THE USE OF ENAMEL MATRIX DERIVATIVE IN THE TREATMENT OF PERIODONTAL DEFECTS: A LITERATURE REVIEW AND META-ANALYSIS

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ABSTRACT: Background—Periodontal disease results in the loss of the attachment apparatus. In the last three decades, an increasing effort has been placed on seeking procedures and materials to promote the regeneration of this tissue. The aim of this paper is to evaluate the effect of enamel matrix derivative (EMD) during regenerative procedures. In addition, a meta-analysis is presented regarding the clinical results during regeneration with EMD, to gain evidence as to what can be accomplished following treatment of intrabony defects with EMD in terms of probing depth reduction, clinical attachment level gain, defect fill (using re-entry studies), and radiographic parameters. Methods—The review includes in vitro and in vivo studies as well as human case reports, clinical comparative trials, and histologic findings. In addition, a meta-analysis is presented regarding the regenerative clinical results. For this purpose, we used 38 studies—including 955 intrabony defects treated with EMD that presented baseline and final data on probing depth, clinical attachment level (CAL) gain, or bone gain—to calculate weighted mean changes in the different parameters. The selected studies were pooled from the MEDLINE database at the end of May, 2003. Results—The meta-analysis of intrabony defects treated with EMD resulted in a mean initial probing depth of 7.94 ± 0.05 mm that was reduced to 3.63 ± 0.04 mm ($p = 0.000$). The mean clinical attachment level changed from 9.4 ± 0.06 mm to 5.82 ± 0.07 mm ($p = 0.000$). These results were significantly better than the results obtained for either open-flap debridement (OFD) or guided tissue regeneration (GTR). In contrast, histologically, GTR is more predictable than EMD in terms of bone and cementum formation. No advantage was found for combining EMD and GTR. Xenograft, or EMD and xenograft, yielded inferior results compared with EMD alone, but a limited number of studies evaluated this issue. Promising results were noted for the combination of allograft materials and EMD. Conclusions—EMD seems to be safe, was able to regenerate lost periodontal tissues in previously diseased sites based on clinical parameters, and was better than OFD or GTR. Its combination with allograft materials may be of additional benefit but still needs to be further investigated.

Key words. Enamel matrix derivative, Emdogain\textsuperscript{®}, meta-analysis, periodontal regeneration.

(I) Introduction

One goal of periodontal therapy is to provide a dentition that functions in health and comfort for the life of the patient (Zander et al., 1976). Studies reporting tooth loss among patients receiving periodontal treatment show that, for the majority of these patients, this goal is a reality (Hirschfeld and Wasserman, 1978; McFall, 1982; Nabers et al., 1988). The validity of this statement is enhanced in view of the contrary results observed among those who were untreated (Becker et al., 1979).

Therapeutic approaches to the treatment of periodontitis generally fall into two major categories: those designed to halt the progression of periodontal attachment loss, and those designed to regenerate or reconstruct lost periodontal tissues (Pihlstrom and Ammons, 1997). Surgical procedures involving root conditioning, autografts, allografts, xenografts, and/or barrier membranes for guided tissue regeneration have been shown to contribute to a successful regenerative outcome (for review, see Garrett, 1996).

Despite the convincing histological evidence that some regeneration may occur in humans following a regenerative surgical approach (Bowers et al., 1989a,b,c), complete and predictable regeneration is still a goal that is difficult to attain. In the last three decades, investigators have increased their efforts to seek procedures and materials to promote periodontal regeneration. Since growth and differentiation factors have been shown to play a key role in wound healing, it was suggested that they could enhance the regenerative process (for review, see Giannobile, 1996). Promising results have been obtained on healing and regeneration of lost attachment with application of recombinant human osteogenic protein-1 (OP-1) in surgically created critical-size class III furcation defects in dogs (Giannobile et al., 1998). Moreover, periodontal regeneration has been demonstrated histologically in humans following the use of purified recombinant human platelet-derived growth factor BB (PDGF-BB) mixed with bone allograft in both Class II furcations and interproximal intrabony defects (Nevins et al., 2003). Although the use of growth factors has demonstrated significant repair and/or regeneration, it is still considered experimental, since no growth factor therapy to treat periodontitis in humans has received approval by the United...
States Food and Drug Administration (FDA).

In 1997, an alternative approach for periodontal regeneration was introduced that was based on embryonic tooth formation (Hammarström, 1997; Heijl et al., 1997). This approach uses an extract of embryonic enamel matrix, termed ‘enamel matrix derivative’ (EMD), thought to induce mesenchymal cells to mimic the processes that take place during the development of the nascent root and periodontal tissues. The present analysis reviews the data on the effect of EMD as a regenerative promoter. It encompasses *in vitro* and *in vivo* studies as well as human case reports, clinical comparative trials, and histologic findings. In addition, a meta-analysis is presented regarding the regenerative clinical results. For this purpose, and to calculate weighted mean changes in the different parameters, we used studies that presented baseline and final data on probing depth, intrabony defect depth and clinical attachment level (CAL) gain, or bone gain.

(II) Literature Review

(II.1) The Enamel Matrix Proteins in the Developing Root

According to the classic theory of root formation and attachment apparatus development, Hertwig’s epithelial root sheath (HERS), which is the apical extension of the enamel organ, induces the mesenchymal cells of the dental papilla to form the mantle predentin before it disintegrates and leaves the root surface. As a result of HERS apoptosis during the embryonic process, the physical barrier it forms between the mesenchymal cells of the dentinal follicle and the forming dentin disintegrates. The mesenchymal cells that have become exposed to the newly formed dentin are induced to differentiate into cementoblasts, and are responsible for cementogenesis. The process of cementum deposition is a prerequisite for the formation of both the periodontal ligament and the alveolar bone, *i.e.*, for the completion of the attachment apparatus development (Armitage, 1991). However, recombinations between slices of root dentin and follicular cells have demonstrated that an exposed dentin surface is not a sufficient stimulus for cementoblast differentiation and cementogenesis (Thomas and Kollar, 1988). Instead, it appears that there is an obligatory intermediate short and specific modulating stage in which the HERS cells secrete enamel-related matrix proteins.

The enamel matrix was generally believed to regulate the initiation, propagation, termination, and maturation of the enamel hydroxyapatite crystallites (Simmer and Sneed, 1995). Other findings indicate that the enamel matrix also has a function outside the developing enamel. Enamel matrix proteins are temporarily deposited onto the dentinal root surface and provide an initial and essential step in the formation of acellular cementum (Slavkin and Boyde, 1975; Slavkin, 1976; Schonfeld and Slavkin, 1977; Owens, 1980). Autoradiographic and scanning electron microscopy studies provide additional evidence that, following apoptosis of HERS cells and deposition of the enamel matrix proteins onto the dentin surface, the cementogenesis process is initiated and kept modulated by these proteins (Lindskog, 1982; Lindskog and Hammarström, 1982; Slavkin et al., 1989a). Subsequently, when cementum has been laid down onto the enamel-matrix-covered dentin surface, an attachment apparatus will develop. Immunological (Slavkin et al., 1989b) and immunohistochemical (Hammarström, 1997) methods both show that enamel matrix proteins are present in acellular cementum, accentuating the importance of these proteins in the cementogenesis process.

(II.2) Composition of the Enamel Matrix Proteins

The major fraction of the enamel matrix proteins is composed of the amelogenins, a family of hydrophobic proteins that account for more than 90% of the organic constituent of the enamel matrix (Brookes et al., 1995). The amelogenins have remained remarkably well-conserved through evolution, suggesting that they may have great functional importance (Brookes et al., 1995).

The second largest component of the enamel matrix proteins is the enamelin (Brookes et al., 1995). Since the enamelin was found to contain serum proteins (Limeback et al., 1989; Strawich and Glimcher, 1990), the more general term "non-amelogenin" is now commonly used to describe this high-molecular-weight fraction (Hammarström et al., 1997). It includes proline-rich enamelin (Fukae and Tanabe, 1987), tuftelin (Deutsch et al., 1991), and tuft proteins (Robinson et al., 1975).

Three matrix proteins, corresponding to amelogenin (Hu et al., 1996), enamelin (Hu et al., 1997b), and shealthin (also called ameloblastin or amelin) (Hu et al., 1997a), and 2 enzymes, corresponding to MMP-20 (Fukae et al., 1998) and EMSP1 (Simmer et al., 1998), have been purified and the cDNA cloned from developing porcine teeth. These proteins are all present in EMD. Although early immunoassay studies could not identify the presence of growth factors in EMD (Gestrelius et al., 1997b), nominal levels of transforming growth factor β1 (TGF-β1) have been detected immunologically (Kawase et al., 2001). In addition, by using the bone morphogenetic protein (BMP) binding protein noggins, investigators have identified BMP-2 and BMP-4 in an osteoinductive fraction of enamel extracts (Iwata et al., 2002). Even though the latter study used non-commercial fractionated enamel extracts from developing pig teeth, it may suggest the presence of these morphogenetic proteins in commercial EMD as well.

(II.3) Emdogain® Formulation

A commercial enamel matrix derivative (Emdogain®, Biora AB, Malmö, Sweden) received FDA approval and is now available for the treatment of periodontal defects. It is a purified acidic extract of developing embryonal enamel derived from six-month-old piglets. Its purpose is to act as a tissue-healing modulator that would mimic the events that occur during root development and to help stimulate periodontal regeneration (Hammarström, 1997; Heijl et al., 1997). The enamel proteins described above are present in Emdogain®.

(II.4) The Emdogain® Vehicle

The amelogenins, which are the hydrophobic constituent of the enamel matrix proteins, aggregate and become practically insoluble at physiological pH and body temperature. They can be dissolved in an acidic or alkaline pH environment and at low temperature. A suitable formulation should thus have a non-neutral pH and allow for gradual re-precipitation of the matrix when physiological conditions are re-established. Using a buccal dehiscence model in monkeys, investigators evaluated several drug vehicles to determine which most effectively allowed the EMD to precipitate on the treated root surface (Hammarström et al., 1997). Regeneration of cementum and
alveolar bone was measured after 8 wks. The results showed that propylene glycol alginate (PGA) was more effective than hydroxyethyl cellulose (HEC) or dextran. PGA appears to be more effective than HEC or dextran. PGA is a propylene glycol ester of alginate, which is commonly used in food and pharmaceuticals as a thickening agent.

To understand the behavior and kinetics of EMD in PGA, investigators have performed in vitro and in vivo studies (Gestrelius et al., 1997a). The neutral pH of PGA in solution was useful for dissolving EMD, even at room temperature. Furthermore, the thixotropic rheology (i.e., the characteristics of a fluid to undergo a decrease in viscosity with time while it is subjected to constant shearing) of PGA permitted the application of EMD as a viscous formulation. When a shear force is applied, such as by means of a syringe, the viscosity of the formulation decreases, which facilitates complete coating of the root surfaces to be treated. The viscosity of PGA decreases under physiological conditions; thus, EMD is "released" to precipitate on the exposed root surfaces in the treated area. In addition, by means of a radiolabeling technique, the PGA vehicle was found to leave the surgical area shortly after the application, thereby facilitating handling. Although the manufacturer recommends a dry environment, once the EMD is applied, slight bleeding seems to be helpful for the precipitation of the product on the root surface (personal communication). (AQ)

Thus, PGA solutions fulfill the essential requirements of a vehicle to facilitate the application of EMD during periodontal surgery.

The first marketed EMD product was supplied in a lyophilized form and was dissolved in an aqueous solution of PGA immediately prior to use. Because mixing EMD with PGA needs extra assistance and time, a new ready-to-use product, Emdogain® Gel (Biora AB, Malmö, Sweden), was developed. It is a pre-mixed formulation of EMD, where the protein has been stabilized by heat treatment prior to being mixed with the vehicle. Both formulations contain 30 mg EMD protein/mL PGA gel, with a viscosity of about 2.5 FAS (shear-thinning rheology).

The clinical and radiographic outcomes of both forms of EMD were compared in one study. Eighty-eight patients with advanced periodontitis were enrolled in a blinded randomized controlled multicenter study. At 8 and 16 months following treatment, a statistically significant reduction of pocket depth and gain of attachment and bone were demonstrated compared with baseline, with no differences between the 2 products (Bratthall et al., 2001).

**II.5 In vitro studies**

**II.5.1 EMD properties**

One of the most important factors that may influence the healing pattern of periodontal tissues after any kind of surgical treatment is the epithelial down-growth along the root surface, which is known to prevent the re-establishment of the normal periodontal architecture (Caton et al., 1980; Nyman et al., 1981). Application of EMD results in limited epithelial down-growth, in contrast to the control sites, where greater epithelial downgrowth takes place (Hammarström et al., 1997). This histologic observation was reinforced by in vitro studies. Addition of EMD to cell culture media resulted in enhanced proliferation of PDL cells, as well as increased protein and collagen production and mineralization. In contrast, EMD had no significant effect on epithelial cell proliferation in vitro (Gestrelius et al., 1997b). It may be concluded that the biochemical environment at the root surface following the application of EMD may prevent the epithelial down-growth in a manner similar to the mechanical prevention achieved with the use of barrier membranes in guided tissue regeneration procedures (Nyman et al., 1982a,b; Gottlow et al., 1986; Stahl et al., 1990).

**II.5.2 Clinical safety of EMD**

Since the commercial formulation of EMD (Emdogain®) is a porcine-derived material (i.e., a xenograft), the potential for it to stimulate an immune reaction when used in humans is of extreme importance. The enamel matrix proteins are highly conserved among mammalian species (Brookes et al., 1995; Slavkin and Diekwisch, 1996, 1997), and exposure to these proteins takes place during tooth development in early childhood. Thus, tolerance should normally be induced and the proteins recognized by the immune system as "self" proteins. Therefore, it is reasonable to assume that they are less likely to act as antigens. In vitro studies showed that EMD does not significantly modify cellular or humoral immune responses. Very high concentrations of EMD induced only a slight increase in the proliferation of human lymphocytes, restricted to the CD25+ (IL-2 receptor) fraction of the CD4+ T-lymphocytes. There was a concomitant decrease of B-lymphocytes, while other cell fractions (CD8+ T-cells, B-cells, and NK(AQ) cells) were not affected, and immunoglobulin and cytokine (IL-2 and IL-6) production was not modified (Peteinaki et al., 1998).

**II.5.3 EMD—mode of action**

To improve their understanding of the possible mechanisms of action of EMD, investigators have studied the in vitro effects of EMD on cells that participate in periodontal regeneration. These studies are reviewed below.

Non-commercial fractionated enamel extracts from developing pig teeth were found to contain low levels of BMP (Iwata et al., 2002). In addition, EMD contains TGF-β1 (Kawase et al., 2001). However, most researchers attribute the benefits of EMD to the enamel matrix proteins. By a variety of techniques (ellipsometry, total internal reflection fluorescence, and biospecific interaction analysis), it has been demonstrated that EMD adsorbs both to hydroxyapatite and collagen and to denuded dental roots. It forms insoluble spherical complexes, and detectable amounts remain at the treated site on the root surface for up to 2 wks, as was shown with radiolabeled protein in rats and pigs (Gestrelius et al., 1997a). This appears to be a sufficient period of time to prevent recolonization by periodontal ligament cells or undifferentiated cells. This assumption was confirmed when scanning electron microscopy of EMD-treated teeth, extracted at different time intervals up to 2 wks after surgery, displayed a progressive colonization of fibroblast-like cells. This observation could not be demonstrated for the control teeth that were sham-operated without application of EMD (Gestrelius et al., 1997a). Immunohistochemical analysis demonstrated that EMD was still present for 4 wks after its application on extracted rat molars that were transplanted to the abdominal wall (Hamamoto et al., 2002). In humans, it was...
demonstrated, by histological and immunochemical methods, that EMD is present on treated root surfaces for up to 4 wks following application during periodontal surgery (Sculean et al., 2002a).

In an attempt to understand the mechanisms by which EMD promotes regeneration of periodontal tissues, investigators evaluated the effect of EMD on periodontal ligament (PDL) cells in culture (Gestrelius et al., 1997b). EMD enhanced proliferation of PDL cells, but not epithelial cells. It increased total protein production by PDL cells and promoted mineralized nodule formation of PDL cells. In contrast, EMD had no significant effect on migration or attachment and spreading of PDL cells. In another study aimed at examining the influence of EMD on the viability, proliferation, and attachment of human PDL fibroblasts to diseased root surfaces in vitro, it was shown that the viability of PDL cells was negatively affected by higher doses of EMD over time, while lower doses elicited no change when compared with control cultures (Davenport et al., 2003). Scanning electron microscopy showed that EMD appeared to increase attachment of periodontal ligament fibroblasts to diseased root surfaces. In addition, amelogenin was shown to have a cell-adhesive activity, which may partially explain the therapeutic effect of EMD in periodontal regeneration (Hoang et al., 2002).

Not all cells involved in periodontal regeneration respond to EMD in a comparable manner. Attachment rate, growth factor production (TGF-β1, IL-6, and PDGF-AB), proliferation, and metabolism of human PDL cells in culture were all significantly increased in the presence of EMD (Lynngstadaas et al., 2001). In contrast, EMD increased cAMP and PDGF-AB secretion in epithelial cell cultures, but inhibited their growth. Results from this and earlier studies suggest that EMD favors mesenchymal cell growth over growth of epithelial cells. Furthermore, it had been shown earlier that EMD also seems to exhibit a cytostatic effect upon cultured epithelial cells (Gestrelius et al., 1997b; Kawase et al., 2000). This may explain EMD’s biological ‘guided tissue regeneration’ effect observed in vivo, analogous to the mechanical prevention of barrier membranes.

The specificity of the effect of EMD on human PDL cells was also demonstrated in an in vitro wound-healing model (Hoang et al., 2000). Wounds were created by 3-mm incisions in cell monolayers across the length of tissue-culture plates made of PDL cells, gingival fibroblasts, or osteosarcoma cells. When the cultured cells were exposed to EMD during a healing period of up to 9 days, an enhanced wound-fill was observed compared with untreated conditions. The PDL wound-fill rates in the presence of EMD at early time points were statistically greater than the rates of the gingival fibroblasts and the osteosarcoma cells that were treated with EMD.

Because early studies did not detect the presence of growth factors in EMD preparations (Gestrelius et al., 1997b), it was postulated that it acts as a matrix enhancement factor, creating a positive environment for cell (osteoblasts and cementoblasts) proliferation, differentiation, and matrix synthesis. The effect of EMD on matrix synthesis was investigated with the use of cultured periodontal fibroblasts (Haase and Bartold, 2001). EMD significantly affected the mRNA levels for matrix proteoglycans (2 were elevated and 1 was decreased) and stimulated hyaluronic acid synthesis.

These results suggest that EMD has the potential to modulate matrix synthesis significantly in vitro in a manner consistent with the changes noted in tissues undergoing repair and regeneration. EMD was found to regulate cementoblast and osteoblast activities (Tokiyasu et al., 2000). In addition, EMD can regulate dental follicle cell activity by increasing matrix protein production and their (AQL) differentiation into cementoblasts and osteoblasts.

This supports the hypothesis that epithelial-mesenchymal interactions may be important during the development of periodontal tissues (Hakki et al., 2001), and that EMD can influence the process at multiple stages of differentiation. A study examining the effect of EMD on osteoblasts showed that EMD has the ability to regulate cells in the osteoblastic lineage (Jiang et al., 2001). The ability to do so depends on the state of maturation within the lineage. EMD induced differentiation of mature well-established osteoblasts; however, it had no effect on undifferentiated mesenchymal cells. These results were in contrast to the effect of BMP-2, which induced the differentiation of undifferentiated cells, 2T9 (osteoblast progenitor cells), in the lineage. This indicates that EMD is an osteoconductive agent (Schwartz et al., 2000), rather than an osteoinductive one.

However, recent in vitro studies suggest that EMD may have the ability to induce osteochondral progenitor cells to differentiate. In a multipotent mesenchymal cell line (C2C12), it was shown that EMD converts the differentiation pathway of the mesenchymal cells into osteoblasts and/or chondroblasts (Ohyama et al., 2002).

EMD may also promote periodontal regeneration by reducing dental plaque. In an ex vivo dental plaque model, it was found that EMD had an inhibitory effect on dental plaque viability (Sculean et al., 2001b). The effect of EMD on the growth of periodontal pathogens was further evaluated in vitro (Spahr et al., 2002). Freshly prepared EMD or its vehicle (PGA) alone was added to calibrated suspensions of microbes. A marked inhibitory effect of EMD on the growth of the Gram-negative periodontal pathogens was demonstrated, and the Gram-positive bacteria were unaffected. It was concluded that EMD has a positive effect on the composition of bacterial species in the post-surgical periodontal wound by selectively restricting growth of periopathogens that can hamper wound healing and reduce the outcome of regenerative procedures.

Results from these in vitro studies indicate that EMD regulates multiple cell types in the healing site, while at the same time modulating the bacterial composition. EMD enhances proliferation rate, metabolism and protein synthesis, cellular attachment rate, and mineral nodule formation of PDL cells and has a similar influence on cementoblasts and mature osteoblasts. In addition, EMD enhances PDL cell attachment. In contrast to its effects on mesenchymal cells, EMD appears to inhibit the proliferation and the growth of epithelial cells. These characteristics partly explain the biological ‘guided tissue regeneration’ effect attributed to EMD.

Most of the effects of EMD are on mature cells rather than on multipotent precursors, suggesting that it may not be capable of controlling the entire regenerative process. At high concentrations, EMD inhibits terminal differentiation of cementoblasts with respect to mineralized module formation (Tokiyasu et al., 2000). This supports the idea that EMD is important for increasing the pool of cells required for periodontal regeneration and for stimulating the early differentiation process, but other factors in the environment for certain cell types may be required to continue the regenerative process in vivo. Other proven abilities of EMD are inhibitory effects on dental plaque viability, which can also contribute to the regenerative result.
The ability of EMD to regenerate acellular extrinsic fiber cementum was first demonstrated in monkeys (Hammarström, 1997). Four lateral incisors from each animal were gently extracted. Immediately after extraction, an experimental cavity was made in each root. The test cavities were treated with crude porcine enamel matrix, and the teeth were re-implanted. Acellular cementum attached to the dentin was induced after 8 wks of healing. The healing of the control cavities, where no enamel matrix was placed prior to replantation, was characterized by deposition of an uneven, thick layer of a cellular, hard tissue that was poorly attached to the denuded dentin.

In another study, with a buccal dehiscence model in monkeys, it was possible to obtain regeneration of 60-80% of the cementum defect by the application of either the whole enamel matrix or the acid extract of EMD to the denuded root surface (Hammarström et al., 1997). New bone formed to a slightly lesser extent. Surgically created buccal dehiscences of 6 mm in both sides of the monkeys’ maxillae were treated either with EMD (following root conditioning with acid), with or without vehicles, or served as controls (conditioned with the acid and given no further treatment). After 8 wks of healing, the monkeys were killed, and tissue blocks were prepared for histologic evaluation. In contrast to the regeneration found in the experimental sites, the amounts of newly formed cementum and alveolar bone in the sham-operated controls were close to zero. This study showed that it is possible to induce regeneration of all the periodontal tissues (acellular cementum, periodontal ligament, and alveolar bone) in a way that mimics the normal development of these tissues. In addition, the periodontal regeneration properties of the enamel matrix were associated with the amelogenin fraction (Hammarström et al., 1997).

The specific characteristics of EMD regarding its bone formation ability (osteoinductive, osteoconductive, or osteogenic) was examined by means of a nude mouse muscle implantation assay (Boyan et al., 2000). No ossicle formation occurred when EMD alone was implanted into cell muscle under conditions that supported osteoinduction by demineralized freeze-dried bovine allograft (DFDBA). If EMD was implanted together with DFDBA that had limited osteoinductive ability, EMD had no detectable effect. However, active DFDBA and EMD above a threshold dose (4 mg) resulted in enhanced bone induction compared with inactive DFDBA or active DFDBA without EMD. It was concluded that EMD is an osteogenic agent. It enhances the osteoinductive potential of the graft material, due in part to its osteoconductive properties, but a threshold concentration is required.

The latter conclusion was further supported in a morphological study in which the effect of locally applied EMD on bone and medullary regeneration was evaluated with the use of rat femurs in a drill-hole injury model (Kawana et al., 2001). The created defects were filled with either EMD (test group) or its carrier, PGA (control group). At 4-28 days post-surgery, the rats were killed, and the dissected femurs were examined by means of various morphological approaches. Bone volume fraction of newly formed bone trabeculae on day 7 post-operatively was significantly higher in the EMD group than in the controls. However, because of active bone remodeling and the marked decrease of bone volume, there was no longer a significant difference in trabecular bone volume between the experimental and control groups on days 14-28. Based on these results, it was suggested that EMD possesses an osteogenic effect on bone and medullary regeneration during wound healing of injured long bones (Kawana et al., 2001).

Results from these in vivo studies indicate that EMD has both osteoconductive and cementoconductive properties. In addition, it has a stimulatory effect on bone growth.

Several animal studies were conducted so that the histological and clinical outcomes following treatment with EMD could be compared with those achieved with guided tissue regeneration (GTR). Critical-size fenestration-type defects produced surgically in the buccal bone of 4 teeth in 3 monkeys were treated with EMD, GTR, or coronally repositioned flap (control) (Sculean et al., 2000a). After 5 months, the monkeys were killed, and descriptive histological evaluation of the healing was performed. The results showed that, in the GTR group, new connective tissue attachment and new bone formation had consistently occurred, whereas, in the defects treated with EMD or with coronally repositioned flaps, new attachment and new bone formed to various extents. Although no quantitative analysis was performed, it was concluded that GTR treatment seems to be more predictable than EMD in terms of periodontal regeneration.

Using a similar research model, the same investigators evaluated the effects of treating intrabony defects with EMD, GTR, or combined EMD and GTR 6 wks after intrabony defects were surgically produced in 3 monkeys (Sculean et al., 2000b). Coronally repositioned flaps were used as the control. After 5 months, the monkeys were killed, and descriptive histological evaluation of the healing was made. In the control group, the healing was characterized by a long junctional epithelium and limited periodontal regeneration at the bottom of the defect. The GTR-treated defects consistently presented periodontal regeneration when the membranes were not exposed, whereas the sites treated only with EMD presented regeneration to various extents. The combined therapy did not seem to improve the results.

Some of the effects seen with EMD may depend on the animal model and the type of defect being studied. No histological benefits in terms of periodontal regeneration were observed when EMD was compared with a combination of EMD and GTR in the treatment of class III furcation defects in dogs (Araujo and Lindhe, 1998). However, in the combination treatment, the cementum that had formed in the apical portion of the furcation defect was cellular, which was different from the corresponding tissue in the coronal portion, and also different from the cementum observed in the GTR group, which was cellular. This cellular cementum formation was attributed to the EMD effect (Araujo and Lindhe, 1998).

Results from these pre-clinical animal studies indicate that EMD has the ability to induce the regeneration of periodontal tissues, i.e., cementum, PDL, and bone (although for the latter the results appeared less in descriptive studies). The ability of EMD to enhance bone formation has been defined as osteogenic. It enhances the osteoinductive potential of graft materials; thus, an osteoinductive material is recommended when bone formation is needed. The periodontal regeneration that is accomplished by the use of EMD appears less predictable than that with GTR in animal studies. The combined use of EMD and GTR in these animal studies does not seem to offer a significant advantage over the use of GTR alone, except for the type of cementum that is formed.
(II.6.2) In vivo human studies

(II.6.2.1) Clinical safety of EMD

The clinical safety of EMD was first evaluated in humans in a multicenter study that assessed the changes in IgE, IgG, IgM, and IgA in 107 patients following multiple periodontal surgical exposures to Emdogain®. There was no increase in those antibodies among the patients (Zetterström et al., 1997). Moreover, a comparison between the test and the control groups (33 patients who underwent flap surgery without Emdogain® application) demonstrated the same types and frequencies of post-surgical experiences, i.e., reactions caused by the surgical procedure itself (Zetterström et al., 1997). In addition, Emdogain® was demonstrated to be a safe product in the treatment of periodontal defects, since multiple applications of Emdogain® did not have any negative impact on periodontal wound healing, as was determined from clinical signs and symptoms reported by the treated patients (Heard et al., 2000).

The clinical safety of EMD was further demonstrated in a study comprised of ten human patients. Only a slight, non-significant activation of the immune system occurred during the first year following Emdogain® application. Neither cellular immunity nor humoral immune response was significantly modified (Nikolopoulos et al., 2002). A review of the literature since the introduction of Emdogain® in 1997 reveals no reports of any complications or adverse reactions following treatment with the enamel proteins. On the contrary, in a split-mouth double-blind randomized study, it was demonstrated that the topical application of Emdogain® in instrumented periodontal pockets with probing depth equal to or exceeding 5 mm enhanced the early healing of the periodontal soft tissue, as was evidenced by gingival condition (gingival index), bleeding on probing, and dentin hypersensitivity tests (Wennström and Lindhe, 2002). These studies also indicated that EMD is safe for periodontal treatment.

The effect of Emdogain® on the early wound-healing process has been evaluated by assessments of the protein levels of matrix metalloproteinases and tissue inhibitors of metalloproteinases in gingival crevicular fluid. It was found that Emdogain®-treated sites showed accelerated wound healing following surgery, compared with placebo-treated sites (Okuda et al., 2001).

(II.6.2.2) Clinical trials

Clinical trials have been conducted for the assessment of the effectiveness of EMD regarding its ability to improve periodontal health. One of the first human studies was a split-mouth randomized multicenter trial undertaken to compare the long-term effect of EMD treatment as an adjunct to modified Widman flap (MWF) surgery vs. MWF plus a placebo (PGA) (Heijl et al., 1997). Thirty-three patients with 34 paired test and control sites (one- or two-wall bony defects ≥ 4 mm deep) were enrolled in the study and monitored for 36 months. The results in the EMD group were better, as shown by a gain in the clinical attachment level, probing depth reduction, and restoration of bone radiographically.

Other studies compared the use of EMD with placebo or open-flap debridement (OFD)/MWF alone in a split-mouth or parallel-group designs and found similar results, i.e., an advantage with EMD in terms of clinical and radiographic findings (Zetterström et al., 1997; Pontoriero et al., 1999; Okuda et al., 2000; Silvestri et al., 2000; Sculean et al., 2001a; Tonetti et al., 2002; Zucchelli et al., 2002). Some case reports have also presented favorable results showing significant improvement in clinical and radiographic parameters following the use of EMD in the treatment of intrabony defects (Heden et al., 1999, 2000; Sculean et al., 1999a; Heard et al., 2000; Parashis and Tsikalakis, 2000; Cardaropi and Leonhardt, 2002; Trombelli et al., 2002). However, it should be noted that when using EMD in a non-surgical approach (all of the above studies were surgical), one might not expect the favorable results demonstrated above. In fact, a histological investigation of the healing of advanced intrabony periodontal defects in humans following non-surgical periodontal therapy with subgingival application of EMD failed to demonstrate regeneration (Sculean et al., 2003c; Gutierrez et al., 2003).

The superiority of surgically treating intrabony defects with EMD compared with open-flap debridement has also been shown with re-entry 12 months post-surgery, where the average defect fill was 2.4 mm greater with EMD (Froum et al., 2001). In a case series study, an average bone fill of 2.54 mm was demonstrated in 21 sites when 13 of them were re-entered 12 months following the use of EMD (Parodi et al., 2000). In most of the studies, the clinical evaluation was performed following a period of at least 6 months. However, even as early as 12 wks post-treatment, better clinical results were obtained following the use of EMD compared with placebo (PGA) (Okuda et al., 2001).

Most of the clinical trials and case reports have used EMD for the treatment of intrabony defects, since horizontal bone loss defects are not likely to exhibit a successful outcome with regenerative treatment (Wikesjö and Selvig, 1999). Nevertheless, EMD was also shown to achieve better clinical improvement in periodontal sites with horizontal bone loss as compared with conventional flap debridement procedures (Yilmaz et al., 2003).

(II.6.2.3) Histologic assessments in humans

The first human histological report assessing the effect of EMD on periodontal regeneration used a mandibular incisor scheduled for extraction due to orthodontic reasons (Heijl, 1997). An experimental surgical procedure, intended to create a buccal dehiscence defect almost reaching the apex of the root, was performed in a setting identical to that of previously reported experimental defects in monkeys (Hammarström et al., 1997). Four months later, the experimental tooth, together with the surrounding soft and hard periodontal tissues, was removed surgically for histological evaluation. Microscopic examination revealed formation of new acellular cementum, new periodontal ligament with inserting and functionally oriented collagen fibers, and associated alveolar bone. The new cementum covered 73% of the original defect. New bone gain was 65% of the pre-surgical bone height (Heijl, 1997).

Other histological reports demonstrating periodontal regeneration following EMD treatment have since been published (Mellonig, 1999; Sculean et al., 2000c, 2003a; Windisch et al., 2002). However, contradictory results have also been observed. In a study of 21 cases treated with EMD, clinical improvement was demonstrated, but in the 2 cases evaluated histologically, there was no evidence of periodontal regeneration (Parodi et al., 2000). In another study, 5 out of 7 intrabony defects that were treated with EMD resulted in a healing char-
acterized by insufficient formation of new bone, while only 2 resulted in true periodontal regeneration (Sculean et al., 1999c). In addition, evaluation of 10 intrabony defects in eight patients treated with EMD revealed histologic evidence of regeneration in only 3 specimens (Yukna and Mellonig, 2000). The healing of the rest of the specimens was characterized by new attachment (connective tissue/adhesion only), or by long junctional epithelium. It was concluded that the use of EMD could result in periodontal regeneration, but on an inconsistent basis.

In summary, EMD treatment in intrabony defects in patients results in enhancement of the outcome in terms of probing depth reduction and gain of attachment, compared with control (open-flap debridement/modified Widman flap). Although the healing is occasionally "true" periodontal regeneration, this cannot yet be considered a predictable and reproducible result.

(II.6.2.4) EMD vs. GTR

GTR is a well-established successful therapeutic method for achieving clinical periodontal regeneration in humans, since both non-resorbable (Nyman et al., 1982b; Gottlow et al., 1986; Stahl et al., 1990) and resorbable barrier membranes (Sculean et al., 1999b) achieve good clinical results based on histological assessments. However, the clinical outcomes of GTR in deep intrabony defects exhibit a high degree of variability. Several factors can directly influence the clinical outcomes of GTR. Among these are factors related to the surgical technique (Cortellini et al., 1995a,b), the clinician's experience and surgical skill (Tonetti et al., 1999), tooth morphology (Lu, 1992), and defect morphology (Tonetti et al., 1993; Trombelli et al., 1997).

Another factor potentially adversely affecting the outcome of every regenerative procedure is bacterial load. Several studies have shown that bacteria may heavily colonize exposed membranes, and that there is a negative relationship between attachment gain and bacterial colonization of the barrier material (Demolon et al., 1993; Machtei et al., 1993; Nowzari and Slots, 1994; Nowzari et al., 1995). As previously mentioned, EMD has a marked inhibitory effect on the growth of the Gram-negative periodontal pathogens, without a similar effect on the Gram-positive bacteria (Spahr et al., 2002). In addition, it was demonstrated to have some antimicrobial effect in vivo (Arweiler et al., 2002). Therefore, one could hypothesize that there is an advantage vis-à-vis the bacterial load in the use of EMD, or EMD with GTR, compared with GTR alone.

Several studies have been conducted for comparison of the effectiveness of these 2 surgical treatment modalities. Although both techniques demonstrate better results than their baseline and/or control within the groups, no significant differences in pocket probing depth reduction have been seen between the EMD and GTR groups (Sculean et al., 1999c,d, 2001a,d; Pontoriero et al., 1999; Minabe et al., 2002; Windisch et al., 2002). Similarly, no statistically significant differences in terms of clinical attachment gain were noticed following treatment with EMD or GTR (Silvestri et al., 2000). However, the results showed a significant interaction between clinical outcome and baseline clinical attachment level. GTR appeared to provide better results than EMD in terms of % clinical attachment gain in patients with a baseline clinical attachment loss ≥ 9 mm. Conversely, EMD appeared to be better than GTR in patients with a baseline clinical attachment loss < 9 mm (Silvestri et al., 2000).

This pilot study was followed by a multicenter controlled clinical trial with 98 patients in whom the treatment efficacy of EMD was compared with the treatment with a non-resorbable membrane (e-PTFE). Once again, no global advantage of one treatment over the other was demonstrated. However, when a regression analysis was applied to a subset of patients with baseline CAL > 8 mm, the CAL gain following GTR was 0.3 mm higher than that following EMD, an increase that has little clinical significance (Silvestri et al., 2003). The only study to date that did find statistically significant differences between the 2 treatment modalities used a titanium-reinforced e-PTFE membrane in the GTR group (Zucchelli et al., 2002). The clinical attachment level gain and the reduction in probing depth were better following GTR, but increased gingival recession was found in the GTR group when compared with the EMD cohort.

Only one study has compared EMD with GTR combined with a bovine-derived hydroxyapatite xenograft. No significant differences in outcomes were found (Pietruska, 2001).

Histologically, a clear advantage for GTR is evident compared with EMD. Almost all of the GTR-treated defects are characterized by true periodontal regeneration to some degree (Sculean et al., 1999c; Windisch et al., 2002). In contrast, EMD-treated defects are generally characterized by new attachment that is not always followed by bone regeneration.

The clinical improvement obtained following treatment with EMD or GTR does not appear to be transient. Both treatment modalities result in outcomes that have been shown, in one study, to be maintained over a four-year period (Sculean et al., 2001d). Results from controlled clinical studies have shown that the stability of gained clinical attachment following conventional and regenerative periodontal therapy is dependent upon stringent oral hygiene and compliance with a maintenance periodontal care program (Weigel et al., 1995; Cortellini et al., 1996). It may be extrapolated, therefore, that, following treatment with EMD as well, it is imperative that the patient be monitored and kept on high standards of oral hygiene, with regular maintenance visits.

(II.6.2.5) The use of EMD in combination with bone grafts

It is well-known that the outcome of any type of regenerative procedure is strongly dependent upon the available space under the mucoperiosteal flap (Garrett and Bogle, 1993; Wikesjo and Selvig, 1999), and that the stability of the wound under the flap during healing is a crucial factor for periodontal regeneration (Wikesjo and Selvig, 1999). Combining bone grafts or bone substitutes with GTR in the treatment of intrabony defects resolves this problem by providing space maintenance (Guillemin et al., 1993; McClain and Schallhorn, 1993).

One of the limitations inherent in the use of early commercially available EMD was related to its physical handling properties (Mellonig, 1999). The EMD formulation was semi-fluid in consistency and lacked the space-maintenance ability of solid graft materials. Because space maintenance is a desirable physical characteristic of a regenerative material, particularly if bone formation is one of the treatment objectives, it was suggested that a combination of demineralized freeze-dried bone allograft (DFDBA) and EMD be used to overcome problems related to EMD fluidity (Mellonig, 1999).

One of the first studies that evaluated the combination of EMD with bone graft used the nude-mouse model to assess the effect of EMD on the osteoinductive activity of DFDBA. DFDBA that demonstrated osteoinductive activity together...
<table>
<thead>
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<th>Study</th>
<th>Design</th>
<th>Participants</th>
<th>Treatment</th>
<th>Outcomes</th>
<th>Defect Morphology</th>
<th>Re-entry/ Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heijl et al., 1997a</td>
<td>RCT**, multicenter, split-mouth, 2 treatment groups, 36 months' duration</td>
<td>33 patients, 26 completed the study, 26 females; mean age, 43 yrs (33-68); 16 smokers</td>
<td>Control, MWF + PGA; Test, MWF + EMD; SPT intervals, NA</td>
<td>△CAL, △PPD, manual probe with acrylic stent; radiographic bone gain</td>
<td>1 or 2 walls</td>
<td>No</td>
</tr>
<tr>
<td>Zetterström et al., 1997a,b</td>
<td>CT, multicenter, parallel groups, 2 treatment groups, 36 months' duration</td>
<td>140 patients, 66 completed the study, 69 females; mean age, 51 yrs (31-78); 87 smokers</td>
<td>Control, MWF; Test, MWF + EMD (2 sites per patient); SPT intervals, every 2 wks</td>
<td>△CAL, △PPD, manual probe, radiographic bone gain</td>
<td>Intraosseous</td>
<td>No</td>
</tr>
<tr>
<td>Heden et al., 1999a</td>
<td>Case series, 12 months' duration</td>
<td>108 patients, 56 females; mean age, 55.8 ± 12.7 yrs; 31 smokers</td>
<td>EMD; SPT intervals, every 2-4 months</td>
<td>△CAL, △PPD, △REC, manual probe, radiographic bone gain</td>
<td>1, 1+2, 2</td>
<td>2+3, 3 walls</td>
</tr>
<tr>
<td>Pontoriero et al., 1999a</td>
<td>RCT, split-mouth, 2 treatment groups, 12 months' duration</td>
<td>10 out of 40 patients, (25 females, age 32-61 yrs); smokers, NA</td>
<td>Control, PGA; Test, EMD; SPT intervals, every 2 wks</td>
<td>△CAL, △PPD, △REC, manual probe</td>
<td>Intraosseous</td>
<td>No</td>
</tr>
<tr>
<td>Sculean et al., 1999b</td>
<td>Case series, 8 months' duration</td>
<td>28 patients; gender, NA; age, 32-60 yrs; smokers, NA</td>
<td>EMD; SPT intervals, every 2 wks</td>
<td>△CAL, △PPD, △REC, manual probe</td>
<td>2,3 walls</td>
<td>No</td>
</tr>
<tr>
<td>Sculean et al., 1999c</td>
<td>RCT, split-mouth, 2 treatment groups, 8 months' duration</td>
<td>16 patients, 6 females; age, NA; smokers, NA</td>
<td>Control, GTR (Resolut); Test, EMD; SPT intervals, after 1st 2 months, monthly</td>
<td>CAL, PPD, REC, manual probe</td>
<td>1,2,3 walls</td>
<td>No</td>
</tr>
<tr>
<td>Sculean et al., 1999c</td>
<td>RCT, parallel groups, 2 treatment groups, 6 months' duration</td>
<td>14 patients; gender, NA; age, NA; smokers, NA</td>
<td>Control, GTR (Resolut); Test, EMD; SPT intervals, after 1st 2 months, monthly</td>
<td>△CAL, △PPD, △REC, manual probe, histologic findings</td>
<td>Intraosseous</td>
<td>Histology</td>
</tr>
<tr>
<td>Heard et al., 2000a</td>
<td>Case series, 6 months' duration</td>
<td>32 patients, 18 females; mean age, 50 yrs (33-69); 12 smokers</td>
<td>EMD in 2 sites for each patient, separated at least 8 wks; SPT intervals, after 1st 6 wks, every 3 months</td>
<td>△CAL, △PPD, △REC, manual probe</td>
<td>Intraosseous</td>
<td>No</td>
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<tr>
<td>Heden, 2000a</td>
<td>Case series, 12 months' duration</td>
<td>61 patients, 31 females; mean age, 56 ± 12 yrs (18-76); 21 smokers</td>
<td>EMD in some cases utilizing the Mod. Papilla preservation technique; SPT intervals, after 1st 6 wks, every 2-4 months</td>
<td>△CAL, △PPD, manual probe, radiographic bone gain</td>
<td>1,2 walls</td>
<td>No</td>
</tr>
<tr>
<td>Lekovic et al., 2000a</td>
<td>RCT, split-mouth, 2 treatment groups, 6 months' duration</td>
<td>21 patients, 13 females; mean age, 39 ± 1 yrs; 12 smokers</td>
<td>Control, EMD; Test, EMD + BDX (Bio-Oss); SPT intervals, after 1st month, every 3 months</td>
<td>△CAL, △PPD, △REC, manual probe with acrylic stent; hard-tissue measurements at re-entry</td>
<td>2,3 walls</td>
<td>Re-entry</td>
</tr>
<tr>
<td>Okuda et al., 2000a</td>
<td>RCT, split-mouth, 2 treatment groups, 12 months' duration</td>
<td>16 patients, 8 females; mean age, 56 ± 11 yrs; smokers, none</td>
<td>Control, PGA; Test, EMD; SPT intervals, after 1st 6 wks, monthly</td>
<td>△CAL, △PPD, △REC, manual pressure-sensitive probe with acrylic stent</td>
<td>1,2,3 walls</td>
<td>No</td>
</tr>
<tr>
<td>Parashis and Tsiklakis, 2000a</td>
<td>Case series, 12 months' duration</td>
<td>15 patients, 9 females; age, 38-67 yrs; 3 smokers</td>
<td>EMD; SPT intervals, after 1st 2 months, monthly for 4 months, then every 3 months</td>
<td>△CAL, △PPD, △REC, manual probe</td>
<td>2,3 walls</td>
<td>No</td>
</tr>
<tr>
<td>Parodi et al., 2000a</td>
<td>Case series, 12 months' duration</td>
<td>21 patients, 10 females; mean age, 53 yrs (41-70); 7 smokers</td>
<td>EMD; SPT intervals, monthly</td>
<td>CAL, △PPD, REC, manual probe, histologic findings</td>
<td>1,2 walls</td>
<td>Histology (in 2 cases); re-entry (in 13 cases)</td>
</tr>
<tr>
<td>Silvestri et al., 2000a,b,c</td>
<td>RCT, parallel groups, 3 treatment groups, 12 months' duration</td>
<td>30 patients, 19 females; mean age, 43.4-48.7 yrs in each group; smokers, none</td>
<td>Control 1, MWF; Control 2, GTR (e-PTFE); Test, EMD; SPT intervals, after 1st 8 wks, every 3 months</td>
<td>△CAL, △PPD, manual pressure-sensitive probe</td>
<td>Intraosseous</td>
<td>No</td>
</tr>
<tr>
<td>Yukna and Mellonig, 2000a</td>
<td>Case series, multicenter, 6 months' duration</td>
<td>8 patients, 3 females; mean age, 52.5 yrs (38-67); 3 smokers</td>
<td>EMD; SPT intervals, every 2-4 months</td>
<td>△CAL, △PPD, △REC, probing technique, NA; histologic findings</td>
<td>1+2, 2, 1+3</td>
<td>Histology</td>
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<tr>
<td>Study</td>
<td>Design</td>
<td>Participants</td>
<td>Treatment</td>
<td>Outcomes</td>
<td>Defect Morphology</td>
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<td>Brathall et al., 2001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>RCT, multicenter, split-mouth, 2 treatment groups, 16 months' duration</td>
<td>88 patients (85 completed), 39 females; mean age, 50 ± 9.6 yrs; smokers, NA</td>
<td>Control, EMD, Test, EMD gel; SPT intervals, after 1st 2-3 wks, at 4, 6, and 12 months</td>
<td>∆CAL, PPD, ∆REC, manual probe, radiographic bone gain</td>
<td>1,2,3 walls</td>
<td>No</td>
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<tr>
<td>Camargo et al., 2001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>RCT, split-mouth, 2 treatment groups, 6 months' duration</td>
<td>24 patients; gender, NA; mean age, 42 yrs ± 7 mos; 18 smokers</td>
<td>Control, OFD; Test, EMD + BDX (Bio-Oss); SPT intervals, after 1st 4 wks, at 3 and 6 months post-surgery</td>
<td>∆CAL, ∆PPD, ∆REC, manual probe with acrylic stent, hard-tissue measurements at re-entry</td>
<td>2,3 walls</td>
<td>Re-entry</td>
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<tr>
<td>Froum et al., 2001&lt;sup&gt;c&lt;/sup&gt;</td>
<td>RCT, split-mouth, 2 treatment groups, 12 months' duration</td>
<td>23 patients; gender, NA; mean age, 45.5 ± 15.9 yrs (19-71); 3 smokers</td>
<td>Control, OFD; Test, EMD; SPT intervals, after 1st 6 wks, monthly</td>
<td>∆CAL, ∆PPD, ∆REC, manual pressure-sensitive probe with acrylic stent, hard-tissue measurements at re-entry</td>
<td>2,3 walls</td>
<td>Re-entry</td>
</tr>
<tr>
<td>Lekovic et al., 2001&lt;sup&gt;d&lt;/sup&gt;</td>
<td>RCT, split-mouth, 2 treatment groups, 6 months' duration</td>
<td>18 patients, 8 females; mean age, 42 ± 12 yrs; 12 smokers</td>
<td>Control, OFD; Test, EMD + BDX (Bio-Oss); SPT intervals, after 1st 4 wks, at 3 and 6 months post-surgery</td>
<td>∆CAL, ∆PPD, ∆REC, manual probe with acrylic stent; hard-tissue measurements at re-entry</td>
<td>2,3 walls</td>
<td>Re-entry</td>
</tr>
<tr>
<td>Lekovic et al., 2001&lt;sup&gt;e&lt;/sup&gt;</td>
<td>RCT, split-mouth, 2 treatment groups, 6 months' duration</td>
<td>23 patients, 10 females; mean age, 45 ± 12 yrs; 9 smokers</td>
<td>Control, EMD + BDX (Bio-Oss); Test, AFFS + BDX (Bio-Oss); SPT intervals, after 1st 4 wks, at 3 and 6 months post-surgery</td>
<td>∆CAL, ∆PPD, ∆REC, manual probe with acrylic stent; hard-tissue measurements at re-entry</td>
<td>2,3 walls</td>
<td>Re-entry</td>
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<tr>
<td>Okuda et al., 2001&lt;sup&gt;f&lt;/sup&gt;</td>
<td>RCT, split-mouth, 2 treatment groups, 12 wks' duration</td>
<td>16 patients; gender, NA; age, NA; smokers, none</td>
<td>Control, PGA; Test, EMD; SPT intervals, after 1st 6 wks, monthly</td>
<td>CAL, PPD; probing technique, NA; gingival fluid evaluations</td>
<td>Intraosseous</td>
<td>No</td>
</tr>
<tr>
<td>Pietruska et al., 2001&lt;sup&gt;g&lt;/sup&gt;</td>
<td>RCT, parallel groups, 2 treatment groups, 12 months' duration</td>
<td>24 patients, 8 females; age, 28-54 yrs; smokers, NA</td>
<td>Control, BDX (Bio-Oss) + GTR (Bio-Gide); Test, EMD; SPT intervals, after 1st 6 wks, bi-monthly</td>
<td>CAL, PPD, REC, manual probe</td>
<td>2,3 walls</td>
<td>No</td>
</tr>
<tr>
<td>Sculean et al., 2001&lt;sup&gt;h&lt;/sup&gt;</td>
<td>RCT, parallel groups, 4 treatment groups, 12 months' duration</td>
<td>56 patients, 32 females; mean age, 36 ± 12.4 yrs (29-68); smokers, NA</td>
<td>Control, OFD; Test 1, GTR (Resolut); Test 2, EMD; Test 3, EMD + GTR (Resolut); SPT intervals, after 1st 2 months, monthly</td>
<td>∆CAL, ∆PPD, ∆REC, manual probe</td>
<td>1+2, 2, 3 walls</td>
<td>No</td>
</tr>
<tr>
<td>Sculean et al., 2001&lt;sup&gt;i&lt;/sup&gt;</td>
<td>RCT, split-mouth, 2 treatment groups, 4 years' duration</td>
<td>16 patients, 12 completed the study, 6 females; mean age, 45 ± 8.5 yrs (37-55); smokers, NA</td>
<td>Control, GTR (Resolut); Test, EMD; SPT intervals, after 1st 2 months, monthly. After 1 yr, every 3 months.</td>
<td>CAL, PPD, REC, manual probe</td>
<td>1,2,3 walls</td>
<td>No</td>
</tr>
<tr>
<td>Sculean et al., 2001&lt;sup&gt;j&lt;/sup&gt;</td>
<td>RCT, parallel groups, 2 treatment groups, 12 months' duration</td>
<td>34 patients, 22 females; age, NA; 7 smokers</td>
<td>Control, EMD; Test, EMD + systemic antibiotics post-op; SPT intervals, after 1st 2 months, monthly</td>
<td>CAL, PPD, REC, manual probe</td>
<td>1,2,3 walls</td>
<td>No</td>
</tr>
<tr>
<td>Cardaropoli and Leonhardt, 2002&lt;sup&gt;k&lt;/sup&gt;</td>
<td>Case series, 12 months' duration</td>
<td>7 patients, 1 female; mean age, 47.6 yrs (35-60); 3 smokers</td>
<td>EMD in deep intrabony lesions (PD &gt; 8 mm); SPT intervals, every 2 wks for the first 6 months, then every month</td>
<td>∆CAL, ∆PPD, ∆REC, manual probe, radiographic bone gain</td>
<td>1, 1-2, 2 walls</td>
<td>No</td>
</tr>
<tr>
<td>Minabe et al., 2002&lt;sup&gt;l&lt;/sup&gt;</td>
<td>RCT, multicenter, parallel groups, 3 treatment groups, 12 months' duration</td>
<td>61 patients, 33 females; age, 38-62 yrs; 12 smokers</td>
<td>Control, GTR (Tissue Guide); Test 1, EMD; Test 2, EMD + GTR (Tissue Guide); SPT intervals, after 1st 6 wks, monthly</td>
<td>CAL, PPD; manual probe</td>
<td>1,2,3 walls</td>
<td>No</td>
</tr>
<tr>
<td>Rosen and Reynolds, 2002</td>
<td>Case series, 2 treatment groups, 6 months' duration</td>
<td>22 patients, 8 females; mean age, 53.1 yrs; smokers, none</td>
<td>Control, EMD + DFBDA + GTR (Atrisorb); Test, EMD + DFBDA + GTR (Atrisorb); SPT intervals, after 1st 2 months, monthly</td>
<td>∆CAL, ∆PPD, manual probe</td>
<td>1, 2, 1+2 walls</td>
<td>Re-entry in several sites</td>
</tr>
<tr>
<td>Scheyer et al., 2002&lt;sup&gt;m&lt;/sup&gt;</td>
<td>RCT, split-mouth, 2 treatment groups, 6 months' duration</td>
<td>17 patients, 11 females; age, 32-73 yrs; 3 smokers</td>
<td>Control, BDX (Bio-Oss); Test, EMD + BDX; SPT intervals, after 1st 2 months, bi-monthly</td>
<td>∆CAL, ∆PPD, ∆REC, manual probe, hard-tissue measurements at re-entry</td>
<td>2, 2+3 walls</td>
<td>Re-entry</td>
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continued on next page
with EMD above a threshold dose (4 mg) resulted in enhanced bone induction, an area of new bone (ossicle area including new marrow), and an area of cortical bone (DFDBA plus bridging new bone) compared with DFDBA, with limited osteoinductive activity, or active DFDBA with EMD in a sub-minimal dose (Boyan et al., 2000). In view of these results, and the fact that there is now a product consisting of EMD and an alloplast material (bioactive glass, BG) (Emdogain® TS, Biora), the studies that evaluated the therapeutic effect of EMD in combination with different bone replacement materials must be reviewed. Currently, several studies have been published regarding the use of EMD combined with bovine-derived bone
(II.6.2.5.1) EMD and xenograft or alloplastic materials

BDX appears to have the ability to augment the effect of EMD in reducing probing depth, improving clinical attachment level, and promoting defect fill when compared with EMD alone or OFD in the treatment of intrabony periodontal defects (Lekovic et al., 2000; Camargo et al., 2001). Similar results were obtained when EMD or autologous fibrinogen/fibronectin system (AFFS) was used in combination with BDX (Lekovic et al., 2001b). Moreover, adding a membrane to the combined treatment of BDX and EMD may even enhance these results (Lekovic et al., 2001a). It should be noted that these studies are limited to a short follow-up period (6 months), and that the clinical results related to the use of EMD alone as control are poor in terms of clinical attachment level gain and probing depth reduction, compared with other published data (Tables 2 and 3).

Other studies have reported conflicting results when EMD and BDX were used in combination compared with BDX alone. No statistically significant differences were found in any of the examined clinical parameters between the 2 treatment groups (Scheyer et al., 2002; Sculean et al., 2002c). Similarly, when EMD was used together with the alloplast material BG, the response was comparable with that for BG alone (Sculean et al., 2002b). EMD plus BDX did not differ from EMD alone with respect to mean reduction in probing depth or in mean gain of attachment. However, gingival recession following treatment with EMD alone was greater than that with the combined therapy, and the bone gain as measured clinically at re-entry surgery was smaller (Velasquez-Plata et al., 2002). A case report study that evaluated the clinical and histological results 6 months following treatment of intrabony defects with BDX alone or EMD+BDX demonstrated a gain in clinical attachment, histologic evidence of new connective tissue attachment, and new bone with both treatment modalities (Sculean et al., 2003a).

(II.6.2.5.2) EMD and allograft bone

The combination of FDBA or DFDBA with EMD, followed by the application of an absorbable polymer barrier of poly(DL-lactide), was studied in 22 patients (Rosen and Reynolds, 2002). Similar clinical results were demonstrated for both therapies (Tables 1 and 2).

The effectiveness of EMD + allograft was not tested directly against that of EMD + BDX in any controlled clinical trial. However, the inclusion of allograft appears to yield a better clinical outcome compared with EMD combined with BDX when the results of the EMD + allograft study (Rosen and Reynolds, 2002) were compared with those from the studies utilizing the EMD + BDX combination (Table 2).

(II.6.2.6) Factors that determine EMD outcomes

Several factors were evaluated in the aforementioned studies for their influence on the clinical or radiographic results obtained following treatment with EMD.

(II.6.2.6.1) Time

Following treatment with EMD, there is a continuous radiographic bone gain over time (through an observation period of 36 months) (Heijl et al., 1997). The control sites (placebo) showed a mean loss of radiographic bone for the entire observation period. The clinical results, however, changed significantly and were maintained from 8 months post-treatment with EMD throughout the observation period.

(II.6.2.6.2) Baseline probing pocket depth/clinical attachment loss

Most of the studies that evaluated the relationship between the initial probing depth and/or clinical attachment level found a positive correlation between these parameters with the clinical attachment level gain and/or probing depth reduction (Zetterström et al., 1997; Heden et al., 1999; Pontoriero et al., 1999; Parodi et al., 2000; Brathall et al., 2001; Tonetti et al., 2002; Trombelli et al., 2002; Zucchelli et al., 2002; Silvestri et al., 2003). One study could not demonstrate any relationship between the baseline attachment loss and the clinical attachment gain (Silvestri et al., 2000). In addition, there was no relationship between defect depth and histologic results (Yukna and Mellonig, 2000).

(II.6.2.6.3) Anatomic location

Two studies assessed the influence of the anatomic location of treatment (mandible or maxilla) on the results obtained following treatment with EMD. There was no agreement in the results of these two studies (Heijl et al., 1997; Brathall et al., 2001).

(II.6.2.6.4) Defect morphology

Conflicting results were obtained regarding the influence of defect anatomy (number of defect walls and its intrabony component). While several studies found a correlation between the number of defect walls and the regenerative success with EMD (Heijl et al., 1997; Tonetti et al., 2002; Silvestri et al., 2003), other studies could not demonstrate such an effect (Heden, 2000; Brathall et al., 2001; Minabe et al., 2002).

(II.6.2.6.5) Defect corticalization

One study found that markedly corticalized and very cancellous bleeding intrabony defects had significantly lower CAL gain than defects with a regular cribiform bony lining (Tonetti et al., 2002).

(II.6.2.6.6) Smoking

Better treatment outcomes were found for non-smokers than for smokers (Heijl et al., 1997; Heden et al., 1999; Heden, 2000; Brathall et al., 2001; Tonetti et al., 2002; Zucchelli et al., 2002). In contrast, some studies could not find significant differences in the treatment outcomes between smokers and non-smokers (Parodi et al., 2000; Sculean et al., 2002b; Trombelli et al., 2002).

(II.6.2.6.7) Gender

One study that evaluated whether gender has any effect found no statistically significant differences in CAL gain between males and females (Parodi et al., 2000).

(II.6.2.6.8) Age

Age was found to have no influence on CAL gain or radiographic bone gain (Brathall et al., 2001).

(II.6.2.6.9) Soft-tissue dimensions and manipulation

CAL gain was significantly influenced by the amount of pre-
<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>N</th>
<th>Initial Probing Depth</th>
<th>Residual Probing Depth</th>
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<th>Initial CAL</th>
<th>Gain</th>
<th>Initial Recal</th>
<th>Change</th>
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<th>Defect Filling</th>
<th>Cristal Bone Resorption</th>
<th>Bone Gain/Resolution</th>
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<td>Heijl et al., 1997</td>
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<td>27</td>
<td>7.8 ± 1.4</td>
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<td>2.3 ± 1.1</td>
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<td>27</td>
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<td>4.0 ± 2.4</td>
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<td>1.4 ± 1.3</td>
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<td>Froum et al., 2002</td>
<td>OFD</td>
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<td>1.42 ± 0.3</td>
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<td>OFD</td>
<td>12</td>
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<td>3.4 ± 0.9</td>
<td>9.3 ± 1.32</td>
<td>5.8 ± 1.16</td>
<td>1.7 ± 0.58</td>
<td>3.0 ± 0.98</td>
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<td>Sculean et al., 2001</td>
<td>OFD</td>
<td>14</td>
<td>8.6 ± 1.8</td>
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<td>Parodi et al., 2000</td>
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<td>7.1 ± 1.2</td>
<td>4.0 ± 1.1</td>
<td>4.3 ± 1.3</td>
<td>10.0 ± 1.7</td>
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surgical interdental supracrestal soft tissue (Trombelli et al., 2002). It was hypothesized that the presence of thick interden-
tal tissues may have facilitated the flap management and sutur-
ing technique, while maximizing the possibility that primary
closure would be achieved in the interproximal area. In addi-
tion, preservation of the interdental soft tissues may limit the
collapse of the flap into the bone defect. Periosteal incisions did
not influence the treatment outcomes (Tonetti et al., 2002). It was hypothesized that the presence of thick interden-
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ing technique, while maximizing the possibility that primary
closure would be achieved in the interproximal area. In addi-
tion, preservation of the interdental soft tissues may limit the
collapse of the flap into the bone defect. Periosteal incisions did
not influence the treatment outcomes (Tonetti et al., 2002).

(II.6.2.6.11) Bleeding on probing

Bleeding on probing during follow-up examinations adversely
influenced the treatment outcomes (Heden et al., 1999; Heden,
2000; Zucchelli et al., 2002; Silvestri et al., 2003).

(II.6.2.6.10) Plaque control

Early plaque formation (0-4 months) was found to have an
adverse effect on radiographic bone gain (Brathall et al., 2001).
In contrast, plaque accumulation was not found to be a deter-
miming factor for CAL gain (Tonetti et al., 2002), although it
should be noted that the plaque scores in this study were very
low and had a small standard deviation.

This study was followed, and its long-term results were published separately (Sculean et al., 2001a). Therefore, its data were excluded from the meta-analysis.

Some of the data from this study were included in the results of another study (Windisch et al., 2002); therefore, they were excluded from the meta-analysis.

Only the data concerning the results following the EMD group were included in the meta-analysis. (EMD gel group results were excluded from the meta-analysis.)

The standard deviation was calculated based on data from the original article.

This study presents the short-term results of a study by Okuda et al. (1999); therefore, its data were excluded from the meta-analysis.
Post-operative administration of drugs

Systemic administration of antimicrobials (amoxicillin and metronidazole) following surgical placement of EMD did not produce statistically superior probing depth reduction or CAL gain compared with treatment with EMD alone (Sculean et al., 2001c). Similarly, the use of non-steroidal anti-inflammatory drugs (COX-2 inhibitors) following regenerative periodontal surgery with EMD did not result in additional clinical improvements when compared with treatment with EMD alone (Sculean et al., 2003b).

(III) Discussion

For meta-analysis purposes, we pooled the experimental studies and case series reported in the medical literature available through the MEDLINE database through the end of May, 2003. Key words for database search included: EMD, enamel matrix derivative, and Emdogain®. Only clinical trials reported in humans with baseline and final data detailing the standard deviation of the results were eligible for inclusion into the meta-analysis. Case reports were excluded.

The meta-analysis was modified according to a previously published method (Machtei, 2001). Weighted mean changes (WMC) were calculated for the following parameters following treatment of intrabony defects with EMD alone or in combination with bone grafts and/or membranes: probing depth, clinical attachment level, and bone level (clinical and radiographically). A formula was used that accounts not only for each sample’s mean value but also for the standard deviations of the changes and the size of the sample.

The following formula was used:

\[
t = \frac{1}{(SD_1^2/n_1 + SD_2^2/n_2 + \ldots + SD_t^2/n_t)}
\]

Likewise, to determine the weighted standard error (WSE) of the changes for the grouped database, we used the following formula:

\[
WSE = \frac{1}{\sqrt{\frac{W_1^2 + W_2^2 + W_3^2 + \ldots + W_t^2}{n}}}
\]

TABLE 3

| Meta-analysis of the Treatment Parameters—Enamel Matrix Derivative Alone |
|-----------------------------|---------------------------|
| Clinical parameters         | Enamel Matrix Derivative  |
| PD initial                  | No. of Defects | No. of Studies |
| 7.94 ± 0.05                 | 883           | 27            |
| PD residual                 | 3.63 ± 0.04* | 643           | 22           |
| P value (t test)            | 0.000         |               |
| CAL initial                 | 9.4 ± 0.06    | 708           | 23           |
| CAL residual                | 5.82 ± 0.07* | 521           | 19           |
| P value (t test)            | 0.000         |               |
| REC initial                 | 1.31 ± 0.05   | 483           | 18           |
| REC residual                | 2.4 ± 0.06*  | 402           | 15           |
| P value (t test)            | 0.000         |               |
| Re-entry                    |               |               |
| Defect fill                 | 3.78 ± 0.03   | 90            | 3            |
| Crestal bone resorption     | 0.46 ± 0.01   | 90            | 3            |
| Radiographic measurements   |               |               |
| Defect resolution           | 2.02 ± 0.08   | 345           | 7            |
| Bone gain                   | 2.37 ± 0.17   | 78            | 3            |

* PD, probing depth; CAL, clinical attachment level; REC, recession.
* Mean ± SEM.
* P < 0.05, significant difference vs. initial measurement.

The reviewed studies that evaluated the use of EMD in the treatment of intrabony periodontal defects compared with flap debridement or placebo are presented in Tables 1 (characteristics of the studies) and 2 (results of the studies). As mentioned earlier, no statistically significant differences in outcomes were found following the use of Emdogain® or Emdogain® Gel (Bratthall et al., 2001); thus, all the meta-analysis calculations were conducted without separating the studies that used either of these products.

A meta-analysis of the effect of EMD in intrabony defects was performed with 28 studies that included 955 intrabony defects (Table 3). The mean initial probing depth of 7.94 ± 0.05 mm was reduced to 3.63 ± 0.04 mm following treatment with EMD. The mean clinical attachment level changed from 9.4 ± 0.06 mm to 5.82 ± 0.07 mm (p = 0.000). The mean gingival recession increased from 1.31 ± 0.03 mm to 2.4 ± 0.06 mm (p = 0.000). The meta-analysis for the re-entry studies resulted in a mean defect fill of 3.78 ± 0.03 mm and a mean crestal bone resorption of 0.46 ± 0.01 mm. The meta-analysis for the radiographic data resulted in a mean defect resolution of 2.02 ± 0.08 mm and a bone gain of 2.37 ± 0.17 mm.

(III.1) EMD

The meta-analysis results obtained following the treatment with EMD were compared with those obtained following open-flap debridement procedures (Table 4). No significant difference was found in the mean initial probing depth between the EMD group and the OFD group (p = 0.849). However, the probing depth reduction following the treatment with EMD was significantly higher in the EMD group (4.82 ± 0.02 mm vs. 2.59 ± 0.06 mm, p = 0.000). Similar results were obtained for the clinical attachment level (CAL) results. Although no significant difference was found in the mean initial CAL between the EMD group and the OFD group (p = 0.579), better clinical attachment gain was obtained in the EMD group (4.07 ± 0.03 mm vs. 2.55 ± 0.04 mm, p = 0.000).

(III.2) EMD vs. OFD

The meta-analysis results obtained following the treatment with EMD were compared with those obtained following open-flap debridement procedures (Table 4). No significant difference was found in the mean initial probing depth between the EMD group and the OFD group (p = 0.849). However, the probing depth reduction following the treatment with EMD was significantly higher in the EMD group (4.82 ± 0.02 mm vs. 2.59 ± 0.06 mm, p = 0.000). Similar results were obtained for the clinical attachment level (CAL) results. Although no significant difference was found in the mean initial CAL between the EMD group and the OFD group (p = 0.579), better clinical attachment gain was obtained in the EMD group (4.07 ± 0.03 mm vs. 2.55 ± 0.04 mm, p = 0.000).
Considering the calculated change in the free gingival margin location, no differences were noted between the 2 groups at the initial examination \( (p = 0.555) \), and lower recession was found following the treatment with EMD \( (0.77 \pm 0.02 \text{ mm} \ vs. \ 1.37 \pm 0.04 \text{ mm}, \ p = 0.000) \).

### (III.3) EMD vs. GTR

A meta-analysis comparison of the results for EMD and GTR is presented in Table 5. While no statistically significant difference was found between the mean initial probing depth of the 2 groups, the mean probing depth reduction was higher in the GTR group \( (4.82 \pm 0.02 \text{ mm} \ vs. \ 5.24 \pm 0.13 \text{ mm}) \). In contrast, while no statistically significant difference was found between the mean initial CAL, CAL gain was higher for the EMD \( (4.07 \pm 0.03 \text{ mm} \ vs. \ 3.64 \pm 0.12 \text{ mm}) \). As expected, these discrepancies are resolved because of the greater increase in recession in the GTR group \( (0.77 \pm 0.02 \text{ mm} \ vs. \ 0.58 \pm 0.06 \text{ mm}) \).

In other clinical studies, the combined therapy (EMD+GTR) had no clinical advantage over EMD or GTR alone (Sculean et al., 2001a; Minabe et al., 2002). In fact, the comparison of the meta-analysis for the results following the treatment with EMD with those obtained following the treatment with the combined treatment of EMD and GTR demonstrates even better clinical results for the EMD alone in terms of probing depth reduction and CAL gain (Table 6). These results should be considered with extra caution, since only 2 studies were eligible for meta-analysis in the EMD+GTR group.

### (III.4) EMD and XENOGRAFT

The comparison of the meta-analysis for the results obtained following treatment with EMD with those obtained following combined treatment of EMD and BDX is presented in Table 7. A higher initial probing depth and probing depth reduction were found with the EMD group compared with the EMD+BDX group \( (7.94 \pm 0.05 \text{ mm} \ vs. \ 7.32 \pm 0.12 \text{ mm} \ and \ 4.82 \pm 0.02 \text{ mm} \ vs. \ 3.94 \pm 0.11 \text{ mm}) \). The CAL gain was higher for the EMD group \( (4.07 \pm 0.03 \text{ mm} \ vs. \ 3.48 \pm 0.12 \text{ mm}) \), although the initial CAL measurements were not available for comparison. In addition, the increase in recession was higher in the EMD group \( (0.77 \pm 0.02 \text{ mm} \ vs. \ 0.58 \pm 0.06 \text{ mm}) \). Similar results were found when EMD was compared with BDX alone: higher initial probing depth and probing depth reduction in the EMD group, along with higher CAL gain (although not significant) and higher increase in gingival recession (Table 8). This latter comparison should be considered with extra caution, since only 2 studies were eligible for the meta-analysis.

### (IV) Conclusions

EMD seems to be a safe and promising product for the treatment of intrabony periodontal defects. Its modifying effects on cells and extracellular matrix have been extensively studied in
vitro, leading to the hypothesis that EMD affects different cells in the healing environment in specific ways. EMD appears to influence PDL cells, cementoblasts, and osteoblasts positively while inhibiting epithelial cells—a characteristic that is favorable for the re-establishment of the periodontal tissues. EMD may not be capable of controlling the entire regeneration process from inception. Rather, its effects appear limited to enhancement of the process in progress. Another important characteristic of this product is its inhibitory effect on the pathogenic dental plaque.

The in vitro studies suggest that this xenograft material may contribute positively to the results of a periodontal regenerative procedure. This hypothesis is supported by the meta-analysis of the in vivo studies, including animal and human trials, case series, and case reports.

The outcome of EMD use in periodontal regenerative treatment has been evaluated in several clinical trials with a variety of experimental designs. One might expect different and even contradictory results due to erratic findings, sampling errors, different methodologies, small differences, or lack of statistical power. The meta-analysis was performed to overcome this inter-study variation. Meta-analysis is a statistical analysis that combines the results from several prior studies in a way that provides increased power for the quantitative identification of both similarities and differences among them. Studies, rather than the individual patient report, are the primary units of analysis for the determination of an overall average. The most accepted method of pooling the results from these different studies is by weighting the inverse of standard errors, since standard errors represent the size of studies and the homogeneity of each study population. The combined data increase the statistical power, and may help overcome the problem of accepting or rejecting the null hypothesis when there are no differences between the study groups. However, it should be noted that meta-analyses are susceptible to clinical heterogeneity, including the different inclusion criteria of study subjects and eligible teeth, and different examiners and operators. In the present meta-analysis, we decided to include case series studies in view of the somewhat limited number of controlled clinical trials. This was done to enhance the statistical power of the calculation, though one must keep in mind that it may allow for the inclusion of some uncontrolled misleading data that may change the final results.

The present meta-analysis for treatment of intrabony defects with EMD consisted of 28 studies on 955 defects. According to our calculations, a mean probing depth reduction of 4.82 ± 0.02 mm may be anticipated when dealing with defects with a mean initial probing depth of 7.94 ± 0.05 mm. This reduction in pocket probing depth was the sum of mean clinical attachment gain of 4.07 ± 0.03 mm and a mean increase of 0.77 ± 0.02 mm in gingival recession. When one compares these results with those from other available meta-analyses that

### TABLE 6
**Comparison of the Meta-analysis Following Enamel Matrix Derivative vs. Enamel Matrix Derivative and Guided Tissue Regeneration**

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<th>EMD</th>
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<td>PD reduction</td>
<td>3.94 ± 0.11</td>
<td>4.82 ± 0.02</td>
<td>0.000</td>
</tr>
<tr>
<td>CAL initial</td>
<td>NA</td>
<td>9.4 ± 0.06</td>
<td>NA</td>
</tr>
<tr>
<td>CAL gain</td>
<td>NA</td>
<td>4.07 ± 0.03</td>
<td>NA</td>
</tr>
<tr>
<td>REC initial</td>
<td>NA</td>
<td>1.31 ± 0.05</td>
<td>NA</td>
</tr>
<tr>
<td>REC increase</td>
<td>0.58 ± 0.06</td>
<td>0.77 ± 0.02</td>
<td>NA</td>
</tr>
</tbody>
</table>

* EMD, Enamel Matrix Derivative; GTR, guided tissue regeneration; PD, probing depth; CAL, clinical attachment level; REC, recession.
|                | Mean ± SEM.      | Mean ± SEM.       | NA      |

* P < 0.05, significant difference vs. EMD + GTR measurement.

### TABLE 7
**Comparison of the Meta-analysis Following Enamel Matrix Derivative vs. Enamel Matrix Derivative and Bovine-derived Bone Xenograft**

<table>
<thead>
<tr>
<th></th>
<th>EMD</th>
<th>EMD + BDX</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD initial</td>
<td>7.94 ± 0.05</td>
<td>7.32 ± 0.12</td>
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</tr>
<tr>
<td>PD reduction</td>
<td>4.82 ± 0.02</td>
<td>3.94 ± 0.11</td>
<td>0.000</td>
</tr>
<tr>
<td>CAL initial</td>
<td>9.4 ± 0.06</td>
<td>4.07 ± 0.03</td>
<td>NA</td>
</tr>
<tr>
<td>CAL gain</td>
<td>4.07 ± 0.03</td>
<td>3.48 ± 0.12</td>
<td>0.000</td>
</tr>
<tr>
<td>REC initial</td>
<td>1.31 ± 0.05</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>REC increase</td>
<td>0.77 ± 0.02</td>
<td>0.58 ± 0.06</td>
<td>NA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>EMD + BDX</th>
<th>EMD + GTR</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD initial</td>
<td>7.32 ± 0.12</td>
<td>7.94 ± 0.05</td>
<td>0.000</td>
</tr>
<tr>
<td>PD reduction</td>
<td>3.94 ± 0.11</td>
<td>4.82 ± 0.02</td>
<td>0.000</td>
</tr>
<tr>
<td>CAL initial</td>
<td>NA</td>
<td>9.4 ± 0.06</td>
<td>NA</td>
</tr>
<tr>
<td>CAL gain</td>
<td>NA</td>
<td>4.07 ± 0.03</td>
<td>NA</td>
</tr>
<tr>
<td>REC initial</td>
<td>NA</td>
<td>1.31 ± 0.05</td>
<td>NA</td>
</tr>
<tr>
<td>REC increase</td>
<td>0.58 ± 0.06</td>
<td>0.77 ± 0.02</td>
<td>NA</td>
</tr>
</tbody>
</table>

* EMD, Enamel Matrix Derivative; BDX, bovine-derived bone xenograft; PD, probing depth; CAL, clinical attachment level; REC, recession.
|                | Mean ± SEM.      | Mean ± SEM.       | NA      |

* Mean ± SEM.
evaluated the treatment of intrabony periodontal defects with EMD, the results are similar. In their meta-analysis, Kalpidis and Ruben (2002) used 12 controlled clinical studies and reported initial probing depth of 7.9 ± 0.8 mm, residual probing depth of 3.9 ± 0.8 mm, and mean pocket depth reduction of 4.0 ± 0.9 mm. EMD improved CAL from 9.4 ± 1.1 mm at baseline to 6.3 ± 1.0, indicating 3.2 ± 0.9 mm of attachment gain. The mean recession increase was 0.9 ± 0.4 mm.

A comparison of the meta-analysis results with EMD treatment with those following OFD revealed an advantage for EMD treatment in all parameters evaluated. Although our meta-analysis results for CAL gain following OFD differ quite remarkably from those obtained in a meta-analysis evaluating treatment of intrabony defects with OFD (Laurell et al., 1998) (2.55 ± 0.04 mm vs. 1.5 ± 0.6 mm), the advantage of EMD over OFD remained statistically significant (CAL gain of 4.07 ± 0.03 mm vs. 2.55 ± 0.04 mm, respectively).

The present meta-analysis results for GTR treatment are similar to those obtained in a meta-analysis study (Cortellini and Tonetti, 2000) evaluating treatment of intrabony defects by GTR. The weighted clinical attachment gain in this analysis was 3.86 mm compared with 3.64 mm in our analysis. Another meta-analysis reported a smaller CAL gain as compared with our calculation (3.64 ± 0.12 mm vs. 4.2 ± 1.3 mm) (Laurell et al., 1998). However, our mean initial probing depth was smaller (7.79 ± 0.13 mm vs. 8.6 ± 0.9 mm), which may explain the lower CAL gain.

In view of the fact that the meta-analysis revealed higher CAL gain for EMD than GTR, it may be postulated that treatment with EMD is preferred over GTR, especially in those cases where fixation of the membrane and the ability to cover it completely and passively with soft tissue are technically challenging. In addition, membrane application is more time-consuming and technique-sensitive than EMD application. Moreover, trimming, suturing, and tight adaptation of the membrane may be difficult, especially in the posterior areas of the mouth. If a non-resorbable membrane is used, a second surgical procedure is required to remove the membrane. Such procedures may cause gingival recession due to marginal necrosis of the flap, thereby creating the need for an additional surgical procedure aimed at harvesting a connective tissue or free gingival graft to cover the newly formed tissue. Furthermore, GTR requires a very intensive follow-up, especially when suppuration at the surgical site of membrane exposure occurs. In contrast, GTR appeared to provide better results than EMD in terms of percent clinical attachment gain when baseline clinical attachment loss is ≥ 9 mm. Histologically, GTR is more predictable in terms of bone and cementum formation as opposed to EMD, which promotes regeneration to a lesser degree.

Based on only 2 studies, there was no evidence to support the therapeutic efficacy of a combination of GTR and EMD. In fact, the meta-analysis revealed that the combination is inferior when compared with EMD or GTR alone. Neither BDX nor the combined therapy of EMD and BDX was better than EMD alone, based on the meta-analysis. However, definitive conclusions should not be drawn, because higher initial probing depth was calculated for EMD alone, which may contribute to the higher probing depth reduction and CAL gain. In addition, only limited studies evaluated these treatment modalities, and further research is needed.

Promising results were obtained in one study that evaluated the use of EMD with DFDBA or FDBA (Rosen and Reynolds, 2002) (Table 2). These results are in accord with those from an animal study that found that EMD is an osteogenic agent that enhances the osteoinductive potential of DFDBA (Boyan et al., 2000). Once again, further research is needed on the combination of EMD and osteoinductive products.

It should be mentioned that all the case series and clinical human trials quoted in this review were performed and/or supervised by periodontists after verifying that the periodontal infection in the dentition was eliminated. This level of periodontal health was achieved by an initial treatment consisting of patient motivation, oral hygiene instructions, scaling, and root planing. Yet, in view of the variability in clinical results in the studies reviewed, one must consider the previously listed determining factors for the treatment outcomes which may partly explain the inconsistent results. These include the anatomic and biological characteristics of the defect, environmental factors such as smoking, the clinician’s experience and surgical skill, and the patient’s behavior, such as complying with the post-operative instructions for oral hygiene.

It can be concluded that, in spite of the variability of outcomes, a meta-analysis revealed an advantage to the use of EMD in the treatment of periodontal intrabony defects. However, in the future, additional well-controlled randomized long-term clinical trials should be conducted and evaluated. Moreover, in vivo and in vitro studies evaluating the mechanism

### Table 8
Comparison of the Meta-analysis
Following Enamel Matrix Derivative vs. Bovine-derived Bone Xenograft

<table>
<thead>
<tr>
<th></th>
<th>EMD</th>
<th>BDX</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Defects</td>
<td>27</td>
<td>29</td>
<td>0.007</td>
</tr>
<tr>
<td>No. of Studies</td>
<td>883</td>
<td>808</td>
<td>0.002</td>
</tr>
<tr>
<td>PD initial</td>
<td>9.4 ± 0.06</td>
<td>7.38 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>PD reduction</td>
<td>4.07 ± 0.03*</td>
<td>4.5 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>CAL initial</td>
<td>1.31 ± 0.05</td>
<td>0.5 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>CAL gain</td>
<td>4.07 ± 0.03*</td>
<td>4.02 ± 0.31</td>
<td>0.688</td>
</tr>
</tbody>
</table>

* EMD, Enamel Matrix Derivative; BDX, bovine-derived bone xenograft; PD, probing depth; CAL, clinical attachment level; REC, recession. Mean ± SEM. * P < 0.05, significant difference vs. BDX measurement.
of action of Emdogain® and its components should be performed. These studies’ results will enhance our understanding of the role of Enamel Matrix Derivative and its clinical indications and contra-indications during periodontal therapy.

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