Effectiveness of Enamel Matrix Derivative in Conjunction with Particulate Autologous Bone in the Treatment of Noncontained Intrabony Defects: A 2-Year Prospective Case Series

Francesco Ferrarotti, DDS1/Federica Romano, DDS2
Andrea Quirico, DDS1/Matteo Di Bella, DDS3
Sara Pallotti, DDS1/Mario Aimetti, MD, DDS4

This case series evaluated the healing of deep intrabony defects treated with a combination of enamel matrix derivative and autologous particulate bone harvested from the buccal and lingual/palatal cortical plate with a Piezosurgery device. A total of 15 defects with a predominantly one- or two-wall component were consecutively treated in 15 patients with advanced chronic periodontitis. In all selected sites, the three-wall component was ≤ 25% of the total defect depth. Clinical and radiographic parameters were recorded at baseline and 12 and 24 months postoperatively. All defects showed favorable clinical and radiographic outcomes at the 24-month follow-up. The probing depth reduction was 4.4 ± 1.6 mm, and more than 50% of the defects presented clinical attachment level gain of at least 5 mm. The bone fill was 3.1 ± 1.6 mm. Int J Periodontics Restorative Dent 2018;38:673–680. doi: 10.11607/prd.3003

Extensive evidence from in vitro experiments and histologic studies performed in animals and humans have shown that the application of enamel matrix derivative (EMD) during periodontal surgery will trigger a cascade of biologic events that positively influence wound healing, eventually leading to periodontal regeneration.1,2 EMD has been used successfully in the treatment of intrabony defects and Class II furcation and recession-type defects.2,3 In intrabony defects, several controlled clinical trials have reported statistically significant higher clinical attachment level (CAL) improvement and probing depth (PD) reduction after application of EMD when compared with open-flap debridement alone.4,5

More conflicting data is available when comparing EMD to guided tissue regeneration using absorbable or nonresorbable membranes.6–8 A recent meta-analysis emphasized the high degree of heterogeneity observed in these trials, partly attributable to differences in the defect morphology.9

Because of its gel-like consistency, application of EMD, especially in noncontained defects, may not prevent the collapse of the flap, thus minimizing the space for regeneration.10 Adequate preservation of supracrestal interdental soft tissues and the combination of EMD with
a grafting material have been suggested as a potential alternative to limit flap collapse and maximize the clinical effect of EMD.\textsuperscript{11,12}

Autogenous bone (AB) is considered the ideal grafting material because it is potentially osteoinductive, bioabsorbable, and easy to handle.\textsuperscript{13,14} Histologic observations from animal models demonstrated the periodontal regenerative potential of bone autograft alone or in combination with EMD in terms of new bone and cementum formation.\textsuperscript{15}

Various systems for collecting intraoral autogenous particulate bone have been studied to guarantee the best quality of the collected material.\textsuperscript{16} The morphology and dimensions of bone chips have been studied in relation to graft success. Dimensions smaller than 125 $\mu$m lead to rapid resorption of fragments and do not guarantee sufficient osteoconductivity for the new bone formation process, whereas fragments larger than 1,000 $\mu$m require longer healing times.\textsuperscript{17} The size of bone chips harvested using a Piezosurgery device seems to be appropriate to achieve regeneration.\textsuperscript{18,19}

No data is available on the combination of EMD and autologous bone graft harvested using a piezoelectric technique in regenerative treatment. Therefore, the aim of this prospective case series was to evaluate the clinical effectiveness of the application of EMD associated with autologous bone collected by means of a piezoelectric device for the treatment of intrabony defects with a predominantly one- or two-wall component.

### Materials and Methods

#### Study Design

Patients with one deep intrabony defect requiring regenerative therapy were consecutively selected among those seeking care for periodontal disease at the Section of Periodontology, CIR Dental School, Department of Surgical Sciences, from May 2011 to April 2012. The study was conducted in accordance with the Helsinki Declaration of 1975 as revised in 2000. All patients gave written informed consent. Criteria for patient selection were diagnosis of severe chronic periodontitis,\textsuperscript{20} completion of etiologic periodontal treatment at least 3 months prior to surgery, a full-mouth plaque score (FMPS) and a full-mouth bleeding score (FMBS) $\leq$ 20% at the time of surgery, and presence of at least one intrabony defect without furcation involvement with PD $\geq$ 6 mm and a radiographic intrabony component of $\geq$ 3 mm. Only sites with a predominantly one- or two-wall component were considered for this study. To be included in this category, intrabony defects should have a three-wall component $\leq$ 25% of the total defect depth. The architecture of the defect was confirmed during the surgical treatment. Patients with systemic diseases contraindicating periodontal surgery or interfering with periodontal healing, smokers, and pregnant or lactating women were excluded.

The following clinical measurements were recorded at the experimental sites at baseline (1 week prior to surgery) and at 12 and 24 months after surgery by the same blinded and calibrated examiner (A.Q.) with a 1-mm marked periodontal probe (PCP-UNC 15, Hu-Friedy): presence/absence of bacterial plaque (Plaque Index [PI]), presence/absence of bleeding on probing (BoP), PD, gingival recession (REC), and CAL. Prior to surgery and 12 and 24 months after surgery, periapical radiographs of the selected sites were taken using the long-cone paralleling technique and an individual film-holder device (RINN XCP Film Holding Instruments, Dentsply). The radiographs were digitized using an image-processing device and analyzed using ImageJ software. The radiographic reference points were the cementoenamel junction (CEJ), the bone crest (BC) level, and the bottom of the bony defect (BD).\textsuperscript{21} The intrabony component (INFRA) of the defect was measured as the distance between BC and BD. The baseline defect angle (RA) was also recorded.

At the end of the experimental procedure, patients were given a 100-mm horizontal visual analog scale (VAS) to record the discomfort/pain experienced during surgery and the first postoperative week.
Surgical Procedure

Teeth with mobility > 1 were splinted before the regenerative treatment. The surgical procedures (Fig 1) were performed by the same experienced clinician (F.F.) with the aid of an operating microscope (S7, Zeiss) at a magnification of ×4 to ×16 according to the papilla preservation surgical technique (Figs 1b and 1c). If required, vertical releasing incisions were made. After defect debridement, the following measurements were taken: the number of residual bony walls, the probing bone level (PBL) as the distance from the CEJ to the apical end of the defect (Fig 1d). The autogenous particulate bone graft was harvested from the buccal and lingual/palatal cortical plate using a piezoelectric device (Piezosurgery, Mectron), and root surfaces were conditioned with EDTA gel (PrefGel, Straumann). After rinsing with sterile saline, the bone graft and EMD (Emdogain, Straumann) were placed to completely fill the defect (Fig 1e). A sandwich technique was adopted as described by Trombelli et al. Finally, flaps were repositioned and secured with vertical or horizontal mattress sutures (W.L. Gore & Associates) (Fig 1f). Care was taken to obtain complete closure of the interdental soft tissue above the treated defect.

Postsurgical Care

Postoperative pain and edema were controlled with analgesic medication for 3 days (600 mg ibuprofen twice daily), and antibiotics were administered for 6 days (1 g amoxicillin and clavulanic acid twice daily). Local plaque control was maintained with 0.12% chlorhexidine mouthrinse three times a day for 4 weeks. Sutures were removed 14 days after surgery. After 4 weeks, subjects discontinued chlorhexidine mouthrinse and resumed conventional hygiene practices with a medium toothbrush and interdental devices. Recall appointments were scheduled monthly during the first year and every 3 months thereafter.

Fig 1 (a) Baseline probing depth (11 mm) of the defect area at the distal aspect of a maxillary canine. (b) Incision. (c) Reflection of the flap with papilla preservation. (d) Intraoperative view of the intrabony defect. (e) Defect filled with enamel matrix derivative and autologous particulate bone graft. (f) Primary closure of the mucoperiosteal flap.
Statistical Analysis

A statistical software program was used for data analysis (SAS version 9.2, SAS Institute). The primary outcome variable was CAL, and secondary outcome variables were the additional clinical parameters and patient perception. Only one measurement per tooth, at the deepest site of the selected defect at baseline, was included in the calculations. Quantitative data were expressed as mean ± SD. To test whether the data were distributed normally, Kolmogorov-Smirnov and Shapiro-Wilk tests were done. Repeated-measures analysis of variance (CAL, REC, INFRA) and the Friedman test (FMPS, FMBS, PD) were used to detect differences over time in clinical and radiographic parameters. When a statistical difference was found, analysis was performed with Newman-Keuls test and Dunn test. A univariate analysis of PD changes from the 12- to the 24-month follow-up was performed to evaluate the effect of baseline and 12-month INFRA values. The experimental level of significance was set as .05.

Results

Figure 2 summarizes the flow of the study. Among 29 individuals assessed for eligibility, 8 were excluded and 21 underwent surgical treatment. Because 6 patients presented intrabony defects with a three-wall component > 30%, only 15 patients (6 women and 9 men; mean age 48.7 ± 9.1 years) were enrolled in this study. One intrabony defect per patient was included in the study. Characteristics of sites based on location, tooth type, and percentage of residual bony walls with respect to defect length are reported in Table 1. The majority of defects were located at mandibular teeth and showed combinations of three-, two-, and one-wall components. The three-wall component was ≤ 10% of the total defect length in 7 sites and between 15% and 25% in the remaining 8 sites. The IBD ranged from 5 to 10 mm. All patients completed the study and complied with the 12- and 24-month re-examinations.

Table 2 presents clinical and radiographic parameters over the 24-month experimental period. All experimental sites achieved and maintained soft tissue primary closure. Healing was uneventful. None of the patients reported intraoperative pain at the end of the surgery. In week 1, slight discomfort was reported by five patients (average VAS value 5.3 ± 8.4). FMPS and FMBS remained < 15% throughout the study period, indicating a good standard of supragingival plaque control. The percentage of surgical sites with PI
and BoP decreased from 26.67% and 20% at baseline to 13.33% and 6.67% at 24 months, respectively. At 12 months, CAL and PD were significantly improved from baseline ($P < .0001$). The 24-month measurements did not significantly change from the 12-month measurements, remaining significantly improved with respect to baseline values ($P < .0001$). At 24 months, there was a PD reduction of $4.4 \pm 1.6$ mm and a corresponding CAL gain of $4.2 \pm 1.5$ mm. Nine defects (60%) experienced complete pocket closure (PD ≤ 3 mm). Only one defect had residual PD > 5 mm. Eight defects (53.33%) presented a CAL gain of at least 5 mm, four defects (26.67%) had a CAL gain of 3 to 4 mm, and three defects (20%) showed a CAL gain of 2 mm. The mean REC change was $0.2 \pm 1.0$ mm.

INFRA values significantly decreased from $5.8 \pm 1.9$ mm at baseline to $2.9 \pm 1.2$ mm at 12 months ($P < .0001$) for an INFRA gain of $2.9 \pm 1.1$ mm. At 2 years, it increased to $3.1 \pm 1.6$ mm corresponding to a radiographic defect fill of $51.8 \pm 18.5$%. Of the defects, 60% had bone fill > 50%. Representative baseline and 24-month clinical and radiographic images of the intrabony defects are presented in Figs 3 and 4.

When defects were stratified according to the baseline INFRA (Table 3), defects with INFRA ≥ 5 mm experienced an average increase in PD of $0.4$ mm at 24 months whereas defects with INFRA < 5 mm yielded an additional improvement of $0.4$ mm compared with 12-month values ($P = .002$). As reported in Table 4, a statistically significant relationship was also detected between residual INFRA ≥ 3 mm at 12 months and PD deterioration between months 12 and 24 ($P = .012$).

**Discussion**

The findings of the present prospective clinical study demonstrated that the use of EMD in association with autogenous particulate bone harvested with a piezoelectric device

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>12 mo</th>
<th>0–12 mo difference</th>
<th>24 mo</th>
<th>0–24 mo difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMPS (%)</td>
<td>$10.9 \pm 6.9^*$</td>
<td>$10.3 \pm 10.1$</td>
<td>$−0.6 \pm 4.2$</td>
<td>$12.0 \pm 2.7$</td>
<td>$−1.1 \pm 7.3$</td>
</tr>
<tr>
<td>FMBS (%)</td>
<td>$7.6 \pm 4.6^*$</td>
<td>$8.3 \pm 3.7$</td>
<td>$−0.7 \pm 4.4$</td>
<td>$8.4 \pm 2.8$</td>
<td>$−0.8 \pm 5.8$</td>
</tr>
<tr>
<td>PD (mm)</td>
<td>$7.9 \pm 1.7^{**}$</td>
<td>$3.4 \pm 0.7$</td>
<td>$4.5 \pm 1.8^{***}$</td>
<td>$3.5 \pm 1.1$</td>
<td>$4.4 \pm 1.6^{***}$</td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>$9.9 \pm 2.1^{**}$</td>
<td>$5.6 \pm 1.8$</td>
<td>$4.3 \pm 1.6^{***}$</td>
<td>$5.7 \pm 2.2$</td>
<td>$4.2 \pm 1.5^{***}$</td>
</tr>
<tr>
<td>REC (mm)</td>
<td>$2.0 \pm 1.7^*$</td>
<td>$2.2 \pm 1.5$</td>
<td>$−0.2 \pm 0.4$</td>
<td>$2.2 \pm 1.8$</td>
<td>$−0.2 \pm 1.0$</td>
</tr>
<tr>
<td>INFRA (mm)</td>
<td>$5.8 \pm 1.9^{**}$</td>
<td>$2.9 \pm 1.2$</td>
<td>$2.9 \pm 1.1$</td>
<td>$2.7 \pm 1.2$</td>
<td>$3.1 \pm 1.6^{***}$</td>
</tr>
</tbody>
</table>

*$P > .05$; $P$ values represent changes among the three time points (Friedman test or analysis of variance [ANOVA]).

**$P < .0001$; $P$ values represent changes among the three time points (ANOVA or Friedman test).

***$P \leq .0001$; $P$ values represent longitudinal changes from baseline (Newman-Keuls test or Dunn test).

FMPS = Full-Mouth Plaque Score; FMBS = Full-Mouth Bleeding Score; PD = probing depth; CAL = clinical attachment level; REC = gingival recession; INFRA = radiographic intrabony defect depth.
resulted in significant improvement in CAL, PD, and INFRA. A statistically and clinically significant CAL gain of 4.2 ± 1.5 mm was observed at 24 months after surgery, with more than 50% of the defects presenting a CAL gain of at least 5 mm. The PD reduction was 4.4 ± 1.6 mm, accompanied by a bone fill of 3.1 ± 1.6 mm.

It was speculated that a combined approach may allow for a combination of the biologic properties of EMD with the tissue-supporting properties of a grafting material. However, data in the literature is etheregenous, and only three trials are available on the clinical benefits of treating intrabony defects with the combination of EMD and AB. These studies reported mean CAL gain ranging from 4.2 ± 1.1 mm to 4.9 ± 1.8 mm and mean PD reduction ranging from 4.7 ± 1.5 mm to 5.6 ± 0.9 mm at 12 months. The radiographic analysis demonstrated a mean defect fill of 4.3 ± 1.3 mm.

In the present study, combined defects with a prevalent one- to two-wall configuration were treated using a minimally invasive surgical technique to achieve and maintain primary interdental wound closure. This was done to enhance the biologic potential of the EMD + AB. The above-mentioned studies did not apply such a technique, and with the exception of one study they selected two- to three-wall defects or did not report on the defect morphology. Moreover, the initial mean depth of the surgically treated defects was about 2 mm more than the mean depth of the bony lesions in the present study. These differences in the defect morphology may partially explain their better outcomes. Recession of the gingival margin was greater than that observed in the present research. Nevertheless, current data were in line with previous systematic reviews on the effectiveness of a combined approach using bone substitutes.

Because no histologic evaluation was performed, no definitive statement can be made concerning the tissue properties achieved. Several histologic studies based on human biopsies indicated a complete periodontal regeneration by using AB grafts in periodontal defects. Experimental studies on nonhuman primates proved that the combined application of EMD and autologous cortical bone resulted in significantly higher bone and cementum formation in comparison with the access flap procedure in small periodontal defects.

Another aspect to evaluate is the intraoral bone harvesting technique used. In the present study, AB was collected from the buccal and lingual/palatal cortical plate with Piezosurgery. In other investigations, a bone scraper or a trephine bur was used to harvest bone from the buccal cortical plate or the retromolar area. Different harvesting techniques and, in particular, different instruments might influence the vitality and viability of harvested bony tissue. This is a relevant issue as the success of bone regeneration depends on, among other factors, the osteogenic potency, vitality, and viability of the grafted osteoblastic cells. However, information from the literature is conflicting.
reported that a Piezosurgery device preserves bone cells’ viability, whereas scrapers and rotary instruments did not. Other investigators observed higher cell viability and expression of osteogenic growth factors in bone harvested with a scraper when compared to bone harvested by Piezosurgery, or failed to confirm it. A limitation of AB grafts is their unpredictable resorption. The dimensions of bone chips influence the resorption rate; however, the ideal dimension of AB chips has not yet been clarified. The presence of bone chips of such varied dimensions as those collected with a Piezosurgery device (mean size 486 ± 355 µm) combines the advantages of early remodeling found with small bone chips and the mechanical support provided by the larger particles. Additional advantages of using a Piezosurgery device are related to efficiency in bone removal, ease of use, and safety on soft tissue. Only five patients reported light morbidity during the first postoperative week.

Significant reduction was found in radiographic defect depth at 24 months, but incomplete bone fill was observed in all treated defects. Clinical outcomes of periodontal regeneration were influenced by the defect depth. Deeper pockets with initial INFRA ≥ 5 mm had significantly more bone gain than shallow pockets but yielded a higher residual intrabony component at 12 months and, in spite of the radiographic stability, experienced a slight worsening in PD values between 12 and 24 months. Defects with residual INFRA ≥ 3 mm at 12 months experienced an increase in PD of about 0.7 mm between 12 and 24 months. Thus, the need for resective treatment to eliminate this residual intrabony component may be anticipated when dealing with defects with initial INTRA ≥ 5 mm.

Conclusions

The treatment of noncontained intrabony defects with EMD in conjunction with AB harvested with a Piezosurgery device led to significantly improved clinical and radiographic parameters. Complete pocket closure was achieved in 60% of the defects, and only one had residual PD > 5 mm. However, when interpreting the present results, it should be kept in mind that patients were treated using a combined therapy, which makes it impossible to draw any conclusions on the contribution of AB to improve the outcomes compared with EMD alone.

Table 3 PD Reductions (Mean ± SD) in Defects with Baseline INFRA < or ≥ 5 mm

<table>
<thead>
<tr>
<th>INFRA (mm)</th>
<th>12 mo (mm)</th>
<th>24 mo (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INFRA &lt; 5 mm (n = 10)</td>
<td>3.8 ± 0.4</td>
<td>6.8 ± 0.8*</td>
</tr>
<tr>
<td>INFRA ≥ 5 mm (n = 5)</td>
<td>6.6 ± 1.9</td>
<td>8.4 ± 1.8*</td>
</tr>
</tbody>
</table>

*P < .002; P values represent differences between groups at this time point.
**P > .05; P values represent differences between groups at this time point.
Bonferroni corrected Mann-Whitney U test.

PD = probing depth; INFRA = radiographic intrabony defect depth; PD 12 to 24 = changes in PD between 12 and 24 months.

Table 4 PD Reductions (Mean ± SD) in Defects with INFRA < or ≥ 3 mm at 12 Months

<table>
<thead>
<tr>
<th>INFRA (mm)</th>
<th>12 mo (mm)</th>
<th>24 mo (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INFRA &lt; 3 mm (n = 6)</td>
<td>1.8 ± 0.4</td>
<td>3.5 ± 0.5*</td>
</tr>
<tr>
<td>INFRA ≥ 3 mm (n = 9)</td>
<td>3.7 ± 0.9</td>
<td>3.3 ± 0.9**</td>
</tr>
</tbody>
</table>

*P > .05; P values represent differences between groups at this time point.
**P = .012; P values represent differences between groups at this time point.
Bonferroni corrected Mann-Whitney U test.

PD = probing depth; INFRA = radiographic intrabony defect depth; PD 12 to 24 = change in PD between 12 and 24 months.
References

Acknowledgments

The authors reported no conflicts of interests related to this study. The study was supported by the local institutions.


