Clinical Comparison of an Enamel Matrix Derivative Used Alone or in Combination With a Bovine-Derived Xenograft for the Treatment of Periodontal Osseous Defects in Humans

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Background: The combination of bone replacement graft materials has been suggested for the treatment of periodontal osseous defects. The purpose of this study was to evaluate the effectiveness of enamel matrix derivative (EMD) combined with a bovine-derived xenograft (BDX) as compared to EMD alone in the treatment of intraosseous defects in patients with moderate to advanced periodontitis.

Methods: Sixteen adult patients with at least 2 intrabony defects were entered in this split-mouth design study. Defects were treated with EMD alone or EMD + BDX. Reentries were performed 6 to 8 months after initial surgery. The following soft and hard tissue measurements were recorded prior to initial surgery and at reentry: probing depth (PD), gingival margin location, clinical attachment level (CAL), depth of defect, and crestal bone level. Statistical analyses were performed to determine changes in PD, CAL, fill of osseous defect, and crestal resorption. Percentages of bone fill (%BF) and defect resolution (%DR) were also calculated.

Results: The most significant results were that gingival recession was greater for the group treated with EMD alone (0.8 ± 0.8 mm) compared to EMD + BDX (0.3 ± 0.6 mm) (P = 0.04) and bone fill was greater for EMD + BDX (4.0 ± 0.8 mm) compared to EMD alone (3.1 ± 1.0 mm) (P = 0.02). The measures for PD reduction, attachment level gain, crestal resorption, %BF, and %DR did not present a statistically significant difference (P >0.10).

Conclusions: This study evaluated the performance of EMD + BDX and EMD alone. The results demonstrated that a significant improvement in clinical parameters was observed. When comparing both modalities, a statistically significant difference was only found for gingival recession and bone fill, yielding a more favorable outcome towards the combined approach. *J Periodontol 2002;73:433-440.*

KEY WORDS

Comparison studies; grafts, bone; enamel matrix derivative; proteins, enamel matrix; periodontal regeneration; follow-up studies.

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Regeneration is defined as the reproduction or reconstitution of a lost or injured part.[1] One of the objectives of periodontal therapy is to regenerate the periodontal attachment apparatus. There must be histological evidence of regeneration of alveolar bone, periodontal ligament, and cementum over a previously diseased root surface to validate true regeneration.[2] This histological evidence is supported by clinical results that include bone fill of osseous defects and gain of clinical attachment levels. Currently, several materials have met these criteria in the human model: intraoral autogenous bone,[3-5] demineralized freeze-dried bone allograft (DFDBA),[6,7] DFDBA with bone morphogenetic protein (BMP),[8] barrier membranes,[9-12] citric acid application,[13] bovine-derived xenograft (BDX),[14,15] and enamel matrix derivative (EMD).[16,17]

EMD and BDX have recently been introduced as part of the armamentarium for regenerative therapy. In the animal model, BDX has shown its ability to become well vascularized and integrated with new host bone,[18,19] and it has been found to be effective in clin-

ical trials.[19-23] A human histologic study demonstrated a new attachment apparatus in a previously diseased root surface in 3 out of 4 specimens, thus indicating that periodontal regeneration is possible following grafting with BDX.[14] Regarding its safety, Cohen et al.[24] reported that a systemic or local immune response does not develop following implantation with BDX. The risk of disease transmission for anorganic bovine bone has been calculated to be 1 in 10.[18,25] A recent study reported that there may be a small amount of protein (average of 11 µg/g of particles) associated to the mineral phase in commercially available preparations of BDX, thus suggesting that this type of grafting material may be contraindicated for individuals who have a history of an immune response to bovine proteins.[26] However, there is no evidence, to date, to indicate that this is a valid statement.

Enamel matrix derivative is obtained from porcine tooth buds.[27-29] It consists primarily of amelogenin and other related proteins that have remained virtually unchanged during evolution,[27,28] maintaining high homogeneity with human enamel proteins.[29] EMD has been reported to be safe and efficient for treating periodontal osseous defects in both animal and human trials.[30-39]

A recent human histological study has shown that the use of EMD will result in a regenerative response.[17] It has been suggested that, due to the limited space-making potential of EMD, it could be mixed with a particle graft to counteract its thixotropic nature.[16] Mixing bone-replacement grafting materials to take advantage of their synergistic effect is not a novel concept.[8,40,41]

The purpose of this study was to evaluate the effectiveness of EMD[§] combined with BDX[‖] as compared to EMD alone in the treatment of intraosseous defects in patients with moderate to advanced periodontitis. A separate study will evaluate the effectiveness of BDX combined with EMD as compared to BDX alone in the treatment of intraosseous defects in humans.[42]

MATERIALS AND METHODS

Sixteen adult patients 36 to 65 years of age (9 female, 7 male; 4 smokers, 12 non-smokers) were entered in this study. Each patient presented evidence of 2 radiographic intrabony defects with associated probing depths of ≥5.0 mm following initial non-surgical therapy. Patients were systemically healthy and had no contraindications for periodontal therapy. Informed consent was obtained after explaining the nature of the investigation being conducted. Both the study and the consent form were approved by an Institutional Review Board.

Initial periodontal therapy consisted of full mouth scaling and root planing utilizing both hand and ultrasonic instruments under local anesthesia. Two visits or more were required, each lasting approximately 90 minutes, with oral hygiene instructions. Occlusal adjustments were provided when required. Four to 6 weeks following the initial phase of treatment, a reevaluation was performed to assess probing depth, attachment level, mobility, and bleeding on probing. An O'Leary plaque score[43] <20% was required for all patients before proceeding with the surgical phase of therapy.

Measurements

All baseline clinical parameters were recorded the day of surgery. Measurements were made with a University of North Carolina periodontal probe and recorded to the nearest millimeter at the mid-facial, mid-lingual, mesial, and distal line angles from the cemento-enamel junction (CEJ) to the free gingival margin (FGM) to evaluate recession, FGM to base of pocket (BP) to evaluate probing depth (PD) changes and CEJ to BP to evaluate attachment level changes. Hard tissue measurements were obtained during surgery as follows: CEJ to alveolar crest (AC) to evaluate crestal height changes, CEJ to base of defect (BD) to measure the amount of defect fill and AC to BD to measure defect depth and evaluate defect resolution. Whenever the CEJ was not present a restoration margin was used instead as a fixed point of reference. Only those defects with an intrabony component (AC-BD) of ≥3.0 mm were included. The highest measurement values were recorded. Measurements were made by a calibrated examiner who was blind to the treatment rendered.

Surgical Procedures

The first site to be treated was randomly assigned either EMD alone or EMD + BDX through the flip of a coin. The alternative graft material was automatically assigned to the remaining defect. Local infiltration of 2% lidocaine containing 1:100.000 epinephrine[¶] was utilized followed by intrasulcular incisions. Full thickness flaps were elevated from both the buccal and lingual aspects preserving as much interproximal tissue as possible. Granulomatous tissue was debrided from the osseous defect and the root surfaces were prepared utilizing ultrasonic and hand instruments removing accretions and altered cementum. All defects were classified as to the number of walls present. After appropriate measurements were taken, the root surfaces adjacent to the defect were bio-modified for 2 minutes with 24% ethylenediaminetetraacetic acid (EDTA) gel (pH 6.7)[#] to remove the smear layer and to facilitate the precipitation of the EMD to the root surface.[44] Following root conditioning, the wound was rinsed thoroughly with sterile saline. A sterile solution of propylene glycol alginate was utilized to reconstitute the lyophilized EMD and it was then applied on the root surfaces in an apico-coronal direction. For the

§ Emdogain, Biora, Chicago, IL.
‖ Bio-Oss, Osteohealth Co., Shirley, NY.
¶ Astra, Westborough, MA.
# PrefGel, Biora AB, Malmö, Sweden.

sites receiving a combination of EMD + BDX (experimental site), the previously described procedure was immediately followed by the insertion of the BDX which had been reconstituted with the remaining EMD. An amalgam carrier was utilized to deliver it into place and condensers were used to pack the graft to the existing level of the alveolar crest. Flaps were repositioned utilizing 5-0 sutures** in a modified vertical mattress fashion over the grafted site. Simple interrupted sutures were utilized in the adjacent areas. Pressure was applied with a moist gauze for 3 minutes postoperatively followed by placement of a periodontal dressing. Each patient was then prescribed oral analgesics (ibuprofen 800 mg every 8 hours as needed), 0.12% clorhexidine gluconate (twice daily) for assistance in plaque control, and antibiotics (doxycycline hyclate 100 mg every 12 hours for 10 days). Postoperative appointments were at 7 days, 14 days, 25 to 30 days, 3 months, and 6 to 8 months (reentry). At each visit the surgical area was debrided and further hygiene instructions were given. At the 3-month recall, a prophylaxis was provided.

During the reentry procedure, clinical soft tissue measurements were collected prior to flap elevation. Hard tissue parameters were recorded in a similar fashion as during initial preparation. Residual defects ≥3.0 mm were grafted with the material originally utilized. Osseous recontouring was performed if defects were <3.0 mm. Flaps were approximated utilizing braided 5-0 polyglactin 910 sutures†† in the same fashion previously described.

Statistical Analysis

This is a split-mouth single-blind design with each patient serving as his/her own control. A power analysis indicated that a minimum of 15 paired defects would be needed in this study to demonstrate statistical significance. A mean and a standard deviation were determined for each clinical parameter in both groups. The baseline and the reentry values were then compared for changes that took place over time such as: probing depth reduction (PDR), gingival recession (REC), attachment level gain (ALG), bone fill (BF), crestal level changes (CR), percent bone fill (%BF), and percent defect resolution (%DR). The paired *t* test was utilized to evaluate differences between the treatment groups. The level of significance for analysis was set at $P < 0.05$.

RESULTS

All 16 patients completed treatment and had no adverse reactions to therapy. Healing was uneventful in the 32 sites involved in this study.

Number of sites based on location, tooth type, number of osseous walls, and treatment rendered are presented in Table 1.

The most significant results were that gingival recession was greater for the group treated with EMD alone (0.8 ± 0.8 mm) compared to EMD + BDX (0.3 ± 0.6 mm) ($P = 0.04$) and bone fill was greater for the combination of EMD + BDX (4.0 ± 0.8 mm) when compared to EMD alone (3.1 ± 1.0 mm) ($P = 0.02$). The measures for probing depth reduction, attachment level gain, crestal resorption, %BF, and %DR did not present a statistically significant difference ($P > 0.10$).

Analyses of soft and hard tissue data are shown in Tables 2 and 3, respectively. The frequency data of defect bone fill greater than or equal to 75%, greater than or equal to 50% and less than 50%. Forty-four percent of the defects treated by both methods gained greater than 50% bone fill. Eighty-eight percent of the defects treated with EMD alone and 100% of the defects treated with EMD + BDX gained greater than 75% bone fill. Clinical cases are shown in Figures 1 and 2.

Table 1.

Number of Sites Based on Location, Tooth Type, Number of Osseous Walls and Treatment

Category	EMD (n = 16)	EMD + BDX (n = 16)
Maxilla	7	7
Mandible	9	9
Anterior Teeth	2	0
Premolars	2	3
Molars	12	13
3 osseous walls	6	7
2-3 osseous walls	10	9

Table 2.

Soft Tissue Responses

Parameter	EMD*	EMD + BDX*	*t* Test *P* Value
Presurgical pockets	6.6 ± 1.3	6.9 ± 0.9	0.37
Postsurgical pockets	2.8 ± 0.8	2.9 ± 0.6	0.61
Probing depth reduction	3.8 ± 1.2	4.0 ± 0.8	0.59
Gingival recession	0.8 ± 0.8†	0.3 ± 0.6†	0.04
Attachment level gain	2.9 ± 0.9	3.4 ± 0.9	0.15

* Patient mean values for mm or percentage.
† Statistically significant difference ($P < 0.05$).

** W.L. Gore & Associates, Inc., Flagstaff, AZ.
†† Vicryl, Ethicon, Inc., Somerville, NJ.

Table 3.

Hard Tissue Responses

Parameter	EMD*	EMD + BDX*	*t* Test *P* Value
Original bone defect	4.9 ± 1.5	5.3 ± 0.9	0.32
Residual bone defect	1.8 ± 1.0	1.3 ± 0.8	0.15
Amount defect fill	3.1 ± 1.0†	4.0 ± 0.8†	0.02
Percent defect fill	64.9 ± 17.6	76.9 ± 14.5	0.10
Crestal resorption	0.6 ± 0.7	0.4 ± 0.5	0.16
Percent defect resolution	76.2 ± 22.3	84.2 ± 16.9	0.31

* Patient mean values for mm or percentage.
† Statistically significant difference ($P<0.05$).

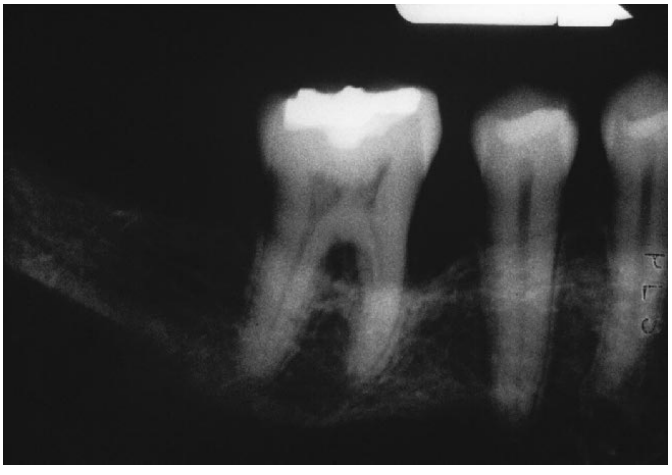

Figure 1A.
Periapical radiograph showing a vertical osseous defect on mesial aspect of tooth #30.

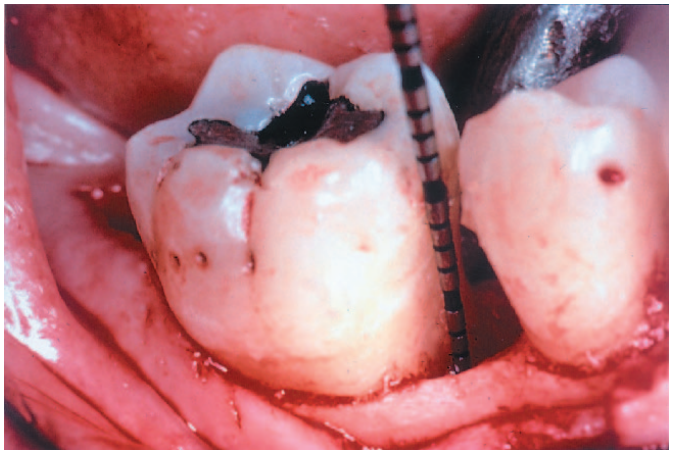

Figure 1B.
Exposure of the 2-3 wall osseous defect measuring 4 mm in depth.

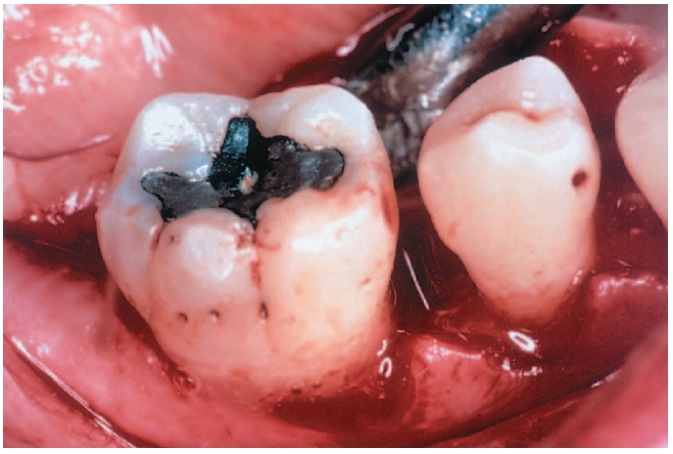

Figure 1C.
Enamel matrix derivative in place prior to suturing.

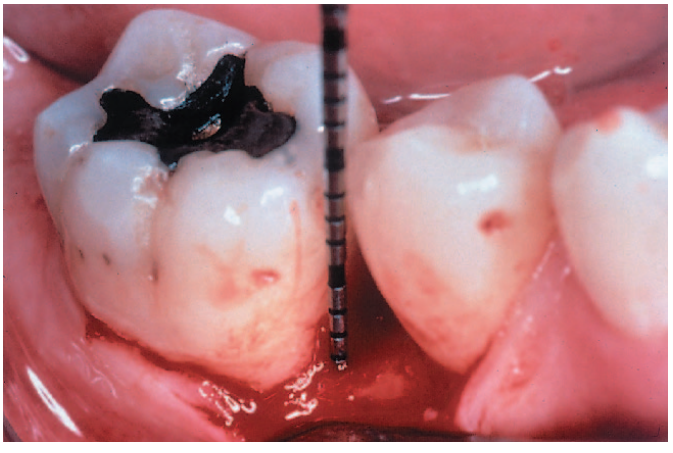

Figure 1D.
Reentry shows no residual defect representing a defect fill of 100%.

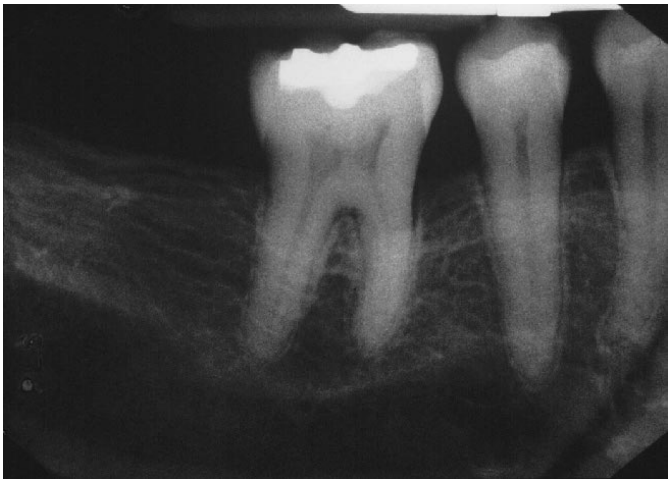

Figure 1E.
Reentry periapical rediograph confirming clinical impression.

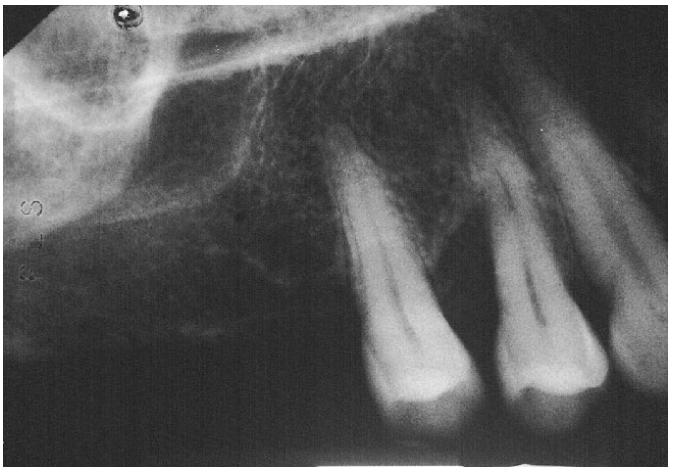

Figure 2A.
Presurgical periapical radiograph.

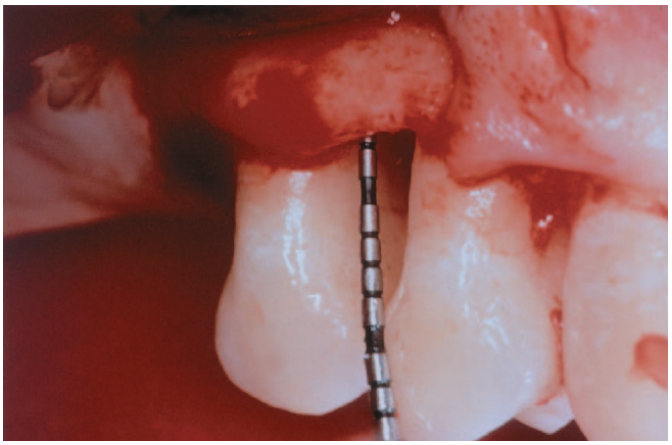

Figure 2B.
Exposure of the 2-3 wall osseous defect measuring 3 mm in depth.

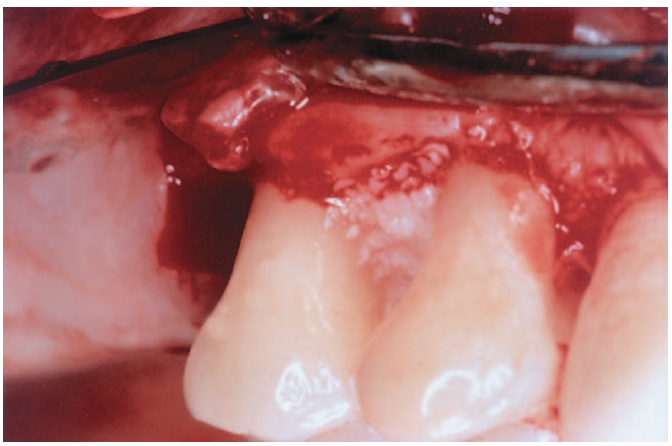

Figure 2C.
Enamel matrix derivative plus bovine-derived bone xenograft in place prior to suturing.

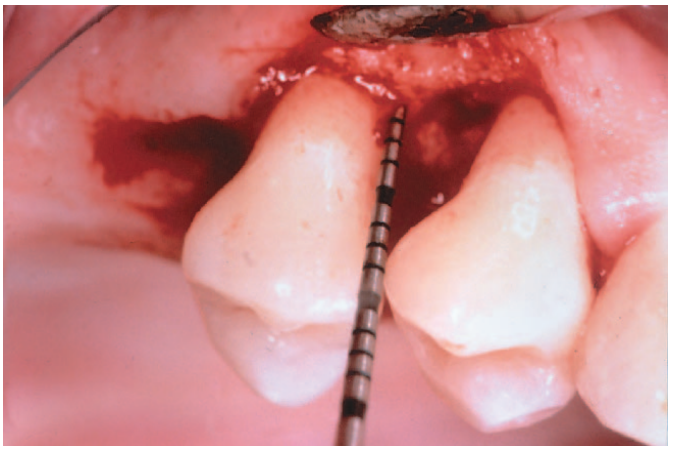

Figure 2D.
Reentry shows no residual defect representing a defect fill of 100% on mesial of tooth #4. Shallow defect on distal of tooth #5 was recontoured prior to suturing.

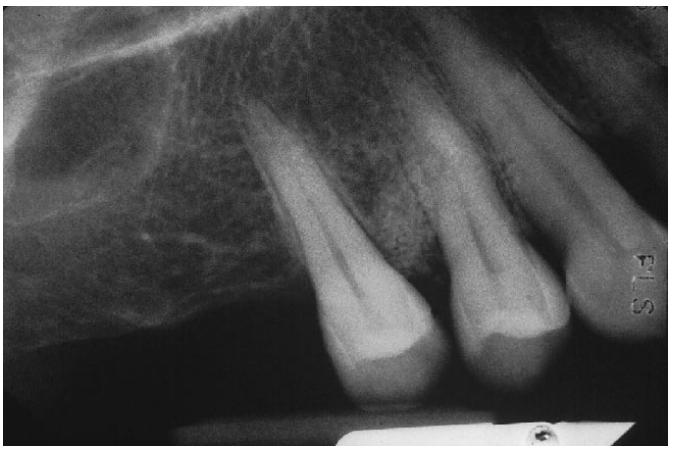

Figure 2E.
Reentry periapical radiograph.

DISCUSSION

This study evaluated the effectiveness of enamel matrix derivatives (EMD) combined with anorganic bovine-derived cancellous bone xenograft (BDX) as compared to EMD alone in the treatment of intraosseous defects in patients with moderate to advanced periodontitis. The results of the statistical analysis of this experiment revealed an overall significant improvement in all clinical parameters for both groups. This confirms previous findings which demonstrate clinical improvements achieved with both EMD and BDX.[14,16,22,23,34,37]

Both groups treated in this study presented very similar baseline conditions. Not only was there an almost identical distribution in anatomical location of the defects (maxillary versus mandibular arch and tooth type) but their remaining osseous walls were comparable (Table 1).

Only 2 clinical responses to treatment showed a statistically significant difference: gingival recession and bone fill. Gingival recession was greater for EMD alone compared to the combined grafting therapy. This might be explained by the lack of space maintaining properties related to the viscous nature of EMD.[16] Regardless of this difference, the attachment level gain was positive for both groups: 2.9 ± 0.9 mm for EMD alone and 3.4 ± 0.9 mm for EMD + BDX. The data for EMD alone compare well with other studies.[33,34,45] Regarding bone fill, the combined grafting group showed a better outcome (EMD + BDX, 4.0 ± 0.8 mm compared to EMD alone, 3.1 ± 1.0 mm). This might be related to the presence of solid graft particles that could enhance the resistance to probe penetration when measuring defect fill. Some of these particles were still observable after 6 to 8 months after the initial surgery.

The exact biologic mechanism of action of EMD has not yet been elucidated. In vitro studies have demonstrated that when EMD is added to cultures of periodontal fibroblasts, protein and collagen production is enhanced as well as stimulation of mineralization.[29,46] It has been suggested that EMD functions as a matrix enhancement factor, creating positive conditions for cell proliferation, migration, differentiation and matrix synthesis.[47-50]

A recent study[26] suggests that the osteogenic potential of BDX may be related to small amounts of protein that are present in it. Some of this extracted material has osteoinductive potential and may contain transforming growth factor-β and bone morphogenetic protein. However, these proteins are present in such small amounts that they may have little or no osteoinductive effect.[51]

According to a literature review article, the mean amount of bone fill of the original defect is about 60% when using any bone replacement graft material.[52] The combination of bone replacement graft materials has been documented to show equal or superior results when compared to non-combined materials.[41,53] In a reentry study, the performance of an anorganic bovine-derived hydroxyapatite bone matrix (ABM) alone and combined with a synthetic cell binding peptide, P-15 (ABM + P-15) was reported.[41] The statistical results showed that P-15 + ABM resulted in generally favorable clinical results (72.9% defect fill) when compared to ABM alone (50.6%). In our study the percent defect fill for the composite graft was 76.9%.

In a similar study to the present one, Lekovic et al.[53] used a combination of EMD and BDX and compared it to EMD alone. These authors found that the addition of EMD augments the effects of BDX in reducing probing depth (EMD + BDX: 3.43 ± 1.32 mm on buccal sites and 3.36 ± 1.35 mm on lingual sites; EMD: 1.91 ± 1.42 mm on buccal sites and 1.85 ± 1.38 mm on lingual sites), improving clinical attachment levels (EMD + BDX: 3.13 ± 1.41 mm on buccal sites and 3.11 ± 1.39 on lingual sites; EMD: 1.72 ± 1.33 mm on buccal sites and 1.75 mm ± 1.37 mm on lingual sites) and promoting defect fill (EMD + BDX: 3.82 ± 1.43 mm on buccal sites and 3.74 ± 1.38 mm on lingual sites; EMD: 1.33 ± 1.17 mm on buccal sites and 1.41 ± 1.19 mm on lingual sites) when compared to presurgical parameters.[53] These values compare favorably with the results of our study.

When adding EMD to BDX, the handling properties of both materials were improved. The viscosity of EMD helped in the delivery of the BDX particles by maintaining them together and somehow making allocation into the defect easier. It has also been speculated that the superior outcome of the combined approach is due to enhanced blood clot stabilization and isolation of the gingival epithelial and connective tissue cells from the defect area.[52]

Reentry surgery to assess bone fill does not distinguish between bone that is attached to the root surface via junctional epithelium or periodontal ligament. Histological evaluation remains the only reliable method to determine the components of the new attachment apparatus and allows for distinction between repair and regeneration. Future studies including human histological evidence are required to determine the nature of wound healing when using a multiple bone graft replacement material approach.

In summary, this study evaluated the performance of 2 materials used in combination (enamel matrix derivatives and anorganic bovine derived cancellous bone xenograft) and one by itself (enamel matrix derivative). The results demonstrated that a significant improvement in clinical parameters was observed when compared to presurgical levels. When comparing both modalities, a statistically significant difference was found only for gingival recession and bone fill, yielding a more favorable outcome towards the combined approach.

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