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 28 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®, 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 Snead, 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 enamelins (Brookes *et al.*, 1995). Since the enamelins were 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 sheathlin (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 noggin, 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 enhance EMD precipitation, thus exposing the periodontal ligament cells to the re-established protein aggregate and allowing the matrix:cell interactions to take place. The other vehicles that were tested, which were stable at neutral pH, appear to exclude the periodontal ligament cells from exposure to the proteins (Hammarström *et al.*, 1997).

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 PAS (and 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 down-growth 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 permit 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 (Lyngstadaas *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**(AQ)** 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.

(II.6) *IN VIVO* STUDIES

(II.6.1) *In vivo* animal studies

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 characteristic 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 support osteoinduction by demineralized freeze-dried bone allograft (DFDBA). If EMD was implanted together with DFDBA that had limited osteoinduction 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 acellular, 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 acellular 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 (Zatterström *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 (Zatterström *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**(AQ)** *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;**(AQ)** Sculean *et al.*, 1999a; Heard *et al.*, 2000; Parashis and Tsiklakis, 2000; Cardaropoli 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 (Wikesjø 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 $\geq$ 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; Wikesjø and Selvig, 1999), and that the stability of the wound under the flap during healing is a crucial factor for periodontal regeneration (Wikesjø 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 1
Characteristics of Reviewed Studies

Study	Design	Participants	Treatment	Outcomes	Defect Morphology	Re-entry/ Histology
Heijl *et al.*, 1997[a]	RCT**, multicenter, split-mouth, 2 treatment groups, 36 months' duration	33 patients, 26 completed the study, 26 females; mean age, 43 yrs (33-68); 16 smokers	Control, MWF + PGA; Test, MWF + EMD; SPT intervals, NA	ΔCAL, ΔPPD, manual probe with acrylic stent; radiographic bone gain	1 or 2 walls	No
Zetterström(**AQ**) *et al.*, 1997[a,b]	CT, multicenter, parallel groups, 2 treatment groups, 36 months' duration	140 patients, 66 completed the study, 69 females; mean age, 51 yrs (31-78); 87 smokers	Control, MWF; Test, MWF + EMD (2 sites *per* patient); SPT intervals, every 2 wks	ΔCAL, ΔPPD, manual probe, radiographic bone gain	Intraosseous	no
Heden *et al.*, 1999[a]	Case series, 12 months' duration	108 patients, 56 females; mean age, 55.8 ± 12.7 yrs; 31 smokers	EMD; SPT intervals, every 2-4 months	ΔCAL, ΔPPD, ΔREC, manual probe, radiographic bone gain	1, 1+2, 2, 2+3, 3 walls	no
Pontoriero *et al.*, 1999[a]	RCT, split-mouth, 2 treatment groups, 12 months' duration	10 out of 40 patients (25 females, age 32-61 yrs); smokers, NA	Control, PGA; Test, EMD; SPT intervals, every 2 wks	ΔCAL, ΔPPD, ΔREC, manual probe	Intraosseous	no
Sculean *et al.*, 1999a[a]	Case series, 8 months' duration	28 patients; gender, NA; age, 32-60 yrs; smokers, NA	EMD; SPT intervals, every 2 wks	ΔCAL, ΔPPD, ΔREC, manual probe	2,3 walls	no
Sculean *et al.*, 1999d*	RCT, split-mouth, 2 treatment groups, 8 months' duration	16 patients, 6 females; age, NA; smokers, NA	Control, GTR (Resolut); Test, EMD; SPT intervals, after 1st 2 months, monthly	CAL, PPD, REC, manual probe	1,2,3 walls	no
Sculean *et al.*, 1999c	RCT, parallel groups, 2 treatment groups, 6 months' duration	14 patients; gender, NA; age, NA; smokers, NA	Control, GTR (Resolut); Test, EMD; SPT intervals, after 1st 2 months, monthly	ΔCAL, PPD, manual probe, histologic findings	Intraosseous	Histology
Heard *et al.*, 2000[a]	Case series, 6 months' duration	32 patients, 18 females; mean age, 50 yrs (33-69); 12 smokers	EMD in 2 sites for each patient, separated by at least 8 wks; SPT intervals, after 1st 6 wks, every 3 months	ΔCAL, ΔPPD, manual probe	Intraosseous	no
Heden, 2000[a]	Case series, 12 months' duration	61 patients, 31 females; mean age, 56 ± 12 yrs (18-76); 21 smokers	EMD (in some cases utilizing the Mod. Papilla preservation technique); SPT intervals, after 1st 6 wks, every 2-4 months	ΔCAL, ΔPPD, manual probe, radiographic bone gain	1,2 walls	no
Lekovic *et al.*, 2000[a,e]	RCT, split-mouth, 2 treatment groups, 6 months' duration	21 patients, 13 females; mean age, 39 ± 1 yrs; 12 smokers	Control, EMD; Test, EMD + BDX (Bio-Oss); SPT intervals, after 1st month, every 3 months	ΔCAL, ΔPPD, ΔREC, manual probe with acrylic stent; hard-tissue measurements at re-entry	2,3 walls	Re-entry
Okuda *et al.*, 2000[a]	RCT, split-mouth, 2 treatment groups, 12 months' duration	16 patients, 8 females; mean age, 56 ± 11 yrs; smokers, none	Control, PGA; Test, EMD; SPT intervals, after 1st 6 wks, monthly	ΔCAL, ΔPPD, ΔREC, manual pressure-sensitive probe with acrylic stent	1,2,3 walls	no
Parashis and Tsiklakis, 2000[a]	Case series, 12 months' duration	15 patients, 9 females; age, 38-67 yrs; 3 smokers	EMD; SPT intervals, after 1st 2 months, monthly for 4 months, then every 3 months	ΔCAL, ΔPPD, ΔREC, manual probe	2,3 walls	no
Parodi *et al.*, 2000[a]	Case series, 12 months' duration	21 patients, 10 females; mean age, 53 yrs (41-70); 7 smokers	EMD; SPT intervals, monthly	CAL, ΔPPD, REC, manual probe, histologic findings	1,2 walls	Histology (in 2 cases); re-entry (in 13 cases)
Silvestri *et al.*, 2000[a,b,c]	RCT, parallel groups, 3 treatment groups, 12 months' duration	30 patients, 19 females; mean age, 43.4-48.7 yrs in each group; smokers, none	Control 1, MWF; Control 2, GTR (e-PTFE); Test, EMD; SPT intervals, after 1st 8 wks, every 3 months	ΔCAL, ΔPPD, manual pressure-sensitive probe	Intraosseous	no
Yukna and Mellonig, 2000[a]	Case series, multicenter, 6 months' duration	8 patients, 3 females; mean age, 52.5 yrs (38-67); 3 smokers	EMD; SPT intervals, every 2-4 months	ΔCAL, ΔPPD, ΔREC; probing technique, NA; histologic findings	1+2, 2, 1+3, 1+2+3 walls	Histology

Study	Design	Participants	Treatment	Outcomes	Defect Morphology	Re-entry/ Histology
Bratthall *et al.*, 2001[a]	RCT, multicenter, split-mouth, 2 treatment groups, 16 months' duration	88 patients (85 completed), 39 females; mean age, 50 ± 9.6 yrs; smokers, NA	Control, EMD; Test, EMD gel; SPT intervals, after 1st 2-3 wks, at 4, 6, and 12 months	ΔCAL, PPD, ΔREC, manual probe, radiographic bone gain	1,2,3 walls	no
Camargo *et al.*, 2001[b,e]	RCT, split-mouth, 2 treatment groups, 6 months' duration	24 patients; gender, NA; mean age, 42 yrs ± 7 mos; 18 smokers	Control, OFD; Test, EMD + BDX (Bio-Oss); SPT intervals, after 1st 4 wks, at 3 and 6 months post-surgery	ΔCAL, ΔPPD, ΔREC, manual probe with acrylic stent; hard-tissue measurements at re-entry	2,3 walls	Re-entry
Froum *et al.*, 2001[a,b]	RCT, split-mouth, 2 treatment groups, 12 months' duration	23 patients; gender, NA; mean age, 45.5 ± 15.9 yrs (19-71); 3 smokers	Control, OFD; Test, EMD; SPT intervals, after 1st 6 wks, monthly	ΔCAL, ΔPPD, ΔREC, manual pressure-sensitive probe with acrylic stent; hard-tissue measurements at re-entry	Intraosseous	Re-entry
Lekovic *et al.*, 2001a[b]	RCT, split-mouth, 2 treatment groups, 6 months' duration	18 patients, 8 females; mean age, 42 ± 12 yrs; 12 smokers	Control, OFD; Test, EMD + BDX (Bio-Oss) + GTR (Bio-Gide); SPT intervals, after 1st 4 wks, at 3 and 6 months post-surgery	ΔCAL, ΔPPD, ΔREC, manual probe with acrylic stent; hard-tissue measurements at re-entry	2,3 walls	Re-entry
Lekovic *et al.*, 2001b[e]	RCT, split-mouth, 2 treatment groups, 6 months' duration	23 patients, 10 females; mean age, 45 ± 12 yrs; 9 smokers	Control, EMD + BDX (Bio-Oss); Test, AFFS + BDX (Bio-Oss); SPT intervals, after 1st 4 wks, at 3 and 6 months post-surgery	ΔCAL, ΔPPD, ΔREC, manual probe with acrylic stent; hard-tissue measurements at re-entry	2,3 walls	Re-entry
Okuda *et al.*, 2001[x]	RCT, split-mouth, 2 treatment groups, 12 wks' duration	16 patients; gender, NA; age, NA; smokers, none	Control, PGA; Test, EMD; SPT intervals, after 1st 6 wks, monthly	CAL, PPD; probing technique, NA; gingival fluid evaluations	Intraosseous	No
Pietruska *et al.*, 2001[a]	RCT, parallel groups, 2 treatment groups, 12 months' duration	24 patients, 8 females; age, 28-54 yrs; smokers, NA	Control, BDX (Bio-Oss) + GTR (Bio-Gide); Test, EMD; SPT intervals, after 1st 6 wks, bi-monthly	CAL, PPD, REC, manual probe	2,3 walls	No
Sculean *et al.*, 2001a[a,b,c,d]	RCT, parallel groups, 4 treatment groups, 12 months' duration	56 patients, 32 females; mean age, 36 ± 12.4 yrs (29-68); smokers, NA	Control, OFD; Test 1, GTR (Resolut); Test 2, EMD; Test 3, EMD + GTR (Resolut); SPT intervals, after 1st 2 months, monthly	ΔCAL, ΔPPD, ΔREC, manual probe	1+2, 2, 3 walls	no
Sculean *et al.*, 2001d[a,c]	RCT, split-mouth, 2 treatment groups, 4 years' duration	16 patients, 12 completed the study, 6 females; mean age, 45 ± 8.5 yrs (37-55); smokers, NA	Control, GTR (Resolut); Test, EMD; SPT intervals, after 1st 2 months, monthly. After 1 yr, every 3 months.	CAL, PPD, REC, manual probe	1,2,3 walls	no
Sculean *et al.*, 2001c[a]	RCT, parallel groups, 2 treatment groups, 12 months' duration	34 patients, 22 females; age, NA; 7 smokers	Control, EMD; Test, EMD + systemic antibiotics post-op; SPT intervals, after 1st 2 months, monthly	CAL, PPD, REC, manual probe	1,2,3 walls	no
Cardaropoli and Leonhardt, 2002[a]	Case series, 12 months' duration	7 patients, 1 female; mean age, 47.6 yrs (35-60); 3 smokers	EMD in deep intrabony lesions (PD 8 mm); SPT intervals, every 2 wks for the first 6 months, then every month	ΔCAL, ΔPPD, ΔREC, manual probe, radiographic bone gain	1, 1-2, 2 walls	no
Minabe *et al.*, 2002[a,c,d]	RCT, multicenter, parallel groups, 3 treatment groups, 12 months' duration	61 patients, 33 females; age, 38-62 yrs; 12 smokers	Control, GTR (Tissue Guide); Test 1, EMD; Test 2, EMD + GTR (Tissue Guide); SPT intervals, after 1st 6 wks, monthly	CAL, PPD, manual probe	1,2,3 walls	no
Rosen and Reynolds, 2002	Case series, 2 treatment groups, 6 months' duration	22 patients, 8 females; mean age, 53.1 yrs; smokers, none	Control, EMD + DFDBA + GTR (Atrisorb); Test, EMD + FDBA + GTR (Atrisorb); SPT intervals, after 1st 2 months, monthly	ΔCAL, ΔPPD, manual probe	1, 2, 1+2 walls	Re-entry in several sites
Scheyer *et al.*, 2002[e,f]	RCT, split-mouth, 2 treatment groups, 6 months' duration	17 patients, 11 females; age, 32-73 yrs; 3 smokers	Control, BDX (Bio-Oss); Test, EMD + BDX; SPT intervals, after 1st 2 months, bi-monthly	ΔCAL, ΔPPD, ΔREC, manual probe; hard-tissue measurements at re-entry	2, 2+3 walls	Re-entry

continued on next page

Study	Design	Participants	Treatment	Outcomes	Defect Morphology	Re-entry/ Histology
Sculean *et al.*, 2002b	RCT, parallel groups, 2 treatment groups, 12 months' duration	28 patients, 15 females; age, NA; 7 smokers	Control, Bioactive Glass (PerioGlas); Test, EMD + Bioactive Glass (PerioGlas); SPT intervals, after 1st 2 months, monthly	CAL, PPD, REC, manual probe	1+2, 2, 3 walls	no
Sculean *et al.*, 2002c[e,f]	RCT, parallel groups, 2 treatment groups, 12 months' duration	24 patients, 14 females; age, NA; 5 smokers	Control, BDX (Bio-Oss); Test, EMD + BDX; SPT intervals, after 1st 2 months, monthly	ΔCAL, ΔPPD, ΔREC, manual probe	1,2,3 walls	no
Tonetti *et al.*, 2002[a,b]	RCT, multicenter, parallel groups, 2 treatment groups, 12 months' duration	172 patients, 166 completed the study, 95 females; mean age, 48 ± 9 yrs; 64 smokers	Control, OFD (PP); Test, EMD (PP); SPT intervals, after 1st 6 wks, every 3 months	ΔCAL, ΔPPD, ΔREC, manual pressure-sensitive probe	1,2,3 walls	no
Trombelli *et al.*, 2002[a]	Case series, 9-12 months' duration (mean, 11.5 ± 0.9)	35 patients, 23 females; mean age, 44.5 yrs (28-61); 11 smokers	EMD with supracrestal soft-tissue preservation (using one of 4 different techniques according to the clinical situation); SPT intervals, monthly	ΔCAL, ΔPPD, ΔREC, manual pressure-sensitive probe, radiographic bone gain	Intraosseous	no
Velasques-Plata **(AQ)** *et al.*, 2002[a,e]	RCT, split-mouth, 2 treatment groups, 6-8 months' duration	16 patients, 9 females; age, 36-65 yrs; 4 smokers	Control, EMD; Test, EMD + BDX (Bio-Oss); SPT intervals, after 1st month, every 3 months	ΔCAL, ΔPPD, ΔREC, manual probe; hard-tissue measurements at re-entry	2+3, 3 walls	Re-entry
Windisch *et al.*, 2002[β,a,c]	RCT, parallel groups/ split-mouth, 2 treatment groups, 6 months' duration	12 patients, 8 females; mean age, 42 ± 5.1 yrs (35-54); 3 smokers	Control, GTR (Resolut); Test, EMD; SPT intervals, every 2 months	ΔCAL, ΔPPD, manual probe, radiographic bone gain, histologic findings	1,2,3 walls	Histology
Zucchelli *et al.*, 2002[a,b,c]	RCT, parallel groups, 3 treatment groups, 12 months' duration	90 patients, 49 females; mean age, 48.2 ± 7.4 yrs (30-61); 34 smokers	Control, OFD (SPP); Test 1, GTR (titanium-reinforced e-PTFE) + SPP; Test 2, EMD (SPP); SPT intervals, after 1st 11 wks, monthly	ΔCAL, ΔPPD, ΔREC, manual pressure-sensitive probe	Intraosseous	no
Sculean *et al.*, 2003b[a]	RCT, parallel groups, 2 treatment groups, 6 months' duration	22 patients, 17 females; age, NA; 3 smokers	Control, EMD; Test, EMD + systemic NSAID post-op; SPT intervals, after 1st 3 months, monthly	CAL, PPD, REC, manual probe	Intraosseous	no
Silvestri *et al.*, 2003[a,c]	RCT, multicenter, parallel groups, 2 treatment groups, 12 months' duration	98 patients, 53 females; mean age, 48.7 yrs; 37 smokers	Control, GTR (ePTFE) (PP or MPP); Test, EMD (PP or MPP); SPT intervals, after 1st 8 wks, every 3 months	ΔCAL, ΔPPD, manual pressure-sensitive probe	Intraosseous	no

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

** RCT, randomized clinical trial; CT, clinical trial; MWF, modified Widman flap; PGA, propylene glycol alginate; EMD, enamel matrix derivative; SPT, supportive periodontal therapy; NA, non-available; r, difference between initial and residual values; CAL, clinical attachment level; PPD, pocket probing depth; REC, gingival recession; GTR, guided tissue regeneration; BDX, bovine-derived bone xenograft; OFD, open-flap debridement; AFFS, autologous fibrinogen/fibronectin system; DFDBA, demineralized freeze-dry bone allograft; FDBA, mineralized freeze-dried bone allograft; BG, bioactive glass; PP, papilla preservation; SPP, simplified papilla preservation; COX2, cyclo-oxygenase-2.

χ This study presents the short-term results of Okuda *et al.*, 2000; therefore, its data were excluded from the meta-analysis.

β Some of the findings from this study have been reported previously (Sculean *et al.*, 1999c).

a Study that was included in the meta-analysis regarding the treatment with EMD alone.

b Study that was included in the meta-analysis regarding OFD alone.

c Study that was included in the meta-analysis regarding the treatment with GTR.

d Study that was included in the meta-analysis regarding the treatment with EMD combined with GTR.

e Study that was included in the meta-analysis regarding the treatment with EMD combined with BDX.

f Study that was included in the meta-analysis regarding the treatment with BDX alone.

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

xenograft (BDX) (Lekovic *et al.*, 2000, 2001a,b; Camargo *et al.*, 2001; Scheyer *et al.*, 2002; Sculean *et al.*, 2002c, 2003a; Velasquez-Plata *et al.*, 2002), alloplastic synthetic bone graft (BG) (Sculean *et al.*, 2002b), and demineralized or mineralized freeze-dried bone allografts (DFDBA/FDBA) (Rosen and Reynolds, 2002).

(II.6.2.5.1) EMD and xenograft or alloplast 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)**.(AQ)**

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**(AQ)** *et al.*, 1997; Heden *et al.*, 1999; Pontoriero *et al.*, 1999; Parodi *et al.*, 2000; Bratthall *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; Bratthall *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**(AQ)** 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; Bratthall *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**(AQ)** 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; Bratthall *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 (Bratthall *et al.*, 2001).

(II.6.2.6.9) Soft-tissue dimensions and manipulation

CAL gain was significantly influenced by the amount of pre-

TABLE 2
Clinical and Radiographic Parameters by Study

Study	Treatment	N	Probing Depth			CAL[b]			REC			Re-entry Results		Radiographic
			Initial	Residual	Reduction	Initial	Residual	Gain	Initial	Residual	Change	Defect Fill	Crestal Bone Resorption	Bone Gain/ Resolution [v]
Heijl *et al.*, 1997	Placebo	27	7.8 ± 1.4[a]	5.2 ± 1.5	2.3 ± 1.1	9.3 ± 2.0	7.1 ± 2.2	1.7 ± 1.3						0 ± 0.7
	EMD	27	7.8 ± 1.1	4.6 ± 1.0	3.1 ± 1.0	9.4 ± 1.5	7.1 ± 1.8	2.2 ± 1.1						2.6 ± 1.7
Zetterström (AQ) *et al.*, 1997	MWF	21	7.4 ± 1.2		3.2 ± 2.0		8.7 ± 1.9		2.2 ± 1.4					0 ± 1.1
	EMD	45	7.4 ± 1.2		3.8 ± 1.8		8.7 ± 1.7		2.9 ± 1.7					2.4 ± 1.4
Heden *et al.*, 1999	EMD	145	8.6 ± 2.14	3.4 ± 1.21	5.2 ± 2.38	10.2 ± 2.23	5.5 ± 2.29	4.6 ± 2.13	1.5 ± 1.6	2.2 ± 2.48	0.6 ± 2.39	2.9 ± 2.1[v]		
Pontoriero *et al.*, 1999	Placebo	10	7.9 ± 1.4	4.4 ± 1.0		8.6 ± 1.3	6.8 ± 1.1		0.7 ± 0.7	2.4 ± 0.7				
	EMD	10	8.0 ± 1.1	3.6 ± 1.0		9.1 ± 1.0	6.1 ± 1.0		0.8 ± 0.6	2.5 ± 0.5				
Sculean *et al.*, 1999a	EMD	32	8.7 ± 1.5	4.3 ± 1.6	4.47 ± 1.59*	10.6 ± 1.9	7.6 ± 1.8	3.0 ± 1.5	1.8 ± 1.2	3.3 ± 0.9	1.47 ± 0.92*			
Sculean *et al.*, 1999d[x]	GTR	16	8.3 ± 1.7	4.3 ± 0.7		10.1 ± 1.9	7.1 ± 1.7		1.8 ± 1.5	2.9 ± 1.5				
	EMD	16	8.1 ± 1.7	4.3 ± 1.2		10.3 ± 1.8	7.2 ± 1.2		2.1 ± 1.3	2.9 ± 1.1				
Sculean *et al.*, 1999c[w]	GTR	7	11.4 ± 2.2	5.6 ± 1.3		13.3 ± 2.3	10.1 ± 1.5	3.6 ± 1.7						
	EMD	7	11.3 ± 1.8	5.6 ± 1.3		12.1 ± 2.0	9.1 ± 1.5	3.2 ± 1.2						
Heard *et al.*, 2000	EMD	64	7.1 ± 1.4	3.3 ± 0.9	3.8 ± 1.5	7.4 ± 1.7	4.7 ± 1.2	2.8 ± 1.7						
Heden, 2000	EMD	72			4.7 ± 2.1			4.2 ± 1.9						
Lekovic *et al.*, 2000[ψ]	EMD	21	7.16 ± 1.2	5.31 ± 1.22	1.85 ± 1.38			1.75 ± 1.37			1.22 ± 1.28	1.41 ± 1.19		
	EMD + BDX	21	7.18 ± 1.28	3.82 ± 1.18	3.36 ± 1.35			3.11 ± 1.39			1.29 ± 1.24	3.74 ± 1.38	0.51 ± 0.69	
	0.44 ± 0.82													
Okuda *et al.*, 2000	Placebo	18	6.22 ± 0.73	4.00 ± 1.03	2.22 ± 0.81	6.83 ± 1.2	6.00 ± 1.28	0.83 ± 0.86	0.61 ± 0.98	1.83 ± 1.15				
	EMD	18	6.33 ± 0.91	3.39 ± 0.85	3.00 ± 0.97	6.72 ± 1.13	4.94 ± 1.00	1.72 ± 1.07	0.39 ± 0.78	1.61 ± 1.09	1.22 ± 0.88			
1.22 ± 0.16														
Parashis and Tsiklakis, 2000	EMD	25	8.4 ± 1.5	4.0 ± 1.1	4.4 ± 1.3	10.2 ± 1.3	6.6 ± 1.2	3.6 ± 1.2	1.8 ± 1.2	2.6 ± 1.2	0.8 ± 0.8			
Parodi *et al.*, 2000	EMD	21	8.09 ± 2.12	3.19 ± 1.47	4.9 ± 1.0	10.38 ± 2.38	6.95 ± 1.83		2.29 ± 1.38	3.76 ± 1.76				
Silvestri *et al.*, 2000	MWF	10	7.7 ± 1.8		1.4 ± 1.3	8.7 ± 2.1		1.2 ± 1.0	1.0 ± 1.1					
	GTR	10	8.1 ± 1.0		5.9 ± 1.1	9.2 ± 1.8		4.8 ± 2.1	1.4 ± 1.1					
	EMD	10	7.7 ± 2.2		4.8 ± 1.6	9.1 ± 3.2		4.5 ± 1.6	1.3 ± 1.5					
Yukna and Mellonig, 2000	EMD	10	7.6 ± 1.43*	3.7 ± 1.49*	3.9 ± 1.66*	10 ± 2.16*	7.6 ± 3.27*	2.4 ± 2.22*	2.4 ± 1.35*	4.0 ± 2.4*	1.6 ± 1.71*			
Bratthall *et al.*, 2001[β]	EMD	85	7.8 ± 1.5	4.2 ± 1.44				2.9 ± 1.57			1.0 ± 1.01			1.0 ± 1.01[v]
	EMD gel	85	7.8 ± 1.69	4.1 ± 1.35				2.7 ± 1.34			0.9 ± 1.06			1.0 ± 1.13[v]
Camargo *et al.*, 2001[ψ]	OFD	24	7.02 ± 1.32	5.48 ± 1.31	1.54 ± 1.34			1.42 ± 1.30			1.21 ± 1.28	1.04 ± 1.06		
	EMD + BDX	24	7.26 ± 1.36	3.44 ± 1.22	3.82 ± 1.38			3.41 ± 1.34			1.28 ± 1.24	3.71 ± 1.51	0.46 ± 0.78	
0.40 ± 0.76														
Froum *et al.*, 2001	OFD	31	7.32 ± 1.48		2.24 ± 0.38[ϕ]			2.75 ± 0.39[ϕ]			1.29 ± 0.31[ϕ]	1.47 ± 0.3f	1.29 ± 0.14	
	EMD	53	7.99 ± 1.46		4.94 ± 0.19[ϕ]			4.26 ± 0.23[ϕ]			0.61 ± 0.15[ϕ]	3.83 ± 0.25[ϕ]	0.46 ± 0.1	
Lekovic *et al.*, 2001a[ψ]	OFD	18	8.43 ± 1.71	5.53 ± 1.12	2.90 ± 0.91			1.48 ± 0.78			1.57 ± 0.34			
	EMD + BDX + GTR	18	8.32 ± 1.87	3.58 ± 0.72	4.74 ± 1.47			3.78 ± 1.14			1.42 ± 0.31			
1.67 ± 0.90	1.16 ± 0.85													
4.81 ± 1.37	0.90 ± 0.83													
Lekovic *et al.*, 2001b[ψ]	EMD + BDX	23	6.49 ± 1.91	3.43 ± 1.02	3.06 ± 1.74			2.86 ± 1.90			0.56 ± 0.48	2.76 ± 0.72		
	AFFS + BDX	23	6.12 ± 1.76	3.33 ± 1.07	2.79 ± 1.70			2.84 ± 1.76			0.52 ± 0.50	2.82 ± 0.68	0.51 ± 0.69	
0.44 ± 0.82														
Okuda *et al.*, 2001[Δ]	PGA	18	6.22 ± 0.73	4.28 ± 0.83		6.83 ± 1.20	6.22 ± 1.00							
	EMD	18	6.33 ± 0.91	3.61 ± 0.98		6.73 ± 1.13	5.50 ± 1.04							
Pietruska *et al.*, 2001	BDX + GTR	12	7.8 ± 1.22	3.4 ± 0.9		9.3 ± 1.32	5.8 ± 1.16		1.7 ± 0.58	3.0 ± 0.98				
	EMD	12	8.0 ± 2.22	4.0 ± 2.2		9.8 ± 3.01	6.8 ± 3.74		1.8 ± 0.73	3.2 ± 1.39				
Sculean *et al.*, 2001a	OFD	14	8.6 ± 1.8	4.9 ± 1.8	3.7 ± 1.4	10.1 ± 1.6	8.4 ± 1.7	1.7 ± 1.5	1.8 ± 1.1	3.5 ± 1.3	1.7 ± 1.1			
	GTR	14	8.4 ± 1.7	4.2 ± 0.7	4.2 ± 1.9	10.3 ± 1.9	7.2 ± 1.8	3.1 ± 1.5	1.9 ± 1.5	3.0 ± 1.5	1.1 ± 1.4			
	EMD	14	8.4 ± 1.9	4.3 ± 1.2	4.1 ± 1.7	10.6 ± 1.8	7.2 ± 1.1	3.4 ± 1.5	2.2 ± 1.3	2.9 ± 1.2	0.7 ± 0.8			
	EMD + GTR	14	8.6 ± 1.5	4.3 ± 1.3	4.3 ± 1.4	10.0 ± 1.7	6.6 ± 1.6	3.4 ± 1.1	1.1 ± 0.6	2.2 ± 1.0	1.1 ± 0.9			

Study	Treatment	N	Probing Depth			CAL[b]			REC			Re-entry Results		Radiographic
			Initial	Residual	Reduction	Initial	Residual	Gain	Initial	Residual	Change	Defect Fill	Crestal Bone Resorption	Bone Gain/ Resolution ([ν])
Sculean *et al.*, 2001d	GTR	12	8.1 ± 1.8	4.7 ± 1.2		9.8 ± 2.3	6.9 ± 1.8		1.7 ± 1.6	2.2 ± 1.0				
	EMD	12	8.1 ± 1.8	4.7 ± 1.2		9.8 ± 2.0	6.8 ± 1.8		1.7 ± 1.0	2.1 ± 1.0				
Sculean *et al.*, 2001c	EMD	17	9.0 ± 1.7	4.3 ± 1.7		10.6 ± 1.6	7.3 ± 1.5		1.6 ± 1.2	2.9 ± 1.4				
	EMD + AB	17	9.1 ± 1.5	4.5 ± 1.1		11.0 ± 1.6	7.5 ± 1.4		1.9 ± 1.1	3.2 ± 1.1				
Cardaropoli and Leonhardt, 2002	EMD	10	10.3 ± 1.05	3.15 ± 0.47	7.15 ± 0.88	11.5 ± 1.96	5.05 ± 1.64	6.45 ± 0.50	1.25 ± 1.44	1.75 ± 1.14	0.50 ± 0.71			4.7 ± 1.34[ν]
Minabe *et al.*, 2002	GTR	23	6.5 ± 1.1	2.4 ± 0.7	3.7 ± 1.2	7.4 ± 1.5	4.7 ± 1.3	2.8 ± 0.9	0.9 ± 0.8					
	EMD	22	6.0 ± 1.3	2.4 ± 0.9	3.8 ± 0.9	8.2 ± 1.6	5.6 ± 1.3	2.6 ± 1.0	1.2 ± 0.8					
	EMD + GTR	24	7.0 ± 2.0	2.9 ± 0.8	4.3 ± 1.6	8.2 ± 2.0	5.3 ± 1.4	3.0 ± 1.3	1.2 ± 0.9					
Rosen and Reynolds, 2002	EMD + DFDBA + GTR	10	8.4 ± 1.6	3.0 ± 0.8	5.4 ± 1.3	9.2 ± 1.3	4.7 ± 1.3	4.5 ± 1.1						
	EMD + FDBA + GTR	12	8.9 ± 2.0	3.2 ± 1.0	5.8 ± 1.6	9.1 ± 1.9	3.8 ± 1.0	5.3 ± 1.7						
Scheyer *et al.*, 2002	BDX	17	7.1 ± 1.0	3.2 ± 0.8	3.9 ± 1.3			3.7 ± 1.5			0.24 ± 0.6	3.0 ± 1.2	0.70 ± 0.5	
	EMD + BDX	17	7.5 ± 1.23	3.2 ± 0.8	4.2 ± 1.1			3.8 ± 0.9			0.41 ± 0.5	3.2 ± 1.4	0.60 ± 0.6	
Sculean *et al.*, 2002b	BG	14	8.07 ± 1.32	3.85 ± 0.66		9.78 ± 1.71	6.71 ± 1.89		1.64 ± 0.74	2.92 ± 1.85				
	EMD + BG	14	8.07 ± 1.14	3.92 ± 0.73		9.64 ± 1.59	6.42 ± 1.08		1.50 ± 1.16	2.50 ± 1.08				
Sculean *et al.*, 2002c	BDX	12	9.7 ± 2.4	3.2 ± 0.7	6.5 ± 2.0	10.1 ± 2.3	5.2 ± 1.2	4.9 ± 2.1	0.5 ± 0.5	2.0 ± 1.0	1.5 ± 1.0			
	EMD + BDX	12	10.0 ± 1.5	4.3 ± 1.4	5.7 ± 1.5	10.9 ± 2.0	6.2 ± 1.9	4.7 ± 1.9	0.9 ± 0.7	1.7 ± 0.8	0.8 ± 0.7			
Tonetti *et al.*, 2002	OFD	83	7.7 ± 1.5		3.3 ± 1.7	9.1 ± 2			2.5 ± 1.5			0.8 ± 1.2		
	EMD	83	8 ± 1.5		3.9 ± 1.7	9.4 ± 2.1		3.1 ± 1.5			0.8 ± 1.2			
Trombelli *et al.*, 2002	EMD	35	8.9 ± 1.7	3.5 ± 0.9	5.4 ± 1.8	10.1 ± 1.6	5.4 ± 1.2	4.7 ± 1.7	1.2 ± 1.0	1.9 ± 1.1	0.7 ± 0.8	3.9 ± 1.8[ν]		
Velasquez-Plata *et al.*, 2002	EMD	16	6.6 ± 1.3	2.8 ± 0.8	3.8 ± 1.2			2.9 ± 0.9			0.8 ± 0.8	3.1 ± 1.0	0.6 ± 0.7	
	EMD + BDX	16	6.9 ± 0.9	2.9 ± 0.6	4.0 ± 0.8			3.4 ± 0.9			0.3 ± 0.6	4.0 ± 0.8	0.4 ± 0.5	
Windisch *et al.*, 2002	GTR	8	10.25 ± 2.77	4.63 ± 1.51	5.62 ± 1.99	12.63 ± 2.72	8.75 ± 2.82	3.87 ± 1.64				0.47 ± 2.63		
	EMD	6	10.33 ± 1.51	5.33 ± 1.37	5.00 ± 0.63	11.17 ± 1.60	8.50 ± 1.97	2.67 ± 1.03				1.05 ± 1.71		
Zucchelli *et al.*, 2002	OFD	30	8.9 ± 0.9	4.4 ± 0.8	4.5 ± 1.0	10.0 ± 1.2	7.4 ± 1.1	2.6 ± 0.8	1.1 ± 0.9	3.1 ± 0.9	1.9 ± 0.8			
	GTR	30	8.9 ± 1.8	2.4 ± 0.7	6.5 ± 1.6	10.3 ± 1.9	5.5 ± 1.3	4.9 ± 1.6	1.4 ± 1.0	3.0 ± 1.2	1.6 ± 1.0			
	EMD	30	9.2 ± 1.0	4.0 ± 0.7	5.1 ± 0.7	9.9 ± 1.4	5.8 ± 1.1	4.2 ± 0.9	0.8 ± 0.8	1.7 ± 0.9	1.0 ± 0.5			
Sculean *et al.*, 2003b	EMD	11	8.6 ± 1.6	4.7 ± 1.8		9.5 ± 1.6	6.5 ± 2.2		0.9 ± 0.9	1.8 ± 1.6				
	EMD + COX2 inhibitor	11	8.7 ± 1.4	4.7 ± 2.0		9.7 ± 2.0	6.5 ± 2.1		1.0 ± 0.9	1.8 ± 1.2				
Silvestri *et al.*, 2003	GTR	49	8.1 ± 1.9		5.6 ± 1.5	8.9 ± 1.9		4.3 ± 1.9	1.1 ± 1.0					
	EMD	49	8.5 ± 1.6		5.3 ± 1.9	9.9 ± 1.4		4.1 ± 1.8	1.8 ± 1.9					

[a] All values are presented in mm.

[b] CAL, clinical attachment level; REC, gingival recession; EMD, enamel matrix derivative; MWF, modified Widman flap; GTR, guided tissue regeneration; BDX, bovine-derived bone xenograft; OFD, open-flap debridement; AFFS, autologous fibrinogen/fibronectin system; AB, antibiotics; DFDBA, demineralized freeze-dried bone allograft; FDBA, mineralized freeze-dried bone allograft; BG; bioactive glass; COX2, cyclo-oxygenase-2.

[ν] Radiographic defect resolution.

* Calculated based on data from the original article.

[χ] 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.

[κ] 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.

[β] 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.)

[ψ] Only lingual site data were included in the meta-analysis.

[φ] Standard deviation was calculated based on data from the original article.

[Δ] This study presents the short-term results of a study by Okuda *et al.* (2000); therefore, its data were excluded from the meta-analysis.

surgical interdental supracrestal soft tissue (Trombelli *et al.*, 2002). It was hypothesized that the presence of thick interdental tissues may have facilitated the flap management and suturing technique, while maximizing the possibility that primary closure would be achieved in the interproximal area. In addition, 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 (Tonneti**(AQ)** *et al.*, 2002).

(II.6.2.6.10) Plaque control

Early plaque formation (0-4 months) was found to have an adverse effect on radiographic bone gain (Bratthall *et al.*, 2001). In contrast, plaque accumulation was not found to be a determining 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.

(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.6.2.6.12) 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 radiographical). 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.

TABLE 3

Meta-analysis of the Treatment Parameters—Enamel Matrix Derivative Alone

		Enamel Matrix Derivative	
	mm$^{\psi}$	No. of Defects	No. of Studies
Clinical parameters			
PD[a] initial	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

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

The following formula was used:

$i = 1.....t$

t = # of studies under analysis

$Wi = 1/(SD_i^2/n_i)$

SD = Standard deviation

n = Number of treated defects

WM Changes =

$$\frac{W_1*Mean_1+W_2*Mean_2+W_3*Mean_3+....W_t*Mean_t}{W_1+W_2+W_3+.....W_t}$$

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

$$WSE = \frac{1}{\sqrt{W_1+W_2+W_3+.....W_t}}$$

(III.1) EMD

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 ($p = 0.000$) 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.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 ± 0.02 mm *vs.* 1.37 ± 0.04 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 ± 0.02 mm *vs.* 5.24 ± 0.13 mm). In contrast, while no statistically significant difference was found between the mean initial CAL, CAL gain was higher for the EMD (4.07 ± 0.03 mm *vs.* 3.64 ± 0.12 mm). As expected, these discrepancies are resolved because of the greater increase in recession in the GTR group (0.77 ± 0.02 mm *vs.* 1.5 ± 0.16 mm).

TABLE 4
Comparison of the Meta-analysis Following Enamel Matrix Derivative *vs.* Open-flap Debridement

	EMD[a]			OFD			P Value
	mm[ψ]	No. of Defects	No. of Studies	mm[ψ]	No. of Defects	No. of Studies	*t* Test
PD initial	7.94 ± 0.05	883	27	7.96 ± 0.09	231	8	0.849
PD reduction	4.82 ± 0.02*	808	22	2.59 ± 0.06	231	8	0.000
CAL initial	9.4 ± 0.06	708	23	9.48 ± 0.13	158	5	0.579
CAL gain	4.07 ± 0.03*	872	22	2.55 ± 0.04	231	8	0.000
REC initial	1.31 ± 0.05	483	18	1.23 ± 0.13	54	3	0.555
REC increase	0.77 ± 0.02*	577	14	1.37 ± 0.04	200	6	0.000

[a] EMD, Enamel Matrix Derivative; OFD, open-flap debridement; PD, probing depth; CAL, clinical attachment level; REC, recession.
[ψ] Mean ± SEM.
* P < 0.05, significant difference *vs.* OFD measurement.

TABLE 5
Comparison of the Meta-analysis Following Enamel Matrix Derivative *vs.* Guided Tissue Regeneration

	EMD[a]			GTR			P Value
	mm[ψ]	No. of Defects	No. of Studies	mm[ψ]	No. of Defects	No. of Studies	*t* Test
PD initial	7.94 ± 0.05	883	27	7.79 ± 0.13	146	7	0.212
PD reduction	4.82 ± 0.02*	808	22	5.24 ± 0.13	134	6	0.000
CAL initial	9.4 ± 0.06	708	23	9.11 ± 0.15	146	7	0.041
CAL gain	4.07 ± 0.03*	872	22	3.64 ± 0.12	134	6	0.000
REC initial	1.31 ± 0.05	483	18	1.19 ± 0.09	138	6	0.247
REC increase	0.77 ± 0.02*	577	14	1.5 ± 0.16	44	2	0.000

[a] EMD, Enamel Matrix Derivative; GTR, guided tissue regeneration; PD, probing depth; CAL, clinical attachment level; REC, recession.
[ψ] Mean ± SEM.
* P < 0.05, significant difference *vs.* GTR measurement.

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 ± 0.05 mm *vs.* 7.32 ± 0.12 mm and 4.82 ± 0.02 mm *vs.* 3.94 ± 0.11 mm, respectively). The CAL gain was higher for the EMD group (4.07 ± 0.03 mm *vs.* 3.48 ± 0.12 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 ± 0.02 mm *vs.* 0.58 ± 0.06 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*

TABLE 6

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

	EMD[a]			EMD + GTR			P Value
	mm[ψ]	No. of Defects	No. of Studies	mm[ψ]	No. of Defects	No. of Studies	*t* Test
PD initial	7.94 ± 0.05	883	27	7.81 ± 0.29	38	2	0.512
PD reduction	4.82 ± 0.02*	808	22	4.3 ± 0.25	38	2	0.000
CAL initial	9.4 ± 0.06	708	23	9 ± 0.3	38	2	0.079
CAL gain	4.07 ± 0.03*	872	22	3.18 ± 0.2	38	2	0.000
REC initial	1.31 ± 0.05	483	18	1.14 ± 0.12	38	2	0.292
REC increase	0.77 ± 0.02	577	14	1.1 ± 0.24	14	1	NA

[a] EMD, Enamel Matrix Derivative; GTR, guided tissue regeneration; PD, probing depth; CAL, clinical attachment level; REC, recession.
[ψ] Mean ± SEM.
* $P < 0.05$, 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

	EMD[a]			EMD + BTX			P Value
	mm[ψ]	No. of Defects	No. of Studies	mm[ψ]	No. of Defects	No. of Studies	*t* Test
PD initial	7.94 ± 0.05	883	27	7.32 ± 0.12	113	6	0.000
PD reduction	4.82 ± 0.02	808	22	3.94 ± 0.11	113	6	0.000
CAL initial	9.4 ± 0.06	708	23	NA	NA	NA	NA
CAL gain	4.07 ± 0.03	872	22	3.48 ± 0.12	113	6	0.000
REC initial	1.31 ± 0.05	483	18	NA	NA	NA	NA
REC increase	0.77 ± 0.02	577	14	0.58 ± 0.06	113	6	0.001

[a] EMD, Enamel Matrix Derivative; BDX, bovine-derived bone xenograft; PD, probing depth; CAL, clinical attachment level; REC, recession.
[ψ] Mean ± SEM.

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

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.

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

	EMD[a]			BTX			P Value
	mm[ψ]	No. of Defects	No. of Studies	mm[ψ]	No. of Defects	No. of Studies	*t* Test
PD initial	7.94 ± 0.05*	883	27	7.38 ± 0.23	29	2	0.007
PD reduction	4.82 ± 0.02*	808	22	4.5 ± 0.28	29	2	0.002
CAL initial	9.4 ± 0.06	708	23	10.1 ± 0.66	12	1	NA
CAL gain	4.07 ± 0.03	872	22	4.02 ± 0.31	29	2	0.688
REC initial	1.31 ± 0.05	483	18	0.5 ± 0.14	12	1	NA
REC increase	0.77 ± 0.02*	577	14	0.5 ± 0.13	29	2	0.001

[a] EMD, Enamel Matrix Derivative; BDX, bovine-derived bone xenograft; PD, probing depth; CAL, clinical attachment level; REC, recession.
ψ Mean ± SEM.
* $P < 0.05$, significant difference *vs.* BDX measurement.

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

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 indication and contra-indications during periodontal therapy.

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