using a multiple regression model, it was statistically significant ($P = 0.0412$). In fact, the absence of surgical complications was associated with 0.85 mm greater CAL gains.

Table 3.
Frequency Distribution of CAL Gain

	Changes in CAL (mm)				
	Loss	0-1	2-3	4-5	≥ 6
Test (EMD)	0%	14%	40%	37%	9%
Control (GTR)	6%	19%	47%	22%	6%

Mantel-Haenszel chi-square $P = 0.563$ (NS).

DISCUSSION

This study failed to demonstrate the superiority of one of the treatments to the other. Both treatments, however, resulted in significant improvements in clinical attachment level gains and probing depths compared to baseline. At 12 months, the results for the EMD group demonstrated an average gain in clinical attachment of 3.1 ± 1.8 mm and an average probing depth reduction of 3.8 ± 1.5 mm. These results compare favorably with those reported in a previous multicenter clinical trial.²⁷ In fact, a recent meta-analysis on the outcomes of 12 randomized controlled clinical trials reported 3.2 ± 0.9 mm for CAL gain and 4.0 ± 0.9 mm for PD reductions³⁴ following treatment of intrabony defects with EMD. The test group of this study, therefore, performed as expected based on the previous evidence.

The GTR group, on the other hand, demonstrated an average gain in CAL of 2.5 ± 1.9 mm and an average reduction in PD of 3.3 ± 1.5 mm. Both these results appear inferior to what has been reported in the majority of previous clinical trials.² In a review on the effect of GTR in intrabony defects,² the meta-analysis carried out in 11 randomized controlled clinical trials rendered a weighted mean of 3.4 ± 0.9 mm for CAL gain. The inferior result in our GTR group may be explained by the high morbidity associated with the use of barrier membranes; i.e., 100% of the GTR cases had at least one complication at one or more of the postoperative visits, while only 6% of the EMD cases recorded any complication. As noted above, a multiple regression analysis indicated that the absence of complications was associated with 0.85 mm greater CAL gain. Membrane exposure was highly prevalent (i.e., 62% of membranes exposed at 3 weeks) despite utilizing a papilla preservation flap. Previous studies have confirmed that membrane exposure, especially in the early stages of wound healing, is associated with reduced outcomes from GTR.^{8,33}

The EMD clinical protocol used in this clinical trial combines both EDTA root conditioning and EMD application. However, in the GTR protocol, root conditioning is not performed. This difference may affect the results, since it is impossible with this research design to assess the differential efficacy of EDTA root conditioning. However, we used this combination in the EMD clinical protocol, since the main aim of this study was to compare, in a practice-based controlled clinical trial, the outcomes obtained following treatment of intrabony defects with papilla preservation flap surgery and two regenerative techniques and, therefore, we used the standard clinical protocol recommended in each of these regenerative surgical procedures.

The employed multivariate analysis took into account other potential sources of variability (Table 2) apart from the treatment effect, such as the center effect,

cigarette smoking, initial probing depth, and the defect depth and its intrabony component. The final model that explained 58% of the observed variability showed that differences between both treatment modalities at 12 months were not statistically significant ($P = 0.07$). However, the impact of the center effect ($P = 0.0021$) and the initial probing depth ($P = 0.021$) were statistically significant. The center effect was the most relevant factor and differences between the best and the worst center in CAL gain were 2.6 mm. This center variability has been observed in similar practice-based multicenter studies^{5,27,35} and may be of important clinical relevance. It may depend on differences in the enrolled patients in terms of social background; form of periodontal disease; response to therapy; persistence of specific pathogens; or differences in technical ability, clinical organization, and experience of the clinicians or a combination of these factors. This confirms the relevance of patient selection and clinician-associated factors in determining periodontal surgical outcomes and also the importance of practice-based multicenter studies to improve validity and external applicability of any form of therapy.

Other recognized sources of variability, such as oral hygiene, control of periodontitis, or cigarette smoking, did not have a significant impact on the outcomes. It should, however, be emphasized that these factors were highly controlled in both treatment groups.

With respect to frequency distributions of the outcomes (Table 3), 6% of GTR-treated sites, but no EMD sites, lost CAL between baseline and 12 months. Moreover, 46% of EMD treated sites gained ≥ 4 mm of attachment compared with 28% in the GTR treated group. These differences however, were not statistically significant ($P = 0.563$).

The following conclusions can be drawn from this investigation:

1. Both EMD and GTR in conjunction with papilla preservation flaps resulted in significant improvements in terms of CAL gains and PD reductions.
2. This study failed to demonstrate the superiority of one of the treatments with respect to the other.
3. The results obtained in the GTR group were heavily influenced by the high frequency of postoperative complications (mostly membrane exposure).

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