# Enamel Matrix Proteins and Guided Tissue Regeneration With Titanium-Reinforced Expanded Polytetrafluoroethylene Membranes in the Treatment of Infrabony Defects: A Comparative Controlled Clinical Trial*

G. Zucchelli, F. Bernardi, L. Montebugnoli, and M. De Sanctis

**Background:** Several studies have documented the clinical efficacy of guided tissue regeneration (GTR) with non-resorbable expanded polytetrafluoroethylene (ePTFE) membranes and enamel matrix proteins (EMP) in the treatment of infrabony defects. The objective of this controlled clinical study was to compare the clinical outcomes of 3 surgical modalities in the treatment of deep interproximal infrabony defects.

**Methods:** Ninety (90) defects in 90 healthy subjects affected by chronic periodontitis were assigned to 1 of 3 treatment groups by blocking to prognostic variables. The test group was treated with the application of EMP and the simplified papilla preservation (SPP) technique; the second group was treated with titanium-reinforced ePFTE membranes and the SPP technique; and the third group was treated with the SPP technique used as access flap control procedure. No differences were observed in terms of baseline oral hygiene and defect characteristics among the 3 groups, indicating that the blocking approach was effective. A stringent infection control program was adopted for 1 year.

**Results:** The 1-year results indicated that: 1) all treatment modalities resulted in clinically significant improvements in clinical attachment levels (CAL) and reduction in probing depth (PD); 2) a statistically significant treatment effect was demonstrated comparing the EMP test, the membrane control, and the flap control groups in terms of CAL gains; 3) both the EMP test and the membrane control groups showed significant CAL gains compared to the flap control group; 4) a statistically significantly greater amount of CAL gain was demonstrated in GTR-treated compared to EMP-treated patients; 5) deeper residual probing depths but smaller increases in gingival recession were demonstrated following EMP therapy; and 6) smoking habits reduced the clinical outcomes of both regenerative procedures.

**Conclusions:** The use of a regenerative procedure is indicated in the treatment of deep vertical bony defects since both the regenerative techniques (GTR and EMD) in the present study resulted in clinically and statistically significant improvements in clinical parameters compared to the access flap procedure. The use of EMP can be helpful in esthetically-sensitive sites and in reducing patient morbidity. *J Periodontol 2002;73:3-12.*

**KEY WORDS**

**Proteins, enamel matrix; guided tissue regeneration; polytetrafluoroethylene/therapeutic use; clinical trials, controlled; comparison studies; membranes, artificial; membranes, barrier; surgical flaps; periodontal attachment; dental papilla; furcation/therapy; follow-up studies.**

* Department of Periodontology, Faculty of Odontology, Bologna University, Bologna, Italy.

The ultimate goal in periodontal therapy is the regeneration of tooth supporting apparatus which has been destroyed due to periodontal disease.[1] Regeneration has been defined as the reproduction or reconstitution of a lost or injured part to restore the architecture and function of the lost or injured tissues.[2] Periodontal regeneration is defined as regeneration of the tooth supporting tissues, including cementum, periodontal ligament, and alveolar bone on a diseased root surface.[2] Several treatment procedures, including the use of various bone graft or bone substitute materials,[3-8] root surface conditioning,[9] guided tissue regeneration (GTR),[10-15] and growth factors,[16,17] have been suggested and utilized with varying degrees of success to achieve this goal. GTR is one of the best documented regenerative approaches. Cumulative evidence indicates that GTR with either non-resorbable or bioresorbable barrier membranes is an efficacious and predictable procedure for the treatment of vertical bony defects.[10-15,18] However, recent articles[15,19] have shown the difficulty in generalizing this technique. A recent meta-analysis[20] on the clinical outcomes following application of guided tissue regeneration to the treatment of deep infrabony defects indicated that: 1) clinically significant attachment level gains can be obtained with GTR (weighted mean 3.7 ± 1.8 mm); 2) these gains are significantly greater than those expected from access flap alone; and 3) clinically similar results are expected with both bioabsorbable and non-absorbable membranes.

A greater amount of clinical attachment gain, with respect to that achieved with a non-reinforced ePTFE membrane, has been demonstrated when the use of self-supporting (titanium-reinforced) barrier membranes was associated with a surgical technique specially designed to preserve interdental soft tissues (modified papilla preservation technique).[12] Furthermore, it has been reported that the use of reinforced barriers might displace the clinical attachment level coronal to the interproximal bone crest.[12]

Another way to address periodontal regeneration is to mimic the process that takes place during the development of the nascent root and periodontal tissues. The discovery of the presence of the enamel matrix layer between the peripheral dentin and the developing cementum, together with the capability of enamel matrix proteins to induce acellular cementum, periodontal ligament, and alveolar bone formation, has provided the fundamental concept for enamel matrix derivative-supported tissue engineering in regenerative periodontal therapy.[21] Findings from various clinical studies[22-28] indicated that topical application of commercially available enamel matrix proteins† on the diseased root surface during access flap surgery promoted clinically significant gains of clinical attachment and bone in infrabony defects. Furthermore, prospective controlled clinical trials[22,25] have demonstrated that these gains are significantly greater than those expected from access flap surgery alone.

Other studies[25,27,28] failed to demonstrate any significant difference between the clinical outcomes after GTR procedures with both resorbable[27,28] and non-resorbable[25] membranes and those achieved following enamel matrix protein (EMP) surgery when conventional soft tissue surgical approaches (Widman flap) were used.

The aim of the present prospective randomized controlled clinical trial was to compare the clinical efficacy (in terms of clinical attachment level gain, probing depth reduction, and variation in gingival recession) of a surgical procedure combining EMP and the simplified papilla preservation (SPP)[29] flap in the treatment of deep infrabony periodontal defects with that observed when the same flap design (SPP) was combined with self-supporting (titanium-reinforced) non-resorbable membranes or was performed as access flap surgery alone.

## MATERIALS AND METHODS

### *Experimental Design*

Three different approaches for the treatment of deep infrabony defects were compared in a randomized controlled clinical trial. The same surgical access to the bony defect (SPP)[29] was performed in all groups. The test group was treated with enamel matrix proteins† (EMP test), the second group with titanium reinforced-ePTFE membrane‡ (membrane control group), and the third group with the access flap only (flap control group). Clinical outcomes were longitudinally followed for 1 year. To avoid randomization imbalances, vertical bony defects were assigned to the 3 treatment groups after controlling for 2 prognostic factors: depth of the infrabony component (INFRA) and clinical attachment level (CAL).[11-13]

### *Subject Population*

Following completion of the initial preparation consisting of oral hygiene instruction and scaling and root planing, 90 subjects (41 males and 49 females, 30 to 61 years of age; mean age 48.2 ± 7.4) with chronic periodontitis were enrolled in this clinical study. Those patients with systemic disease who smoked more than 20 cigarettes per day, received antibiotics in the 6 months preceding the start of the study, or had a full mouth plaque score and full mouth bleeding score greater than 25% after cause-related therapy were excluded from the study. All patients gave informed consent to participate in this controlled clinical trial.

† Emdogain, Biora AB, Malmo, Sweden.
‡ Gore-Tex Regenerative Material, W.L. Gore & Associates, Inc., Flagstaff, AZ.

One tooth site per patient, located in the interproximal area, associated with an angular bony defect (infrabony component deeper than 3 mm) and a clinical attachment loss greater than 7 mm, was identified. Defects did not extend into a furcation.

The tooth population (90 teeth) consisted of 40 incisors, 28 cuspids, 12 bicuspids, and 10 molars. Fifty-two (52) teeth were located in the maxillary arch.

Baseline full mouth plaque score was 11.2 ± 1.8; baseline full mouth bleeding score was 10.8 ± 2.0.[30]

### *Clinical Characterization and Selected Sites*

Full mouth plaque scores (FMPS) were recorded as the percentage of total surfaces (4 aspects per tooth) which revealed the presence of plaque.[31] Bleeding on probing was assessed dichotomously at a force of 0.3 N with a manual pressure-sensitive probe. Full mouth bleeding scores (FMBS) were recorded as the percentage of total surfaces (4 aspects per tooth) which revealed the presence of bleeding upon probing.

The following clinical measurements were taken 1 week before the surgery and at the 1-year follow-up: 1) clinical attachment level (CAL), measured from the cemento-enamel junction (CEJ); 2) probing depth (PD), measured from the gingival margin; and 3) marginal gingival recession (REC), measured from the CEJ to the gingival margin.

A single investigator, blinded with respect to the treatments, performed the clinical measurements at baseline and at 1 year.

Measurements were performed at 6 sites around all teeth; the study, however, reports only local measurements at the deepest interproximal point of the selected defects. All measurements were performed by means of a manual pressure-sensitive probe and were rounded up to the nearest millimeter.

### *Clinical Measurements at Time of Surgery*

The following clinical measurements were taken at the time of the surgery immediately after debridement of the defects:[11] distance from the CEJ to the bottom of the defect (CEJ-BD); and distance from the CEJ to the most coronal extension of the bone crest (CEJ-BC). The infraosseous component of the defects (INFRA) was defined as INFRA = (CEJ-BD) – (CEJ-BC).

### *Randomization*

Before surgery, assignment to the 3 treatment regimens (30 patients/group) was performed using a custom-made program based on balanced permuted blocks.[15] Blocking to control for the effects of the prognostic variables INFRA and CAL was used to decrease outcome variability.[11-13,32,33] These 2 variables were categorized to make blocks as follows: CAL ≤ or >10 mm and INFRA ≤ or >6 mm. For randomization purposes, INFRA was estimated before surgery on radiographs and by performing transgingival bone sounding. Furthermore, to reduce the chance of unfavorable splits between test and control groups in terms of key prognostic factors, the randomization process balanced smoking status and location of the defect at the upper first premolars in the test and control groups.[15]

### *Surgical Procedures*

Access to the bony defects of all patient groups was achieved with the SPP technique described by Cortellini et al.[29] In brief, an oblique submarginal horizontal incision was made at the level of the interdental papilla covering the infrabony defect. The horizontal incision was continued intrasulcularly in the buccal aspect of teeth neighboring the defect.

At the level of the interproximal space with the bony defect the buccal flap was raised up with a split-full-split approach in the coronal-apical direction: the interdental tissue (surgical papilla) was dissected split-thickness up to the level of the buccal bone crest to preserve the supra-crestal connective tissue over the defect. Then, full-thickness flap elevation continued to expose at least 3 to 5 mm of buccal bone. The most apical portion of the flap was elevated split thickness to facilitate the coronal displacement of the flap itself.

The remaining soft tissues of the defect-associated papilla were dissected from the root surfaces of the 2 neighboring teeth. A bucco-lingual incision was then performed at the base of the papilla as close as possible to the bone crest. Intrasulcular incisions were performed in the lingual/palatal aspect of the 2 teeth neighboring the defect and extended to the interdental papillae of the adjacent interdental spaces. The entire interproximal papillary tissues covering the bony defect were moved palatally/lingually and then elevated, full thickness, with the palatal/lingual flap. Flap (buccal and palatal/lingual) elevation was considered adequate when the entire vertical bone defect was accessible for instrumentation.

**EMP group.** Following careful scaling, root planing, and debridement of the bony defect, the exposed root surface was conditioned with a 24% EDTA gel for 2 minutes to remove the smear layer. The root was subsequently rinsed with saline. A solution composed of a powder of EMD† (30 mg) mixed with 1 ml of propylene glycol alginate (PGA) gel was applied twice: first immediately after root conditioning on the exposed root surface and left in place for 3 minutes during which bleeding was controlled with the use of gauze; and second immediately before tying the last suture which joined the interproximal papillary tissues to the buccal flap.

**GTR group.** Following scaling, root planing, and debridement of the defect, the exposed root surface was conditioned with a 24% EDTA gel for 2 minutes. After rinsing with saline, an interproximal titanium reinforced non-resorbable ePTFE membrane was cut and

reshaped to permit its precise adaptation to the interdental zone and the bony defect. Afterwards, the membrane was positioned at the level of, or coronal to, the bone crest to completely cover the defects, overlapping at least 3 mm of the residual bone. Membrane positioning, at the level of the bone crest or coronal to it, was chosen in relation to the width of the interdental space and the amount of supracrestal interdental soft tissue; in the presence of a wide interdental space (>2 mm) and/or a thick/wide suprabony component, membranes were positioned coronal to the bone crest (close to the CEJ); conversely when no diastema was present or when the supracrestal connective tissue was thin/narrow, membranes were sutured at the level of the bone crest. This facilitated primary soft tissue coverage above the membrane material.

**Control group.** The same mechanical and chemical (24% EDTA gel for 2 minutes) treatment to the root surface was performed in this group, but neither EMD nor membranes were applied to the defects.

A blunt dissection into the vestibular lining mucosa was then carried out to eliminate muscle tension and to permit coronal displacement of the buccal flap.

In the defect-associated interdental space, the interdental papilla was moved again buccally and a horizontal internal mattress suture[34] was used to improve flap adaptation above the bony defect and to bring the interdental papilla as close as possible to the buccal flap. A single interrupted suture was then used to achieve complete primary closure of the interdental tissues over the defect. Single interrupted sutures were used in each interproximal area neighboring the defect area.

### *Infection Control*

Patients were given antibiotics[§] (amoxicillin plus clavulanic acid 1g/day) starting the day before surgery and for 6 days thereafter.

All patients were instructed to rinse the mouth with a 0.12% solution of chlorhexidine twice a day for 11 weeks. During this period, they were recalled once a week for professional tooth cleaning.

### *Membrane Removal*

Six weeks after surgery, patients treated with ePTFE membranes underwent a second surgery in order to remove the barrier material. The same surgical approach (SPP)[29] was used to gain access to the membrane and to achieve complete soft tissue closure above the regenerated tissue.

### *Plaque Control*

When chlorhexidine was discontinued, full mechanical interproximal cleaning in the surgically-treated area was reinstituted. Patients were recalled for professional tooth cleaning and reinforcement of self-performed oral hygiene measures at 1-month intervals up to the 1-year reevaluation. No attempt at probing or deep scaling was made before the 1-year follow-up.

### *Data Analysis*

Statistical application software[‖] was used for statistical analysis. Data were expressed as mean value ± standard deviation. The following outcome and predictor variables were defined as: 1) CAL gain = baseline CAL – 1 year CAL; 2) PD reduction = baseline PD – 1 year PD; and 3) REC increase = 1 year REC – baseline REC.

The normality assumption was verified and the presence of any randomization imbalance between the 3 experimental groups was tested by one-way analysis of variance and chi-squared analysis.

General linear models were fit relating CAL gain, REC increase, and PD reduction to 3 categorical (technique, smoking status, and tooth type [anterior versus posterior]) and 6 continuous (FMPS, FMBS, LBS, PD, REC, INFRA) factors as covariates (analysis of covariance). In the case of significance, Bonferroni *t* test was applied as a multiple comparison test.

One-way ANOVA was used to evaluate differences in the clinical parameters at 1 year in the 3 experimental groups. Bonferroni *t* test was applied as a multiple comparison test. Unpaired Student *t* test was used in the GTR group to evaluate differences in the clinical outcomes between sites with and without membrane exposure, and between sites with different membrane positioning at the time of surgery.

## RESULTS

### *Experimental Population*

Mean ages in the EMP test, membrane control, and flap control groups were 50.2 ± 5.3, 47.2 ± 7.1, and 48.8 ± 6.8, respectively. In the EMP test group, 18 patients were female, in the membrane control group 15 were female, and in the flap control group 16 were female.

The tooth population consisted of: 13 incisors, 11 cuspids, 3 bicuspids, and 3 molars in the EMP test group; 14 incisors, 9 cuspids, 4 bicuspids, and 3 molars in the GTR group; and 13 incisors, 10 cuspids, 3 bicuspids, and 4 molars in the flap control group.

Ten patients (10) were "smokers" in the EMP group and 12 in the GTR and flap control groups (chi-square, 0.43, NS). None of the selected patients dropped out before the termination of the study.

### *Baseline Oral Hygiene and Defect Characteristics*

Baseline oral hygiene and defect characteristics are shown in Table 1. No statistically significant difference was observed among the 3 clinical parameters, indicating that the randomization process was effective. Baseline FMPS were 11.1 ± 1.4 in the EMP test group, 10.4 ± 1.8 in the GTR group, and 11.4 ± 2.4 in the flap

§ Augmentin, Smith Klein Beecham, S.p.a., Milan, Italy.
‖ SAS version 6.12, SAS Institute, Cary, NC.

control group. Similarly, FMBS were 10.4 ± 1.1, 9.8 ± 1.2, and 10.2 ± 2.2 for the 3 groups, respectively. Baseline CAL was 9.9 ± 1.4 in the EMP test group, 10.3 ± 1.9 in the GTR group, and 10.0 ± 1.2 in the flap control group. The depths of the infrabony components (INFRA) of the defects were 6.1 ± 1.3, 6.6 ± 1.2, and 6.2 ± 1.0 in the 3 groups, respectively.

### *Early Healing Event*

All sites healed uneventfully. Membrane exposure occurred in 10 out of 30 cases (33%); in all cases the amount of barrier exposure did not exceed 2 $mm^2$ and was limited to a small portion of the interproximal area. Plaque accumulation over the exposed ePTFE membrane was controlled with a topical chlorhexidine application. In all sites with membrane exposure, gingival inflammation was minimal; thus, membrane removal was postponed to 6 weeks. No wound edge necrosis or flap dehiscence were observed in the flap control and EMP test groups. Lower patient morbidity was obviously observed in the EMP-treated group (compared to the GTR group) since it was not necessary to perform a second surgery for membrane removal.

### *Clinical Parameters at 1 Year*

The results from ANOVA of the 3 experimental groups are summarized in Table 2. No significant difference between the 3 groups was found in the FMPS and FMBS mean values at 1 year. FMPS was 9.9 ± 0.9 in the test group, 10.0 ± 1.4 in the GTR group, and 10.2 ± 1.4 in the flap control groups. Similarly, FMBS was 9.4 ± 1.1 in the test group, 9.6 ± 1.8 in the GTR group, and 9.8 ± 1.8 in the flap control group. A comparison between the individual mean plaque and bleeding scores calculated from the baseline and the 12-month follow-up revealed that, during the course of the trial, no marked change had occurred in the oral hygiene status in any of the 3 experimental groups (data not shown).

Statistically significant differences in terms of CAL, residual PD, and gingival recession were observed among the 3 groups. Both the EMP test and the GTR groups showed significantly smaller CAL at 1 year than the flap control group. Differences in CAL between the EMP group and the GTR control group did not reach a statistically significant value. In all groups, the 1-year CAL remained located within the baseline infrabony component of the defects.

Both the EMP test and the flap control groups showed significantly deeper PD at 1 year than the membrane control group. Differences in PD between the EMP group and the flap control group were not statistically significant.

A statistically significant greater amount of gingival recession at 1 year was found in the membrane control and in flap control groups compared to the EMP test group. No statistically significant differences were found in the amount of gingival recession between the 2 control groups.

### *Clinical Changes at 1 Year*

The significance of factors affecting 1-year CAL gain, PD reduction, and REC increase was evaluated by adopting a general linear model.

**CAL gain.** Sum of squares, degrees of freedom, mean square, F value and *P* level for each variable entering the model were reported in Table 3.

The R-squared statistic indicates that the model as fitted is highly significant and explains 73.9% of the variability in CAL gain. The most significant variable entering the model and affecting the CAL gain at 1

**Table 1.**

## Baseline Clinical Parameters and Defect Characteristics

| | EMP (n = 30) | GTR (n = 30) | Control (n = 30) | F | *P* |
|---|---|---|---|---|---|
| FMPS (%) | 11.1 (1.4)* | 10.4 (1.8) | 11.4 (2.4) | 0.67 | NS |
| FMBS (%) | 10.4 (1.1) | 9.8 (1.2) | 10.2 (2.2) | 0.73 | NS |
| CAL (mm) | 9.9 (1.4) | 10.3 (1.9) | 10.0 (1.2) | 0.55 | NS |
| PD (mm) | 9.2 (1.0) | 8.9 (1.8) | 8.9 (0.9) | 0.44 | NS |
| REC (mm) | 0.8 (0.8) | 1.4 (1.0) | 1.1 (0.9) | 0.18 | NS |
| CEJ-BD (mm) | 11.6 (0.8) | 11.7 (1.9) | 11.5 (1.1) | 0.22 | NS |
| CEJ-BC (mm) | 5.5 (1.2) | 5.1 (1.2) | 5.2 (1.1) | 0.70 | NS |
| INFRA (mm) | 6.1 (1.3) | 6.6 (1.2) | 6.2 (1.0) | 1.39 | NS |

*Mean (SD).

**Table 2.**

## Clinical Parameters at 1 Year

| | EMP (n = 30) | GTR (n = 30) | Control (n = 30) | F |
|---|---|---|---|---|
| FMPS (%) | 9.9 (0.9) | 10.0 (1.4) | 10.2 (1.4) | 0.43 NS |
| FMBS (%) | 9.4 (1.1) | 9.6 (1.8) | 9.8 (1.8) | 1.05 NS |
| CAL (mm) | 5.8 (1.1) a | 5.5 (1.3) a | 7.4 (1.1) b | 25.2 |
| PD (mm) | 4.0 (0.7) a | 2.4 (0.7) b | 4.4 (0.8) a | 54.3 |
| REC (mm) | 1.7 (0.9) b | 3.0 (1.2) a | 3.1 (0.9) a | 17.4 |

a,b = Different letters indicate a statistically significant difference between groups for each parameter.
*Means (SD).
NS = not significant.

year was the type of periodontal procedure (F = 55.38). In particular a CAL gain of 4.2 ± 0.9 (range 3 to 6 mm) was obtained in the EMP-treated group, a CAL gain of 4.9 ± 1.6 (range 2 to 9 mm) in the membrane control group, and a CAL gain of 2.6 ± 0.8 (range 1 to 4 mm) in the flap control group. Bonferroni *t* test analysis showed significant differences among all 3 procedures.

Among the defect characteristics, the initial probing depth was a highly significant covariate (F = 36.35). Among patient characteristics, smoking status was a highly significant covariate (F = 4.49). Smoking status, in particular, was a negative predictor for both regenerative procedures while it did not reach statistical significance in the flap control group.

In the GTR group, Student *t* test analysis comparing the clinical outcomes obtained in sites with (N = 10) and without (N = 20) membrane exposure indicated a statistically significant smaller amount of attachment gain (3.6 ± 1.0 mm versus 5.5 ± 1.5 mm, df = 26, $t$ = 4.23, $P$ = 0.0002) and greater increase in gingival recession (2.5 ± 0.8 mm versus 1.2 ± 0.6 mm, df = 13, $t$ = 4.51, $P$ = 0.0005) in the sites with membrane exposure.

Similarly sites with membranes placed coronal to the bone crest (17 sites) at the time of surgery showed a greater amount of CAL gain (5.6 ± 1.5 versus 3.8 ± 1.0; df = 27, $t$ = 3.89, $P$ = 0.0005) compared to sites in which the membrane was positioned at the bone crest (13 sites).

**PD reduction.** The periodontal procedure was the most significant variable affecting 1-year PD reduction (F = 58.55). The mean reduction of PD was 5.1 ± 0.7 in the EMP test group (range 4 to 7 mm), 6.5 ± 1.6 in the GTR group (range 4 to 10 mm), and 4.5 ± 1.0 in the flap control group (range 3 to 7 mm). Bonferroni *t* test indicated that both the EMP test and the GTR group showed significantly greater PD reduction than the flap control group. Differences in PD reduction between the EMP group and the GTR group were also statistically significant: a greater reduction in probing depth was demonstrated in the membrane-treated sites compared to the EMD-treated sites.

Further significant variables affecting 1-year PD reduction were baseline PD (F = 47.18) and local bleeding score (F = 4.62).

**REC increase.** Once again, the periodontal procedure was the most significant variable affecting 1-year CAL gain (F = 14.16). The mean increase in gingival recession was 1.0 ± 0.5 in the EMP test group (range 0 to 2 mm), 1.6 ± 1.0 in the GTR group (range 0 to 4 mm), and 1.9 ± 0.8 in the flap control group (range 1 to 4 mm). A statistically significant greater increase of gingival recession was found in the GTR group and in the flap control group compared to the EMP test group. No statistically significant difference was found in the increase in gingival recession between the 2 control groups. Further significant variables affecting 1-year REC increases were baseline PD (F = 5.42) and smoking status (F = 6.26).

**Table 3.**

## CAL Gain at 1 Year

General Linear Models

Number of dependent variables: 1
Number of categorical factors: 3
Number of quantitative factors: 6

Analysis of Variance for CAL gain

| Source | Sum of Squares | Df | Mean Square | F Ratio | P |
|---|---|---|---|---|---|
| Model | 146.106 | 10 | 14.6106 | 22.39 | 0.0000 |
| Residual | 51.5494 | 79 | 0.652524 | | |
| Total (corrected) | 197.656 | 89 | | | |

Type III Sums of Squares

| Source | Sum of Squares | Df | Mean Square | F Ratio | P |
|---|---|---|---|---|---|
| Technique* | 72.2705 | 2 | 36.1352 | 55.38 | 0.0000 |
| Smoking† | 2.93135 | 1 | 2.93135 | 4.49 | 0.0372 |
| Tooth‡ | 1.60953 | 1 | 1.60953 | 2.47 | 0.1203 |
| FMPS (%) | 0.258098 | 1 | 0.258098 | 0.40 | 0.5312 |
| FMBS (%) | 0.961647 | 1 | 0.961647 | 1.47 | 0.2284 |
| LBS (%) | 0.0912469 | 1 | 0.0912469 | 0.14 | 0.7094 |
| PD (mm) | 23.719 | 1 | 23.719 | 36.35 | 0.0000 |
| REC (mm) | 1.39957 | 1 | 1.39957 | 2.14 | 0.1470 |
| INFRA (mm) | 1.31094 | 1 | 1.31094 | 2.01 | 0.1603 |
| Residual | 51.5494 | 79 | 0.652524 | | |
| Total (corrected) | 197.656 | 89 | | | |

*Technique = EMP versus GTR versus flap.
†Smoking = yes versus no.
‡Tooth = anterior versus posterior.
LBS = local bleeding score.

### *Frequency Distribution*

Table 4 displays the frequency distribution of CAL, PD, and REC for the 3 treatment groups.

**CAL.** Sites treated with EMP and non-resorbable membranes gained 4 mm or more in 76.6% and 83.3% of the cases, respectively. In the membrane control group, 33.3% of sites gained 6 mm of CAL or more. This compared favorably with 6.6% of sites from the group treated with EMP.

**PD.** Of sites treated with GTR, 87% showed a PD ≤3 mm at 1 year and none had residual pockets deeper than 5 mm. Conversely, 20% of sites treated with EMP had shallow pockets (≤3 mm) at 1 year and 7% showed residual pockets deeper than 5 mm.

**REC.** The great majority (87%) of sites treated with EMP experienced a very small increase in gingival recession (≤1 mm), while about half (47%) of the GTR-treated sites showed a 2 mm or more increase in gingival recession.

## DISCUSSION

The findings from the present controlled clinical study demonstrated that regenerative therapy, including either the use of enamel matrix proteins or the application of self-supporting barrier membranes in deep infrabony defects, enhanced outcome variables such as probing depth reduction and clinical attachment level gain. In fact, a statistically and clinically significant greater amount of CAL gain and PD reduction was demonstrated in EMP- and membrane-treated patients compared to access flap-treated patients.

The significance of the treatment effect on CAL gain was evaluated with a multivariate analysis (Table 3) taking into account the potential sources of variability such as the treatment modalities, the patient, and defect characteristics. The final model explained 74% of the observed variability. The most significant variable entering the model was the type of periodontal procedure. Among the measured variables, smoking habits and baseline probing depths were significantly associated with the expected amounts of CAL gains. The analysis revealed that smoking habits reduced the amount of clinical attachment gain of both regenerative procedures. These data confirm the detrimental effect of smoking on the clinical outcomes following regenerative therapy.[14,30]

Other factors found relevant in previous investigations,[11,30,35] such as FMPS, FMBS, and depth of the infrabony component of the defect, were not significant. The lack of significance of FMPS and FMBS on the clinical outcomes in the present study can be attributed to the infection control protocol and the strict plaque control regimen adopted. Baseline and 1-year FMBS and FMPS were about 10%,[30] thereby reducing the range of the values of these potential covariates. The lack of significance of the baseline infrabony component of the defect[11,35] is probably due to the impact of probing depth in the statistical model; PD and depth of the infrabony component of the defect are highly correlated.

The present data indicated that GTR therapy with titanium-reinforced barriers and EMP therapy bring different changes in clinical attachment level, probing depth, and gingival recession. A statistically significant greater amount of CAL gain (4.9 mm versus 4.2 mm) was demonstrated in GTR-treated compared to EMP-treated patients. However, the 2 regenerative procedures were almost equally satisfactory in terms of efficacy and reproducibility, since a similar and very high percentage (76.6%, EMP and 83.3%, GTR) of treated defects gained 4 mm of CAL or more. Thus, clinically highly significant improvements in CAL levels were accomplished even with a more simple, less risky (no risk of membrane exposure), and less invasive (1 versus 2 surgeries) EMP surgical technique.

The amount of clinical attachment gained with the EMP procedure in the present study compares favorably with that reported in previous clinical trials.[22-28] The difference can be explained, at least in part, in that a specifically designed flap to preserve interdental soft tissue was performed. This is also confirmed by the fact that control sites in the present study, which were treated with the same flap design (SPP), gained more clinical attachment than control sites of other controlled clinical studies[22,25] on EMP in which a conventional modified Widman flap procedure was used as an access flap to the bony defects. Also, defect selection (the depth of the infrabony component of the present defects was greater than that of previous clinical studies) and the relative number of smoking patients might have contributed to better clinical outcomes in the present study.

The CAL gains obtained in the GTR-treated patients in the present study were similar to those reported in a previous study,[12,35] in which a similar surgical technique (modified papilla preservation flap) and the same membrane material were utilized; however, the present 1-year CAL remained located within the original infrabony component of the defect. The differ-

**Table 4.**

### Frequency of Clinical Characteristics at 1 Year

| CAL Gain (mm) | EMP (%) | GTR (%) | Control (%) |
|---|---|---|---|
| <2 | 0 | 0 | 3.3 |
| ≤2 to >4 | 23.3 | 16.7 | 76.7 |
| ≤4 to >6 | 70.0 | 50.0 | 0 |
| ≥6 | 6.7 | 33.3 | 0 |
| PD | | | |
| ≤3 | 20.0 | 87.0 | 10.0 |
| 3<PD≤5 | 73.3 | 13.0 | 70.0 |
| ≥6 | 6.6 | 0 | 20.0 |
| REC Increase (mm) | EMP (%) | GTR (%) | Control (%) |
| 0 | 16.7 | 3.3 | 0 |
| 1 | 70.0 | 50.0 | 30.0 |
| 2 | 13.3 | 33.3 | 50.0 |
| >2 | 0 | 13.3 | 20.0 |

ence can be explained, at least in part, by variation in the surgical technique. In the present study 13 (out of 30) membranes were positioned at the level of the bone crest because of the presence of narrow interproximal spaces and/or the absence of a thick suprabony component associated with the infrabony defect which did not allow for membrane positioning coronal to the bone crest. Conversely, in the study by Cortellini et al.,[12] membranes were positioned close to the CEJ and the more coronal shift of the CAL can be explained by the greater amount of space available under the membrane.[12,35] Separate analysis comparing sites with membranes placed at or coronal to the bone crest indicated a greater amount of CAL gain (5.6 ± 1.5 versus 3.8 ± 1.0) in sites in which the membrane was positioned close to or at the CEJ. Furthermore, only in these latter sites was the 1-year CAL displaced within the suprabony component of the original defects.

In the present study, EDTA was used to condition the root surface before membrane placement in GTR patients to minimize differences in the surgical approaches between groups. Since prior GTR studies did not use EDTA, it cannot be excluded that the use of root conditioning might contribute to the different clinical outcomes found in the present study compared to that of Cortellini et al.[12,35] Furthermore, the relative number of smoking patients in the present study compared to other GTR studies might have contributed to explaining the differences in clinical outcomes.

A primary goal of periodontal therapy is to reduce probing depth in order to limit the risk of local reinfection. Shallow pockets have a strong negative predictive value for future disease progression, while deep pockets in treated patients are a risk indicator for periodontal disease progression.[36] In our study, a highly significant difference was demonstrated in the 1-year PD and in the reduction of PD between the 2 groups treated with regenerative procedures. GTR-treated sites resulted in 2.4 mm PD at 1 year that compares favorably with the 4.0 mm observed in the EMP group. Furthermore, the reduction of PD was clinically and statistically greater in GTR-treated compared to EMP-treated (6.5 mm versus 5.1 mm) sites. These differences are even more clinically meaningful if one considers that 87% of sites treated with GTR showed a PD ≤3 mm at 1 year and none had residual pockets deeper than 5 mm. Conversely, only 20% of sites treated with EMP had shallow pockets (≤3 mm) at 1 year and 7% showed residual pockets deeper than 5 mm (Table 4).

Another common outcome following periodontal surgery is the recession of the gingival margin. This may represent a patient concern when an esthetically-sensitive site is treated. In the present study, GTR therapy was associated with a greater increase in gingival recession compared to EMP therapy (1.6 mm versus 1.0 mm). From a clinical standpoint, it is even more significant to observe that the great majority (87%) of sites treated with EMP experienced a very small increase in gingival recession (≤1 mm), while about half (47%) of the GTR-treated sites showed a 2 mm or more increase in gingival recession (Table 4).

Different clinical outcomes that can be expected following EMP and GTR therapy allow us to make some speculations: GTR therapy is best indicated when the main goal to be achieved by the regenerative procedure, together with the increase in functional tooth support, is a shallow residual pocket; conversely, EMP is the treatment of choice when minimal gingival recession is desired. This is the case of esthetically-sensitive sites where even a small increase in gingival recession can represent an esthetic problem for the patient. In these clinical situations even a less favorable result in terms of residual probing depth but with minimal change in the position of the gingival margin could be the treatment objective if combined with a clinically significant increase in the attachment level and if achieved with a simpler, less invasive (1 versus 2 surgeries), and less risky surgical approach. In fact, the risk of membrane exposure must be considered when a GTR approach to the periodontal defect is selected. Separate analysis comparing the clinical outcomes obtained in the present study between sites with and without membrane exposure indicated a statistically and clinically significant smaller attachment gain (3.6 ± 1.0 mm versus 5.5 ± 1.5 mm) and greater increase in gingival recession (2.5 ± 0.8 mm versus 1.2 ± 0.6 mm) in the sites with membrane exposure.

Membrane exposure and the consequent bacterial colonization have been indicated as major complications of guided tissue regeneration therapy.[37-42] The reported prevalence of membrane exposure was in the 70 to 80% range.[20] Membrane exposure has been reported to be highly reduced (range 40 to 50%) with the use of access flap specifically designed to preserve interdental tissues. In the present study, a simplified papilla preservation flap has been used in association with the use of a non-resorbable titanium-reinforced membrane. Even so, membrane exposure occurred in 33% of the cases. A similar percentage was reported by Cortellini and co-workers[29] in a study in which 18 patients were treated with the same surgical technique adopted in the present study. The presence of a wide interdental space and/or the presence of a consistent suprabony component associated with the infrabony component has been reported to reduce the risk of membrane exposure, limit gingival recession, and thereby prevent esthetic damage.[20] The reason for this is that, in the presence of these anatomical situations,

membranes can be positioned in a coronal position with respect to the bone crest and can be completely and predictably covered by the coronally advanced soft tissues. In the present study 13 out of 30 defects had no diastema or thick suprabony component. The absence of a consistent suprabony component was due to the presence of an intact height of the interproximal bone crest at the adjacent tooth and/or to the presence of preoperative interdental soft tissue recessions. In such clinical cases membranes were positioned at the level of the bone crest in order to reduce the risk of membrane exposure. Despite this, membrane exposure occurred in one-third of the treated cases and was responsible for the increased gingival recession after GTR therapy.

Therefore, if an esthetically sensitive site must be treated with a regenerative procedure and if the defect has an adequate suprabony component or it is located between diastematic teeth, a titanium-reinforced ePTFE membrane is the material of choice because it can be positioned close to the CEJ and thus permit a greater amount of CAL gain and a more coronal displacement of the clinical attachment level. Conversely, in cases where adequate membrane fixation and soft tissue coverage can hardly be performed, EMP might be preferred to GTR to reduce the risk of postoperative gingival recession.

The use of a bioabsorbable barrier membrane has been advocated in order to spare the patient a second surgery to remove non-resorbable membranes. The same advantage can be ascribed for the use of EMP. The clinical outcomes obtained in the EMP-treated patients of the present study are similar to those achieved in other controlled clinical trials[13] or case series[43] in which similar flap design was associated with the use of bioabsorbable barrier membranes. Furthermore, no clinically and statistically significant differences were demonstrated in randomized split-mouth clinical studies[27,28] comparing EMP and resorbable membranes in the treatment of vertical bony defects. Since it is easier to apply a gel than position a membrane around a defect, and since both bioabsorbable and non-resorbable membranes can become exposed resulting in gingival recession and unesthetic outcomes, it seems logical to consider EMP rather than a bioabsorbable membrane when use of a non-resorbable membrane is not recommended.

Several conclusions can be drawn from this controlled clinical trial:

The use of a regenerative procedure was indicated in the treatment of deep vertical bony defects since both the regenerative techniques (GTR and EMP) in the present study resulted in clinically and statistically significant improvements in clinical attachment levels compared to access flap procedures.

A statistically significant greater amount of CAL gain was demonstrated in GTR-treated compared to EMP-treated patients.

The 2 regenerative procedures were almost equally satisfactory in terms of efficacy and reproducibility; however, EMP therapy was technically more simple, less risky (no membrane exposure and infection) and less invasive (only 1 surgery) than GTR therapy.

Better results in terms of PD reduction and depth of the residual pocket could be expected following GTR therapy with titanium-reinforced ePTFE membranes.

The increase of gingival recession after surgery was more limited with the use of EMP instead of titanium-reinforced ePTFE membranes.

The use of EMP was helpful in favoring resolution of deep infrabony defects, especially in esthetically sensitive sites, and in reducing patient morbidity.

Smoking habits reduced the clinical outcomes of both regenerative procedures.

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Send reprint requests to: Dr. Giovanni Zucchelli, Department of Periodontology, Faculty of Odontology, Bologna University, Via S. Vitale 59, 40125 Bologna, Italy. Fax: 3951 225208; e-mail: giovanzu@tin.it.

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