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Abstract

Title of Thesis: Comparison of Enamel Matrix Derivative Alone or in Combination with Bone Replacement Graft Materials in the Treatment of Intrabony Defects: A Systematic Review

Ahad Soleymanzadeh, Master of Science, 2015

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The ultimate goal of any periodontal therapy is to regenerate all lost tissues of the periodontium: bone, cementum and periodontal ligament.

The purpose of this systematic review was to compare the outcome when BRG was combined with EMD vs. EMD alone for the treatment of periodontal intrabony defects.

A comprehensive literature search through February 11, 2015 was performed using Medline and EMBASE for all literature related to intrabony defects treatment using EMD with and without bone graft.

Either EMD or combination of EMD with BG resulted in improved clinical outcomes. In conclusion, most studies report differences for gingival recession with the addition of bone graft but this trend was not statistically significant. Bone fill, as measured at reentry, was the only variable yielding more favorable outcome with the combined approach. Mean defect fill 2.36 ± 3.9 mm and 3.78 ± 0.7 mm ($p < 0.05$) for EMD alone and combination therapy, respectively.

Comparison of Enamel Matrix Derivative Alone or in Combination with Bone
Replacement Graft Materials in the Treatment of Intra-bony Defects:
A Systematic Review

By
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I. Introduction

A. A Historical Perspective

Periodontitis is one of the most prevalent inflammatory conditions that affects 47% of adults, with moderate severity, in the United States¹. Periodontitis results in destruction of the supporting structures of the dentition—namely bone, cementum and periodontal ligament (PDL). Historically, the primary goal of therapy was to eliminate periodontal pockets, thereby facilitating control of bacterial biofilm formation and reducing the risk of disease progression. This surgical approach was intended to provide access to root surfaces for scaling and root planing, to the alveolar bone for re-contouring, and to enable re-positioning of the gingiva closer to the underlying bone. Schluger (1949) introduced osseous surgery as a means of achieving this goal by altering the underlying bone topography; however, this technique might result in the unnecessary bone removal around the affected and adjacent teeth². The unintended consequence of osseous surgery is longer teeth, due to root exposure, which risk of hypersensitivity and root caries. Ochsenbein further developed the principles and clinical guidelines for performing osseous surgery³; this resective technique has remained a fundamental procedure for pocket reduction. Despite the inherent destructive nature of this surgical approach, the clinical outcome provided a periodontium maintainable in health by the patient and periodontist.

Given the inherent limitations of osseous surgery, clinical and research efforts turned to strategies for regenerating the periodontium lost to disease. Early efforts focused on

developing surgical techniques as well as sources of bone graft materials. Autogenous bone was harvested in the form of osseous coagulum and bone blend from intraoral sites and iliac crest autogenous marrow from extraoral sources.⁴⁻⁶ Iliac cancellous bone and marrow has been shown to support crestal bone apposition, with stable long-term outcomes over 20 years^{6,7,8}. Histologic evaluation of teeth removed 2 to 8 months after grafting with iliac cancellous bone and marrow revealed crestal bone apposition as well as formation of a new attachment apparatus, including new bone cementum, and periodontal ligament⁹. However, a follow up report revealed that 2.8% of the cases developed root resorption following grafting with fresh iliac bone autografts¹⁰. Cancellous bone harvested from the maxillary tuberosity, edentulous ridges, and extraction sites has been reported to provide treatment outcomes comparable to autogenous iliac crest grafts¹¹. Although these procedures offered predictable and consistent long-term results, the widespread use of autogenous bone was restricted because of limitations in donor bone available from intraoral sites and morbidity associated with harvesting bone from extraoral sites. These early studies provided pioneering evidence that periodontal regeneration was achievable.

Non-human graft materials have been evaluated as an alternative to autogenous bone for achieving regeneration. Coralline-derived hydroxylapatite (Durapatite™), for instance, has been shown to reduce clinical probing depths when used to graft intrabony defects; however, histologic examinations of treated defects showed no new periodontal attachment, osteogenesis, or cementogenesis¹². Coralline-derived hydroxylapatite appears to function as “filler”, resulting in repair rather than regeneration.

Hydroxyapatite was also evaluated as particulate graft material for treating periodontal defects. Clinically, hydroxyapatite was found to reduce clinical probing depths; however, histologically, the particles of the graft were completely encapsulated and surrounded by dense fibrous connective tissue with no inflammatory infiltrate¹³. In the absence of a biologic agent, synthetic bone grafts have been found to support only periodontal repair. Although there is proof-of-principle evidence that multiple graft materials, such as xenogeneic bone, support periodontal regeneration¹⁴, the most compelling evidence of periodontal regeneration is available for demineralized freeze-dried bone allograft¹⁵⁻¹⁷.

Clinical efforts have also focused on strategies to achieve periodontal regeneration without a bone replacement graft. The treatment of 3-wall intrabony defects with root debridement, defect degranulation, and flap resection has been shown to result in about 60% hard tissue defect fill¹⁸. The concept of promoting bone and periodontal regeneration by excluding soft tissue from osseous defects provides the basis for guided tissue regeneration (GTR)¹⁹, in which a barrier membrane is used to partition the soft tissue from the underlying bone during healing. The formation of a new attachment apparatus on a previously diseased root surface has been attributed to the ability of a barrier membrane to prevent apical migration of epithelium and to promote migration of PDL cells into the defects site²⁰.

The first commercially marketed barrier membrane was composed of expanded polytetrafluoroethylene (ePTFE), a non-resorbable material requiring removal 6-8 weeks after surgical placement. Histologic studies demonstrate that GTR using an ePTFE

barrier membrane supported periodontal regeneration²¹. Comparable clinical results were later shown for bioabsorbable barrier membranes, such as collagen and synthetic polymers, which eliminated the need for a second surgical procedure for retrieval and resulted in less patient morbidity. Similar results have been reported for polylactic acid-based barriers, such as Guidor[®] Matrix Barrier²². In general, treatment outcomes with different GTR barrier membranes have been shown to be comparable and superior to outcomes with Modified Widman flap surgery²³.

Several studies suggest that the application of combined regenerative approaches, such as barrier membrane and graft material²⁴, may provide clinical outcomes superior to either technology alone²⁵. More advanced osseous defects, such as 1, 2-wall intrabony and class II furcation defects, appear to respond most favorably to combination therapy²⁶. Moreover, longitudinal evidence suggests that combination therapy using GTR and graft material provides stable long-term clinical outcomes, particularly in furcation defects²⁷.

Although GTR barrier membranes and non-cellular bone replacement grafts support periodontal regeneration, these biomaterials are generally thought to lack substantial bioactive properties. Enamel matrix derivative (EMD), in contrast, is a biologic agent that has been shown to promote periodontal regeneration^{28, 29} without a barrier membrane or graft material, which contribute to clot formation and stability.

Considerable clinical interest, therefore, has emerged in examining whether the delivery of EMD using bone grafts will enhance periodontal regeneration. EMD has been combined with different bone replacement grafts, including allografts, xenografts, and

synthetic materials. The results of several studies suggest that the combination of EMD and bone graft supports regenerative outcomes superior to EMD alone³⁰⁻³³.

B. Enamel Matrix Derivative (EMD): An Introduction

EMD is composed primarily of two enamel matrix proteins, proteins, amelogenins and enamelin.³⁴ Amelogenins have been demonstrated to play an active role on root surfaces during dental development prior to cementogenesis³⁵. Hertwig's epithelial root sheath cells synthesize and secrete enamel matrix-like products, such as ameloblastin, as well as mineralized extracellular matrix resembling acellular cementum³⁶. The role of enamel matrix proteins, particularly amelogenins, in root formation provided the biological basis for examining their potential to promote periodontal regeneration³⁷. Emdogain[®] is a commercially available enamel matrix derivative enriched with amelogenins proteins, with only trace amounts of enamelin or amelin³⁸. Given the hydrophobic nature of Emdogain[®], this biologic is delivered using a propylene glycol alginate (PGA), which allows for its precipitation on the root surface³⁷.

C. Cellular Effects

EMD exerts pleiotropic effects on multiple cells in the periodontium. Numerous studies have examined the effect of EMD on inflammatory cells and biomarkers as well as wound healing. EMD has been shown to significantly decrease interleukin-1 β (IL-1 β) and RANK-L expression, increase prostaglandin E₂ (PGE₂) and osteoprotegerin (OPG) expression, increase proliferation and migration of T lymphocytes, induce monocyte differentiation, and increase cellular clearance of bacterial and tissue debris^{39,40}. In addition, EMD has been found to increase angiogenesis by inducing endothelial cell

proliferation, migration and capillary-like sprout formation⁴¹. EMD appears to promote bone formation by modulating IL-7 and PGE₂ production⁴². Moreover, EMD stimulates connective tissue growth factor (CTGF) expression in PDL cells, which has a stimulatory effect in proliferation and differentiation of fibroblastic cells⁴³.

Of particular relevance, EMD has been shown to modulate the multi-lineage differentiation of the human PDL cells. EMD induces PDL cells to undergo osteogenic⁴⁴, adipogenic, neovasculogenic and chondrogenic differentiation while suppressing adipogenesis, neurogenesis and gliogenesis⁴⁵. EMD also appears inhibit epithelial proliferation, including the down-growth of epithelium⁴⁶ during periodontal wound healing and regeneration. EDM has antibacterial effect on dental biofilm vitality^{47,48}.

In summary, EMD exhibits the biologic potential to attenuate the inflammatory, reduce bone resorption, enhance bone formation, promote vascularization, as well as regulate key molecules in tissue repair that stimulate maturation of extracellular matrix.

D. Root Conditioning: A Prerequisite

Scaling and root planing results in the formation of smear layer on the root surface⁴⁹. In order to remove smear layer and expose collagen fibers, the topical application of pH neutral 24% ethylenediaminetetraacetic acid (EDTA) gel is recommended as a root condition agent prior to applying EMD to the root surface. EDTA (15% to 24%) has been reported to enhance regenerative outcome with EMD by effectively removing the smear layer⁵⁰, and mineral from the root surface to expose collagen matrix⁵¹, thereby producing a more biocompatible surface. In contrast to other root conditioning agents, such as citric acid, EDTA offers the advantage of not causing superficial necrosis of the

periodontal tissues⁵⁰.

E. Experimental and Human Studies

Clinical studies generally demonstrate the effectiveness of EMD in improving gains in clinical attachment level and reductions in periodontal probing depth. In a placebo-controlled study, Heijl et al. compared the effectiveness of EMD to a vehicle control in 1- and 2-walled defects after 3 years. EMD supported significantly greater reductions in probing depth (3.1 mm versus 2.3 mm) and gains in attachment level (2.2 mm versus 1.7 mm) than control defects⁵². Moreover, long-term studies of EMD therapy have shown stable reductions in probing depth and gains in clinical attachment after treatment in intrabony defects⁵³.

The treatment of periodontal defects with EDM in animal models results in the formation of new cementum, bone and periodontal ligament^{37,31}. However, histologic studies in humans provide only proof-of-principle evidence that EMD supports periodontal regeneration. Parodi et al. examined the use of EMD in the treatment of deep intrabony defects. Despite significant improvements in clinical attachment level, the radiographic analysis failed to demonstrate corresponding evidence of defect fill. Moreover, upon histologic examination, two defect sites did not show evidence of new attachment⁵⁴. Yukna and Mellonig⁵⁵ evaluated 10 intrabony defects treated with EMD after notching the apical extent of calculus. Histologic evaluation of the region coronal to the base of the calculus notch showed evidence of regeneration (new cementum, new bone, and new periodontal ligament) in only 3 specimens after 6 months.

F. Safety of Enamel Matrix Derivative

In vitro assays evaluating the safety of EMD have not demonstrated a significant effect of the agent on cellular or humoral immune responses. Similarly, serologic analysis of subjects treated with EMD fail to demonstrate a significant immunogenic responses to the agent ⁵⁶.

G. Enamel Matrix Derivative vs. GTR: An Observation

EMD and GTR support periodontal regeneration through different biologic mechanisms. GTR functions through use of a barrier that provides clot protection and space maintenance, which allows for cells to populate the defect⁵⁴. In contrast, EMD proteins promote periodontal regeneration primarily by stimulating periodontal ligament and cementum formation⁵⁴. EMD is still considered a novel treatment modality that requires further studies to evaluate the mechanism of therapy⁵⁷.

II. Purpose

The objective of this systematic review was to compare the effectiveness of EMD alone versus EMD plus bone graft in supporting improvements in clinical parameters—namely, clinical attachment level gain, probing depth reduction, and defect fill. A secondary purpose was to compare clinical outcomes associated with different bone graft materials when used in combination with EMD.

III. Material and Methods

This systematic review included only RCTs with a follow-up of at least 6 months.

A. Search Protocol

English language publications were searched through May 2015 using MEDLINE (PubMed) and EMBASE using the following search criteria: EMD, emdogain, enamel matrix proteins, enamel protein, dental enamel proteins AND periodontology, GTR, guided tissue regeneration, periodontal defect, angular defect, infrabony defect, intrabony defect and infrabony, intrabony, intraosseous, graft, transplant, particulate matter, bone transplantation, bone allograft, allogeneic bone, bone substitutes, BioOss, anorganic bone, autogenous bone, bone replacement graft, FDBA, and DFDBA.

Field 1: Emdogain OR amelogenin OR "enamel matrix proteins" OR "enamel proteins" OR enamel matrix derivative OR enamel matrix derivatives

(AND) Field 2: infrabony OR intrabony OR intraosseous

(AND) Field 3: particulate matter OR allogenic bone OR bone substitutes OR bioOss OR anorganic bone OR autogenous bone OR bone replacement graft OR bone graft OR transplant OR bioOss OR anorganic bone OR FDBA OR DFDBA

(AND) Field 4: randomized controlled trials

The citations from the existing systematic reviews that were submitted after 1995 were also analyzed. Relevant citations were extracted and included in this study

Only articles published in the English language and human studies were included.

B. Selection Criteria

The selection criteria and outcome variables are described below. Randomized clinical trials comparing EMD versus EMD plus various types of bone or bone substitute grafting procedures (BG) for the treatment of human intrabony defects.

Criteria for including a study were as follows: 1) non-surgical therapy completed before regenerative therapy, 2) PD \geq 6 mm and/or intrabony defect \geq 3 mm, 3) no systemic diseases, and 4) a good level of oral hygiene 5) randomized controlled trials

The exclusion criteria were: 1) studies in which EMD was compared with non-EMD included tests 2) non-randomized controlled trials 3) lack of EMD alone arm 4) case studies, case series and histologic reports 5) studies that lacked mean change in PD, CAL or REC measurements were excluded.

As a primary outcome the change in clinical attachment level (CAL), change in probing depth (PD), change in gingival recession (REC) was explored. The secondary outcome measures included change in radiographic bone levels (RAD), % bone fill and bone sounding.

SEARCH STRATEGY

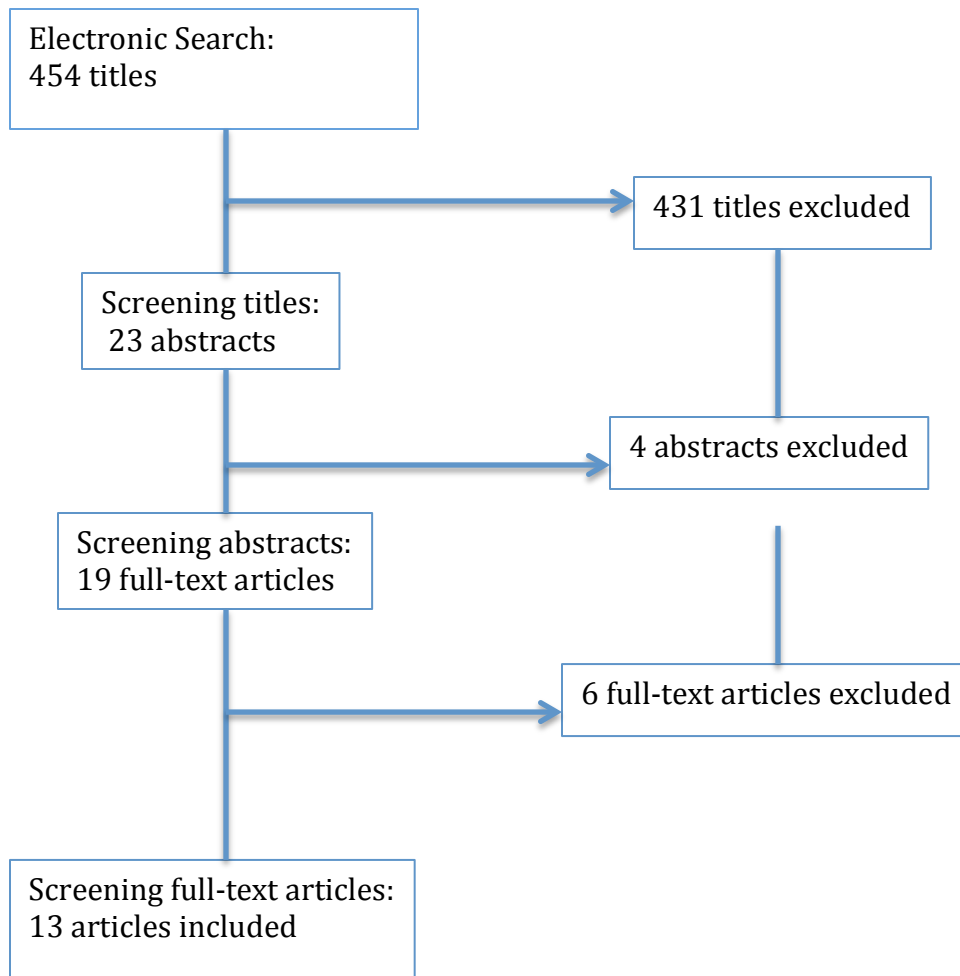


Figure 1. Data Flowchart depicting initial search results to final studies

C. Data Collection and Analysis

Selection of Studies: Two independent reviewers evaluated the titles and abstracts of all studies meeting the inclusion and exclusion criteria. If the abstract did not provide sufficient data to establish qualification for inclusion, the full report was obtained and reviewed. Study characteristics were identified and recorded in a table. Characteristics recorded included a study design, number of subjects, age, assessment interval, treatment description, clinical parameters, defect morphology, re-entry/radiographic assessment.

Data Extraction: Two independent reviewers extracted study information and results using a data extraction form.

Data Statistical Analysis: Descriptive statistics were expressed as mean and standard of deviations. Analysis of variance (ANOVA) was used to detect intergroup differences of PD, CAL, REC, bone fill and radiographic bone gain.

A subgroup analysis was performed separately for mammalian and synthetic bone graft materials. A significant level of 0.05 was used in all statistical comparison.

Quality Assessment of Evidence: The protocol for grading the level of evidence was based on the Strength of Recommendation Taxonomy (SORT) from the Journal of American Family Physician. Assessment was based on Strength-of-Recommendation (Figure 2) and Quality of Evidence (Figure 3). Table 1 provides the characteristics of the reviewed studies.

Study	Design	Participants	Treatment	Outcome	Defect Morphology	Re-entry/Histology	Level of Evidence
Lekovic et al., 2000	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, ΔBF, manual probe with acrylic stent; hard-tissue measurements at re-entry	2,3 walls	Re-entry	1A
Velasquez-Plata et al., 2002a	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, ΔBF, manual probe; hard-tissue measurements at re-entry	2+3, 3 walls	Re-entry	1A
Zucchelli et al., 2003	RCT, 2 treatment groups, 1 year duration	60 patients, 26 males, 34 females, age 34-62 yrs, 20 smokers (10 in each group)	Control, EMD; Test, EMD + BPBM (bovine porous bone mineral), SPT interval, 1-month	ΔCal, ΔPPD, ΔREC, manual probe, Radiographic Examination	Angular defects not categorized.	None	2A
Kuru et al., 2006	RCT, 2 treatment groups, 1 year duration	23 patients, mean age 44.7 years	Control, EMD; Test, EMD + Perioglas; SPT intervals every two weeks for the first two months, and then once a month for next 6 months	ΔCal, ΔPPD, ΔREC, ΔRBG, manual probe and radiographs	1, 2-walled	Radiographic	2A
Bokan et al., 2006	RCT, 2 treatment groups, 8 months duration	56 patients, 29 female, 27 males	Control, EMD; Test EMD + TCP tricalcium phosphate); SPT intervals, for 6 month, every 4	ΔCal, ΔPPD, ΔREC, manual probe	1/2-walled, 2/3-walled	None	2A
Jepsen et al., 2008	RCT, 2 treatment groups, 6 months duration	75 patients, 23 males, 52 female, median age of 48.2; 12 occasional smokers	Control, EMD; Test, EMD + SBC, SPT intervals, 3 months and 6 month	ΔCal, ΔPPD, ΔREC, manual probe with bone sounding	1, 2 and combined 1/2 walls	None	2A
Meyle et al., 2011	Multicenter, RCT, 2 treatment groups, 12 months duration	73 patients, 23 males and 50 females, with a mean age of 46.9 years, 12 of the patients were occasional smokers.	Control, EMD; Test EMD + SBC (bone CERAMIC) graft), SPT intervals every 3 months	ΔCal, ΔPPD, ΔREC, ΔRBG, manual probe with acrylic stent, bone sounding	1 and 2 walled	Radiographic	2A
De Leonardis et al., 2013	RCT, splitmouth, 3 treatment groups, 24 month duration	34 patients, 15 males, 21 females, mean age was 45.3 ± 5.9	Control, EMD; Test, EMD + HA/TCP; SPT intervals, weekly for the first 6 weeks, then every 3 month	ΔCal, ΔPPD, ΔREC, ΔRBG, manual probe, Radiographic Examination	1 and 2 walled	Radiographic	1A

Table 1. Characteristics of the reviewed studies

Study	Design	Participants	Treatment	Outcome	Defect Morphology	Re-entry/Histology	Level of Evidence
Gurinsky et al., 2004	RCT, 2 treatment groups, 6 months duration	40 patients, 17 males, 23 females, ages 19-76 years	Control, EMD; Test, EMD + DFDBA, SPT intervals, 7, 10, 25 and 30 days, 3 months and 6 months	Δ Cal, Δ PPD, Δ REC, Δ BF, manual probe	1, 2, 3, and combination	Re-entry	2A
Guida et al., 2007	RCT, 2 treatment groups, 1 year duration	27 patients, 13 males, 14 females, mean age of 46.3, 4 smokers	Control, EMD; Test, EMD + ACBP (autogenous cortical bone particulate), SPT interval, monthly	Δ Cal, Δ PPD, Δ REC, manual probe, Radiographic Examination	1 and 2 walled	Radiographic	2A
Yilmaz et al., 2010	RCT, 2 treatment groups, 1 year duration	40 patients, 24 males, 16 females, aged 30-50 years	Control, EMD; Test, EMD + AB (autogenous bone), SPT intervals 2 weeks for the first 2 months, then once a month for	Δ Cal, Δ PPD, Δ REC, manual probe	2 and 3 walled	None	2A
Sculean et al., 2005	RCT, 2 treatment groups, 1 year duration	28 patients, 13 males, 15 females, no age range given	Control, EMD; Test, EMD + Bioactive glass, SPT interval every second week during the first 2 months after surgery and once a month the rest of the year	Δ Cal, Δ PPD, Δ REC, manual probe, Clinical bone level	1-2, 2, 3 walled	None	2A
Sculean et al., 2005	RCT, 2 treatment groups, 1 year duration	28 patients, 13 males, 15 females, no age range given	Control, EMD; Test, EMD + Bioactive glass, SPT interval every second week during the first 2 months after surgery and once a month the rest of the year	Δ Cal, Δ PPD, Δ REC, manual probe, Clinical bone level	1-2, 2, 3 walled	None	2A
Ogihara et al., 2014	RCT, 3 treatment groups, duration 3 years	69 patients, 14 males, 55 females, mean age of 54 years	Control, EMD; Test, EMD + FDBA, EMD + DFDBA, SPT interval, weekly for the first month and every month for up to 6 months.	Δ Cal, Δ PPD, manual probe	2, 3, combination	None	2A

Table 1. (Continued) Characteristics of the reviewed studies

Table 1. Strength-of-Recommendation Grades

<i>Strength of recommendation</i>	<i>Basis for recommendation</i>
A	Consistent, good-quality patient-oriented evidence*
B	Inconsistent or limited-quality patient-oriented evidence*
C	Consensus, disease-oriented evidence,* usual practice, expert opinion, or case series for studies of diagnosis, treatment, prevention, or screening

*—Patient-oriented evidence measures outcomes that matter to patients: morbidity, mortality, symptom improvement, cost reduction, and quality of life. Disease-oriented evidence measures intermediate, physiologic, or surrogate end points that may or may not reflect improvements in patient outcomes (e.g., blood pressure, blood chemistry, physiologic function, pathologic findings).

Figure 2. The Basis For Strength of Recommendation⁵⁸

Table 2. Assessing Quality of Evidence

<i>Study quality</i>	<i>Diagnosis</i>	<i>Treatment/prevention/ screening</i>	<i>Prognosis</i>
Level 1: good-quality, patient-oriented evidence	Validated clinical decision rule SR/meta-analysis of high-quality studies High-quality diagnostic cohort study*	SR/meta-analysis or RCTs with consistent findings High-quality individual RCT† All-or-none study‡	SR/meta-analysis of good-quality cohort studies Prospective cohort study with good follow-up
Level 2: limited-quality patient-oriented evidence	Unvalidated clinical decision rule SR/meta-analysis of lower quality studies or studies with inconsistent findings Lower quality diagnostic cohort study or diagnostic case-control study	SR/meta-analysis of lower quality clinical trials or of studies with inconsistent findings Lower quality clinical trial Cohort study Case-control study	SR/meta-analysis of lower quality cohort studies or with inconsistent results Retrospective cohort study or prospective cohort study with poor follow-up Case-control study Case series
Level 3: other evidence	Consensus guidelines, extrapolations from bench research, usual practice, opinion, disease-oriented evidence (intermediate or physiologic outcomes only), or case series for studies of diagnosis, treatment, prevention, or screening		

*—High-quality diagnostic cohort study: cohort design, adequate size, adequate spectrum of patients, blinding, and a consistent, well-defined reference standard.

†—High-quality RCT: allocation concealed, blinding if possible, intention-to-treat analysis, adequate statistical power, adequate follow-up (greater than 80 percent).

‡—In an all-or-none study, the treatment causes a dramatic change in outcomes, such as antibiotics for meningitis or surgery for appendicitis, which precludes study in a controlled trial.

(SR = systematic review; RCT = randomized controlled trial)

Figure 3. The Basis For Grading The Quality of Evidence⁵⁸

IV. Results

The search strategy yielded 454 articles. A total of 13 articles met the criteria for inclusion in the final analysis. (Fig. 1)

Summary of Findings: All of the included studies were randomized clinical trials^{30, 33, 59-69}. Three studies involved split-mouth design (3 studies)^{30, 60, 68}.

Characteristics of Participants: The ages of study participants of the included studies ranged from 19-76 years (Table 1). Some studies failed to report the mean age (7 studies)^{33, 47, 59, 62, 63, 68, 69} while others failed to report a range of the participant's ages (9 studies)^{30, 59-61, 63-67, 69}. One study did not report either age range or mean age of its participants⁶⁷. One study did not specify the number of male or female subjects⁶⁴.

Sample Size: The sample size ranged from 16 to 75 participants (Table 1).

Assessment Interval: The clinical parameters were measured at baseline and post-surgery at either 6 months^{30, 62, 63, 67} (4 studies), 6-8 months⁶⁸ (1 study), 8 month⁶⁴ (1 study), 6 and 12 months^{61, 65} (2 studies), 12 months^{33, 59, 69} (3 studies), 12 and 24 months⁶⁰ (1 study), 12, 24 and 36 months⁶⁶ (1 study). Post-surgery re-entry assessment was performed at 6 month^{30, 62, 66} (3 studies) and 6-8 months⁶⁸ (1 study). Bone sounding to assess bone fill was performed six months after surgery^{63, 65} (2 studies). Radiographic assessments were evaluated post-surgery at 6 months^{62, 67} (2 studies), 8 months⁶⁴ (1 study), 12 months^{33, 60,}

^{61, 65} (4 studies), and 12 and 35 months⁶⁶ (1 study). One study performed histological analysis 6 months after surgery.⁶⁷

Subject Inclusion Criteria: The majority of the studies selected subjects with no systemic disease^{33, 59-62, 64, 66-69}. Four studies selected subjects with a good level of oral hygiene^{62, 63, 67, 69} while one study accepted subjects with adequate oral hygiene⁵⁹. The majority of studies required intrabony defects probing at least 5 mm^{62, 68} or 6 mm^{59-61, 64, 66, 67, 69}. Nine studies included radiographic defects measuring at least 3 mm^{59, 60, 62, 67, 69} or 4 mm^{61, 63-65}. Two studies required patients to comply with a maintenance program^{67, 69}.

Subject Exclusion Criteria: Only three studies excluded subjects that had smoking habits^{60, 66, 69}. Two studies excluded subjects that smoked more than 30 cigarettes/month^{63, 65}, whereas one study excluded subjects who smoked >20 cigarettes/day³³. Two studies indicated that they excluded subjects with acute infection in the surgical area^{62, 63}. Seven studies excluded females who were pregnant or lactating⁶⁰⁻⁶⁶. Two studies excluded subjects with poorly or uncontrolled diabetes and unstable conditions^{63, 65}. One study reported that subjects with exposed root surfaces were excluded⁶⁴, and one subject excluded endodontically treated teeth⁶⁴. Four studies excluded subjects with full mouth plaque score > 20%^{33, 60, 65, 68}.

A. Characteristics of Interventions

Bone Graft Material

Bone graft materials from mammalian and synthetic sources were combined with EMD to treat intrabony defects. Three studies used bovine derived xenograft^{30, 33, 68}, while six studies used synthetic graft material (bioactive glass, β -tricalcium phosphate (TCP), bone ceramic (biphasic calcium phosphate), hydroxyapatite/TCP)^{59, 60, 63-65, 67}. Four studies used either autogenous bone^{61, 69} or allogeneic bone (FDBA, DFDBA)^{62, 66}.

Intrabony Defect Characteristics

Surgical evaluation of the intrabony defect revealed 1, 2, 3-walled or combination morphology. Five studies reported 1 and 2-walled defects^{60, 61, 63-65}. Four studies reported only 2 and 3-walled defects^{30, 66, 68, 69}. One study failed to characterize defect morphology³³. The remaining studies included intrabony defects with a wide-range of defect morphology^{59, 62, 67}.

Incision and Flap Management

The incision designs used in these studies included intrasulcular, simplified papilla preservation, and/or modified papilla preservation technique. Eight studies used intrasulcular design^{30, 61, 62, 64, 66-69}. Simplified papilla preservation technique was utilized by four studies^{33, 59, 60, 65}, one of which also used modified papilla preservation technique

⁶⁰. All of the studies used full thickness flap to access the intrabony defects. After treating the defects with EMD alone EMD plus graft, the flaps were either repositioned or coronally advanced to achieve primary closure. Nine studies reported flap repositioning^{30, 59, 60, 62-68}. Coronally advance flaps were performed in two studies^{33, 69}. One study replaced the flap at the presurgical level or coronally advanced the flap in order to achieve primary closure⁶¹. Primary closure of the flap margins was reported in all studies, with the exception of 2 studies that did not report closure technique^{65, 68}.

Root Surface Treatment

With 2 exceptions^{65, 66}, all studies reported using 24% EDTA for 2 minutes to remove smear layer. Minocycline (10mg/ml) was used for 3 minutes to treat the root surfaces in one study⁶⁶, whereas no root conditioning was used in another study⁶⁵. Only two studies report attempting to control bleeding from defect prior to application of EMD^{33, 63}.

Quality of Evidence

A clear and succinct method of classifying the strength and quality of evidence has been provided by the JEBDT guidelines. Strength is given an A, B or C rating. An A denotes consistent, quality patient oriented evidence, B is limited quality or inconsistent patient oriented evidence, and C denotes disease oriented evidence or expert opinions⁷⁰. When assessing the quality of evidence, the JEBDT guidelines first distinguish the nature of a study: whether it is a diagnostic study, a treatment/prevention/screening study, or a

prognosis study. The types of studies that qualify for each level vary depending on the nature of the study, as seen in Figure 3.

The majority of studies evaluated (ten) were grade 2A-all randomized clinical trials (RCT) studies using high quality patient-oriented evidence. This patient oriented evidence measures outcomes that matter to patients, morbidity, mortality, symptom improvement. The remaining three studies were grade 1A in the sense that they involved split-mouth design and were high quality RCT in nature⁷⁰.

B. Characteristics of Outcome Measures

The following table describes the characteristics of the studies reviewed in this review (Table 2). (Note: The numbers 1-13 as they appear on the X-axis of the following graphs correspond to the respective studies as they are numbered in the Table 1.)

#	Study	Duration of Study	Bone Graft Material Used
1	Lekovic et al., 2000	6 months	Bovine porous bone mineral
2	Valesquez-Plata et al., 2002	6-8 months	Bovine derived xenograft
3	Zucchelli et al., 2003	12 months	Bovine porous bone mineral
4	Kuru et al., 2006	12 months	Bioactive glass
5	Bokan et al., 2006	8 months	Beta-tricalcium phosphate
6	Jepsen et al., 2008	6 months	Biphasic calcium phosphate
7	Meyle et al., 2011	12 months	Biphasic calcium phosphate
8	De Leonardis et al., 2013	24 months	Hydroxyapatite/beta-TCP
9	Gurinsky et al., 2004	6 months	DFDBA
10	Guida et al., 2007	12 months	Autogenous cortical bone particulate
11	Yilmaz et al., 2010	12 months	Autogenous bone
12	Sculean et al., 2005	12 months	Bioactive glass
13	Ogihara et al., 2014	36 months	FDBA/DFDBA

Table 2. Study description

Probing Depth Change

Comparative analysis (ANOVA) was performed to evaluate differences between control (EMD) and test (EMD + BG) among all studies on their clinical parameter outcomes.

Each outcome parameter is compared individually among all studies in order to clearly demonstrate any potential difference (Figure 4).

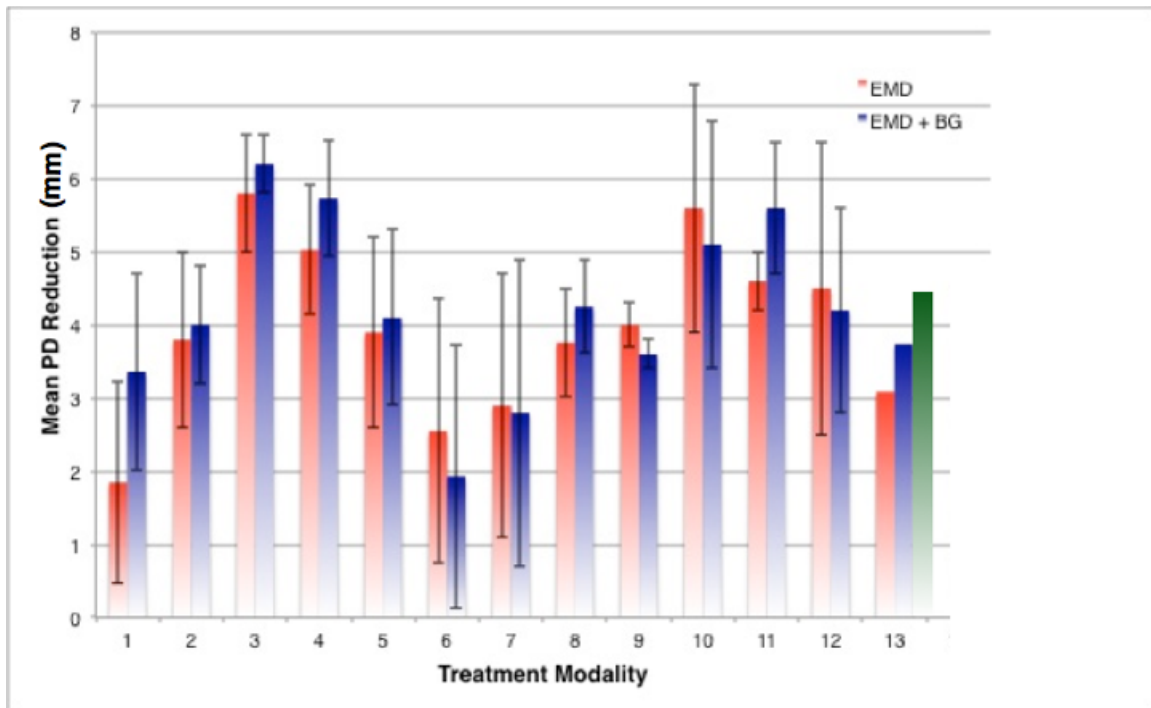


Figure 4. Comparison of Mean PD Reduction among EMD groups and EMD + BG group in all studies

All studies present with comparable pocket depth reduction for intrabony defects treated with EMD alone and EMD plus bone graft. Ogihara et al. reported improvement in the pocket depth reductions for EMD plus bone graft, particularly with FDBA⁶⁶ (green column); however, there were no standard deviation provided to statistically evaluate the observed difference. Lekovic et al. reported significantly greater reduction in probing depth in defect sites treated with EMD plus graft (buccal defects: 3.4 ± 1.3 mm; lingual

defects 3.4 ± 1.4 mm) compared sites treated with EMD alone (1.9 ± 1.4 mm on buccal sites and 1.9 ± 1.4 mm on lingual sites)³⁰. Yilmaz et al. reported statistically significant reduction in the mean probing pocket depth (5.6 ± 0.9 mm, $p < 0.001$) defects treated with EMD and bone graft⁶⁹.

Clinical Attachment Level Changes

All studies present with comparable clinical attachment gain for both EMD groups and EMD + bone graft groups (Figure 5).

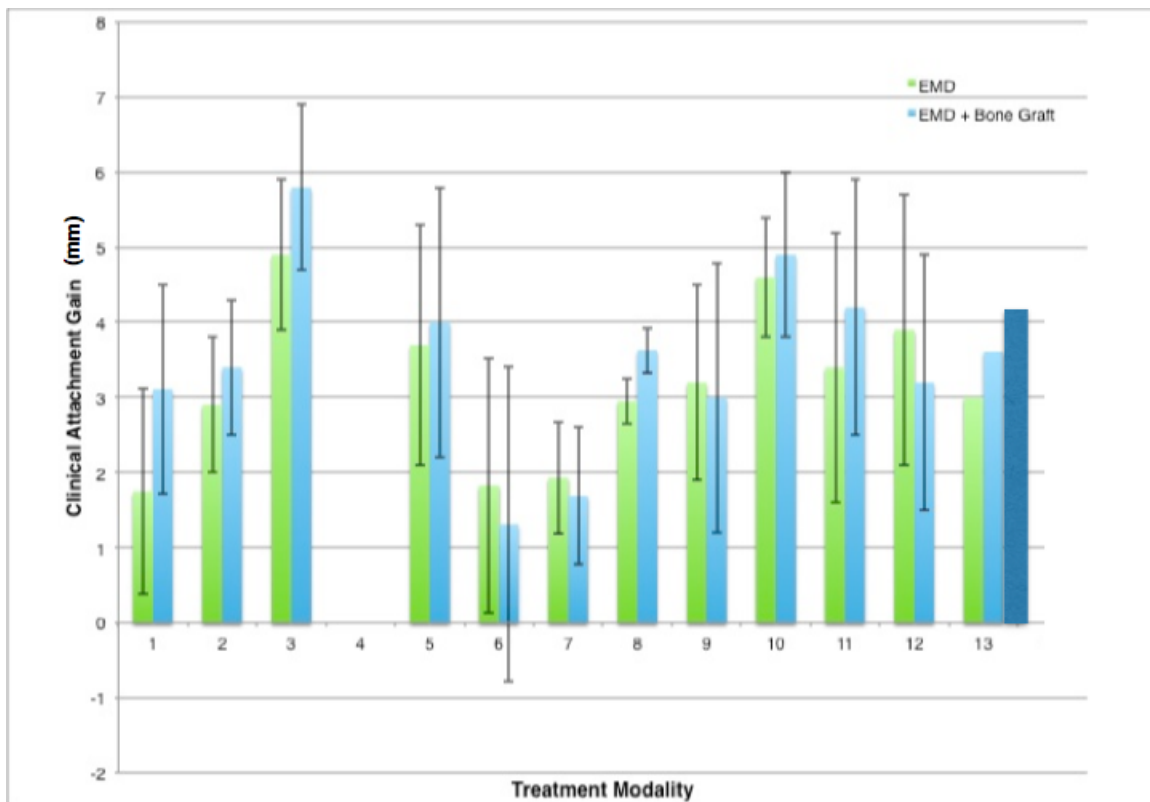


Figure 5. Comparison of Mean Clinical Attachment Gain (CAL) between EMD Groups and EMD + Bone Graft Groups

For studies reporting changes in CAL, comparable gains in clinical attachment level were found for defects treated with EMD alone and EMD plus bone graft. Lekovic et al. reported that combination group presented with significantly more attachment gain (buccal defects: 3.1 ± 1.4 mm; lingual defects: 3.1 ± 1.4 mm) than the EMD group (buccal defects: 1.7 ± 1.3 mm; lingual defects: 1.8 ± 1.4 mm)³⁰.

Clinical Recession Changes

The following figure depicts the mean recession change among EMD groups and EMD plus bone graft groups (Figure 6).

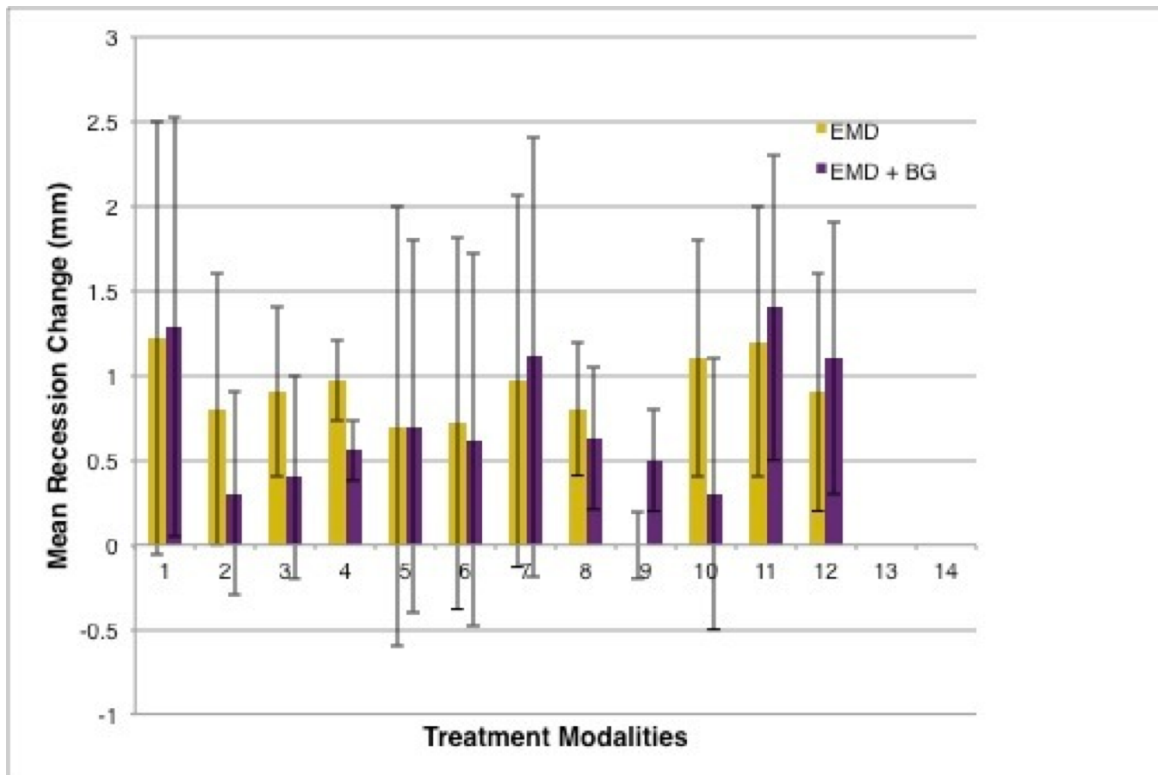


Figure 6. Comparison of Mean Recession Change among EMD groups and EMD + BG group in all studies

In this analysis, the reported mean REC change is statistically significant only in study #10. The authors concluded that addition of bone graft resulted in a decrease in post-surgery recession⁶¹. Study #13 did not report REC changes.

Re-entry Bone Fill Changes

The following figure depicts the mean re-entry bone fill changes among EMD groups and EMD plus bone graft groups (Figure 7).

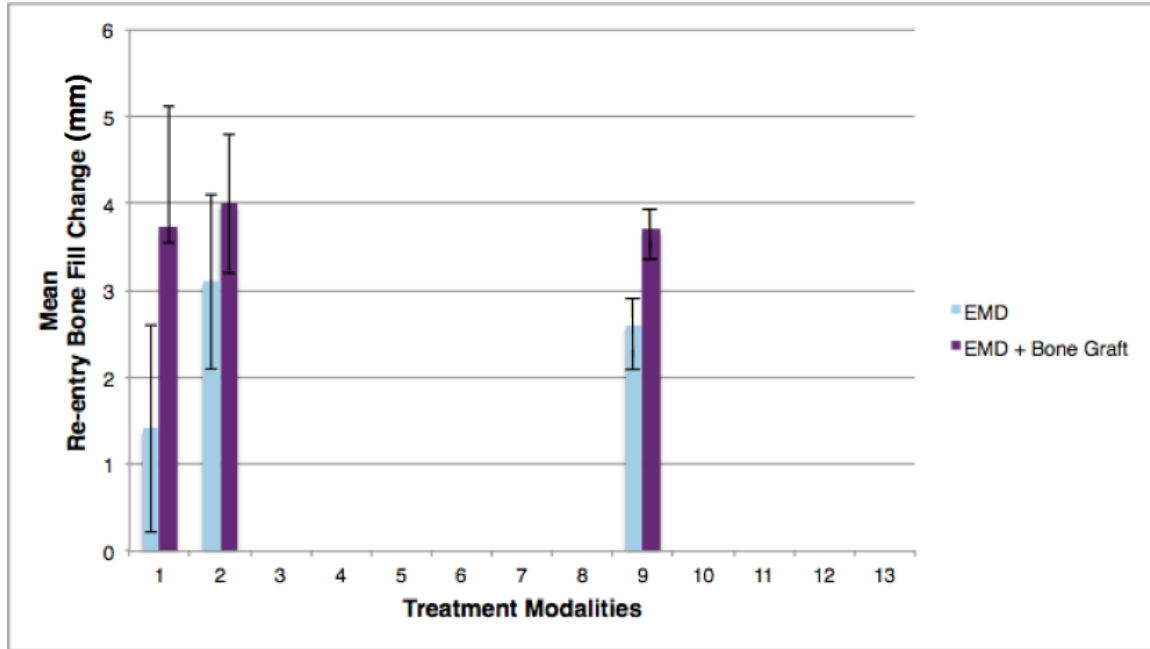


Figure 7. Comparison of Mean Re-entry Bone Fill Change among EMD groups and EMD + BG group in all studies

Based on the reported results from all studies, only three studies completed re-entry bone fill assessment^{30, 62, 68}, whereas 4 studies conducted radiographic bone gain assessment^{33, 60, 64, 65}. Lekovic et al. reported statistically significant PD reduction, attachment gain and defect fill (3.8 ± 1.4 mm on buccal sites of the intrabony defects and 3.7 ± 1.4 mm on lingual sites of the intrabony defects for combination therapy versus 1.3 ± 1.2 mm on buccal sites of the intrabony defects and 1.41 ± 1.19 mm on lingual sites of the intrabony defects) for combination therapy³⁰. Velasques-Plata et al. reported that bone fill was greater for combination therapy (4.0 ± 0.8 mm) compared to EMD alone (3.1 ± 1.0 mm, $P < 0.02$)⁶⁸. Gurinsky et al⁶² reported statistically significant changes between two

treatment modalities. The mean value for bone fill in the EMD plus DFDBA group was 3.7 ± 0.2 mm compared to 2.6 ± 0.4 mm for the EMD alone group. Therefore, all three studies that included surgical re-entry as part of their study methods confirmed that combination therapy significantly improved the bone fill in intrabony defects in comparison to EMD treated defects.

Radiographic Bone Gain Changes

The following figure depicts the mean radiographic bone gain changes among EMD groups and EMD plus bone graft groups (Figure 8).

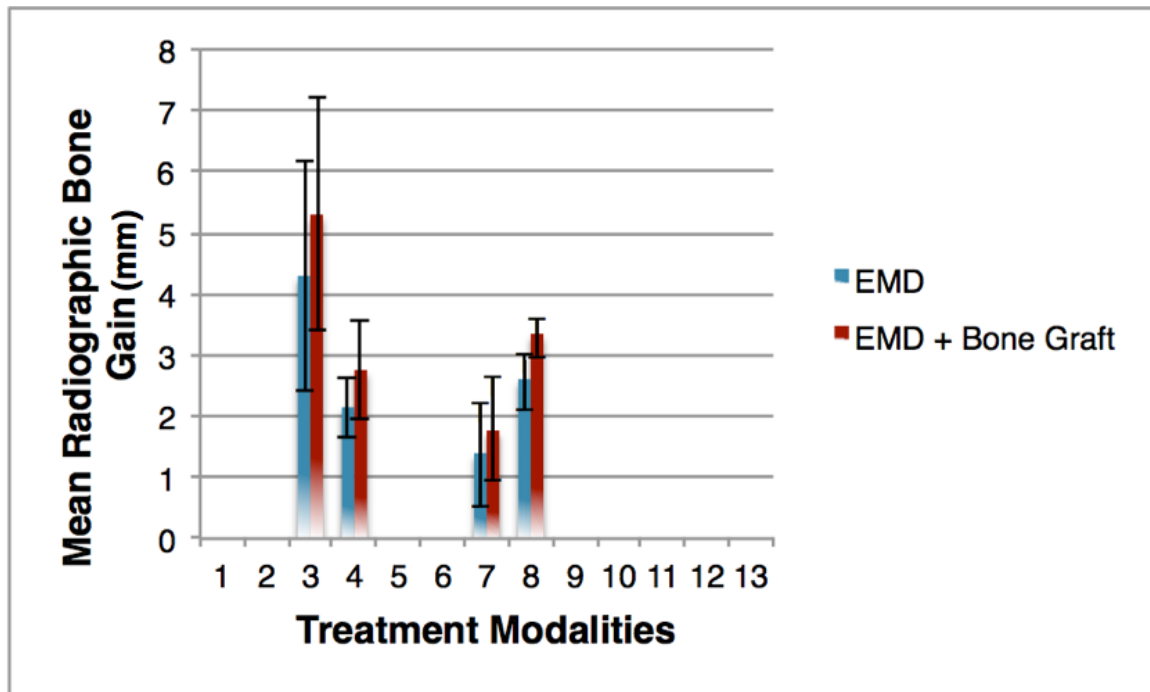


Figure 8. Comparison of Mean Radiographic Bone Gain Change among EMD groups and EMD + BG group in all studies

Three studies completed re-entry bone fill assessment^{30, 62, 68}, whereas 4 studies reported radiographic bone fill^{33, 60, 64, 65}. Zucchelli et al. reports statistically greater gain in radiographic bone (DEPTH) level in the EMD plus bone graft group (5.3 ± 1.1 mm) compared to EMD alone group (4.3 ± 1.5 mm)³³. Kuru et al. reported a gain in radiographic bone fill of 2.15 ± 0.42 for EMD alone group and 2.76 ± 0.69 mm for combination group⁶⁴. Meyle et al. reported comparable outcomes in radiographic bone gain for both treatment modalities (EMD plus bone graft: 2.7 ± 1.9 mm; EMD alone: 2.8 ± 1.6 mm)⁶⁵. Similarly, De Leonardis et al reported a greater gain in radiographic bone

fill for EMD plus bone graft (3.35 ± 0.80 mm) compared to EMD alone (2.61 ± 0.49 mm)

60

Differences in Clinical Parameter Outcomes for EMD and EMD + Bone Graft

A comparison of weighted (sample size) mean differences between EMD alone and EMD plus bone graft was performed for each clinical parameter across studies. These outcomes included changes in pocket depth, clinical attachment level, recession, defect fill and radiographic bone gain (Figure 9).

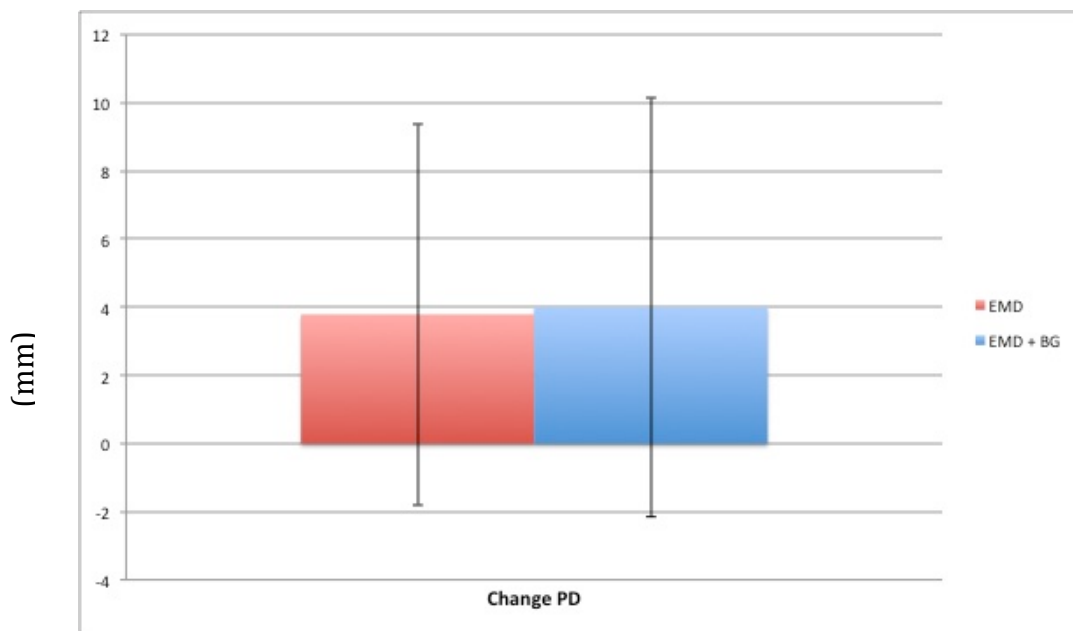


Figure 9. Comparison of Weighted Mean Pocket Depth Change Between EMD only and EMD + Bone Groups

A comparison of probing depth reduction based on a weighed mean differences analysis revealed no difference between treatment groups (EMD alone: 3.79 ± 5.6 mm; EMD plus bone graft: 4.0 ± 6.15 mm, NS).

The following figure depicts the weighted mean clinical attachment level changes between EMD groups and EMD plus bone graft groups (Figure 10).

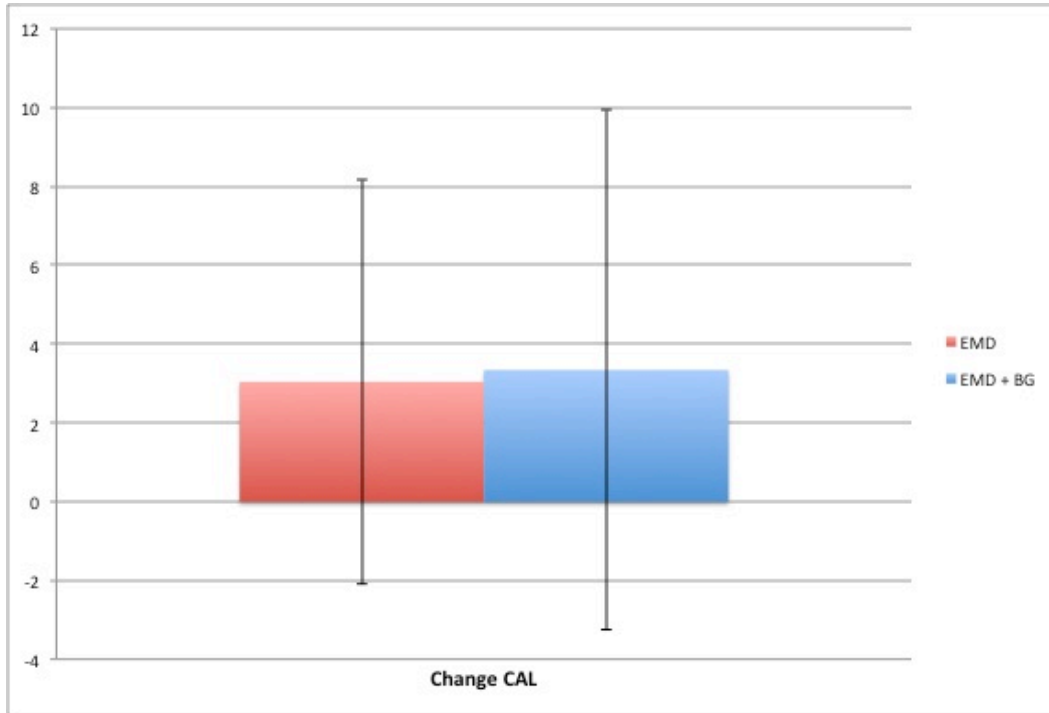


Figure 10. Comparison of Weighted Mean Clinical Attachment Levels Change Between EMD only and EMD + Bone Groups

Comparable gains in clinical attachment level were also found for treatment groups (EMD alone: 3.0 ± 5.1 mm; EMD plus bone graft: 3.3 ± 6.6 mm, NS).

The difference between the two treatment groups was not statistically significant for post-surgical recession reduction although both groups improve this clinical parameter similarly (0.4 ± 4.11 for EMD group versus 0.49 ± 6.3 for EMD + BG, $p > 0.05$) (Figure 11).

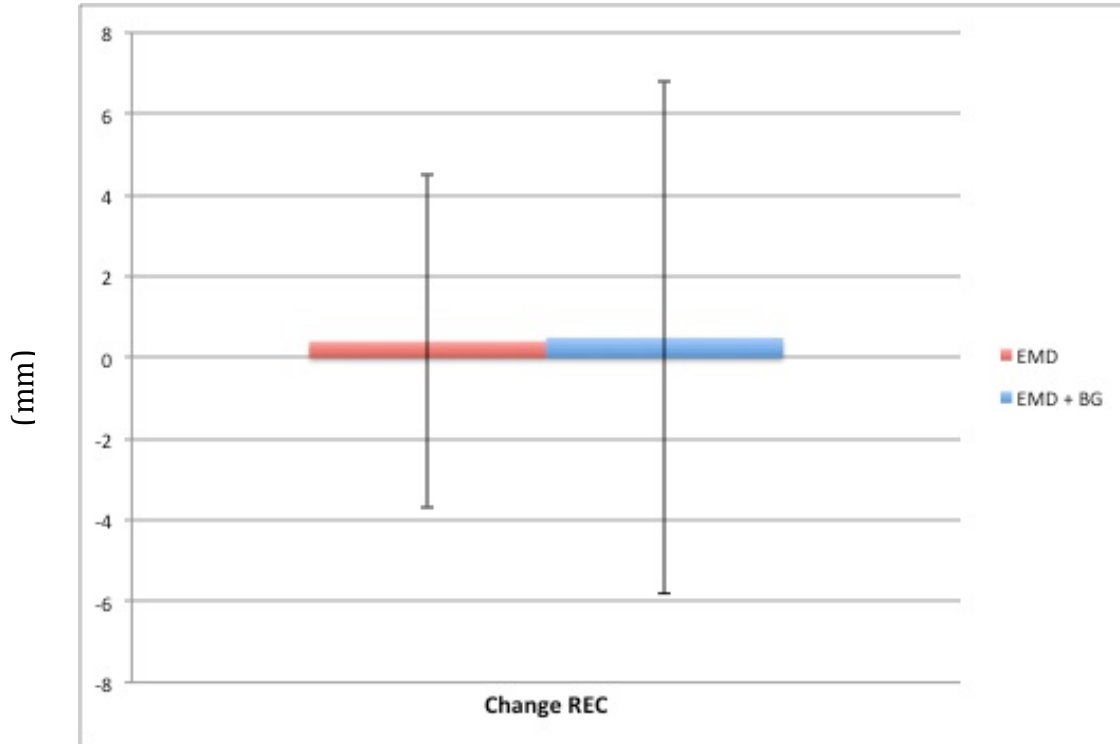


Figure 11. Comparison of Weighted Mean Recession Change Between EMD only and EMD + Bone Groups

Treatment with EMD plus bone graft was associated with a significantly greater increase in bone fill than with EMD alone (3.8 ± 0.7 mm versus 2.4 ± 3.9 mm, $p < 0.05$) (Figure 12).

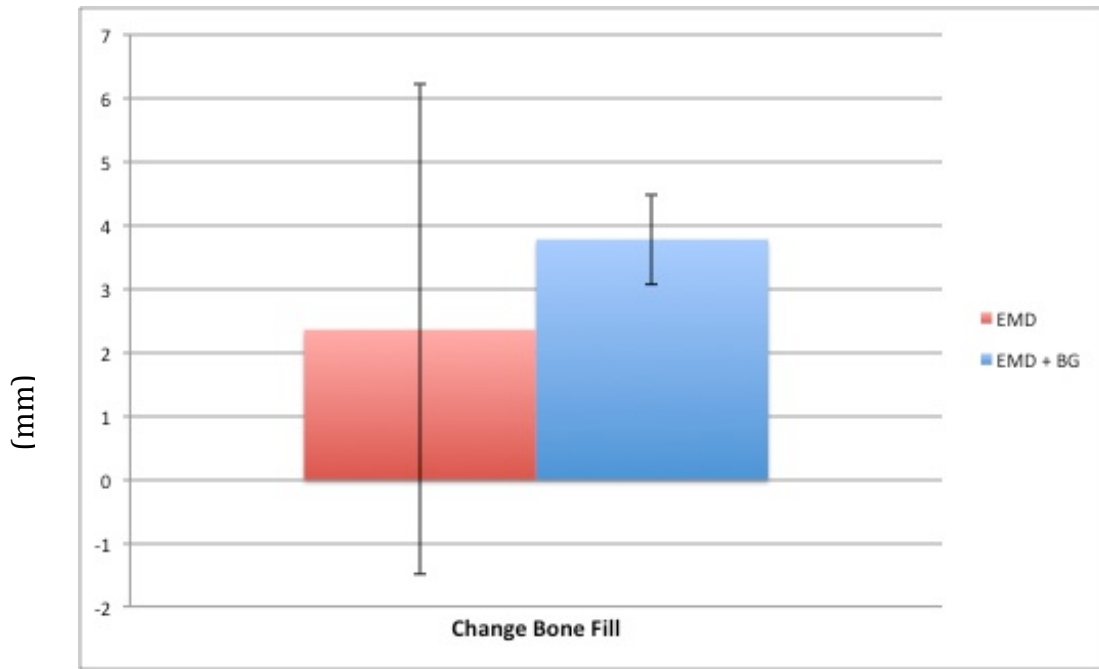


Figure 12. Comparison of Weighted Mean Bone Fill Change Between EMD only and EMD + Bone Groups ($P < 0.05$)

Similar gains in radiographic bone fill were observed between treatment groups (EMD alone: 2.9 ± 9.3 mm; EMD plus bone graft: 3.0 ± 6.1 mm, NS) (Figure 13).

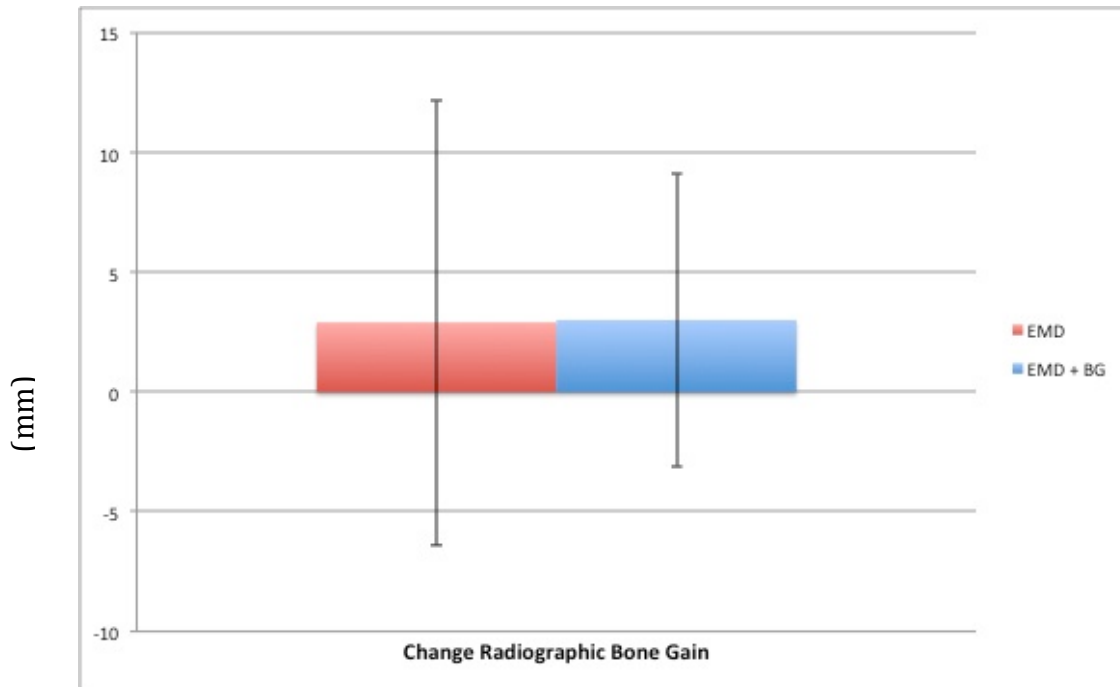


Figure 13. Comparison of Weighted Mean Radiographic Bone Gain Change Between EMD only and EMD + Bone Groups

Differences in Clinical Parameter Outcomes for Mammalian Bone Group and Synthetic Bone Group

In a subgroup analysis, differences in clinical outcome measures were compared separately for studies comparing EMD and mammalian bone grafts versus EMD and synthetic bone grafts (Figure 14).

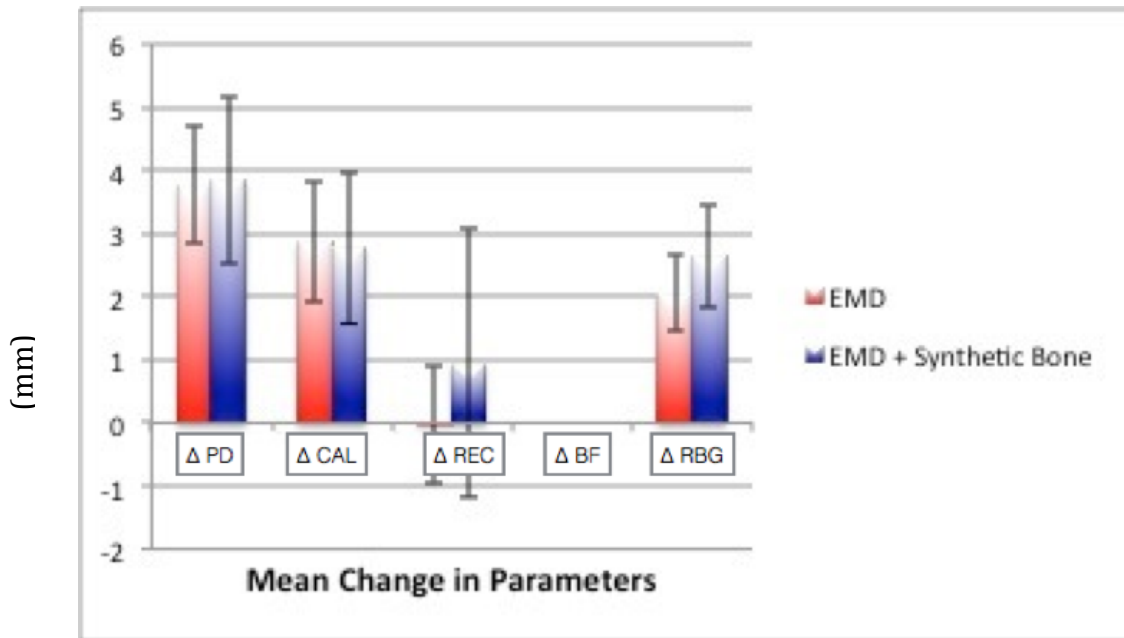


Figure 14. Mean Change in Parameters in EMD only Group and EMD + Synthetic Bone Group

Improvements in clinical outcome measures were similar for EMD combined with either mammalian or synthetic bone grafts. No statistically significant differences in outcomes were found when comparing mammalian and synthetic grafts. Changes in mean bone fill could not be evaluated due to lack of a single re-entry surgery for any of synthetic bone graft studies.

In studies comparing EMD alone to EMD plus mammalian bone graft, a significant trend of greater defect fill with EMD plus bone graft (3.8 ± 0.2 mm) compared to EMD alone (2.4 ± 0.9) ($p < 0.05$) (Figure 15).

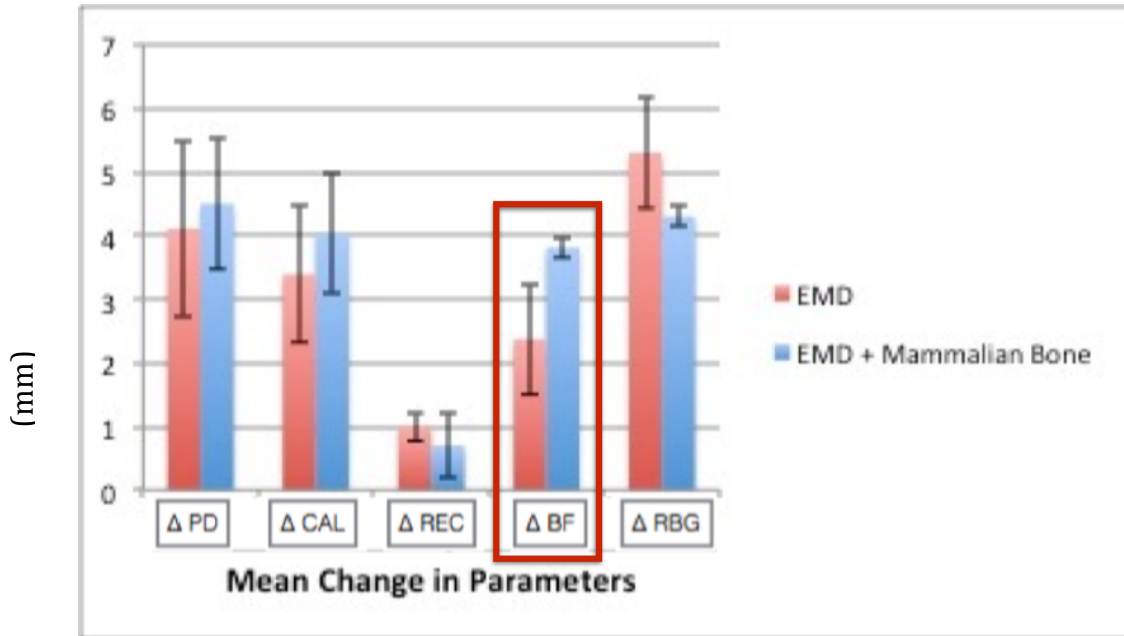


Figure 15. Mean Change in Parameters in EMD only Group and EMD + Mammalian Bone Group

According to this figure, the mammalian-derived bone group enhanced all changes in clinical parameters when compared to the synthetic group (Figure 16).

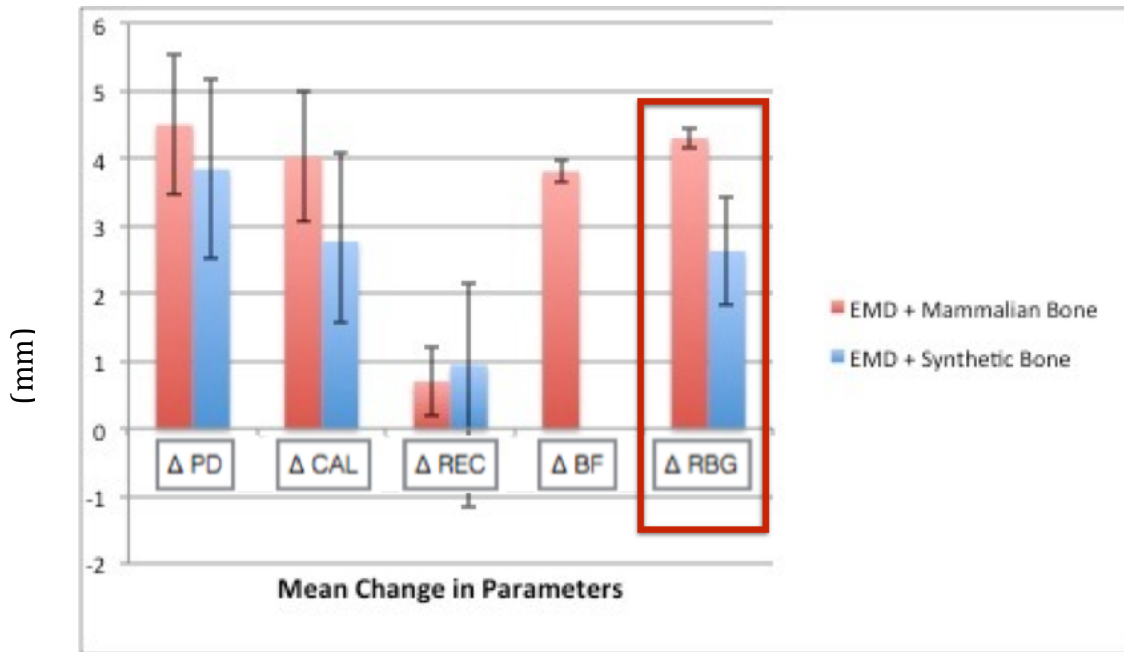


Figure 16. Mean Change in Parameters in EMD + Mammalian Bone Group and EMD + Synthetic Group

A significantly greater gain in radiographic bone fill was observed for EMD plus mammalian bone (4.3 ± 1.5 mm) compared to EMD plus synthetic bone graft (2.6 ± 0.8). Although mammalian bone is able to significantly improve the mean bone fill changes, there was no synthetic group that could be compared to assess this difference.

V. Discussion

This systematic review compared the clinical outcomes obtained in studies comparing EMD alone and EMD combined with bone replacement grafts in intrabony defects. Overall, these studies reported changes in pocket depth, clinical attachment levels and recession. EMD alone or in combination with a bone graft support significant CAL gains and reductions in PD. EMD plus bone graft supported greater gains in re-entry bone defect fill than EMD alone.

Gains in CAL and defect fill are surrogate measures for periodontal regeneration. The formation of a long junctional epithelium, the reestablishment of the connective attachment, and an increase in bone fill are associated with improvements in CAL⁷¹. Bone fill is often considered more accurate surrogate measure of periodontal regeneration than CAL. Listgarten (1979) and Nyman (1982), for example, have argued that only surgical re-entry can evaluate the bone fill⁷². Nevertheless, histological analysis is currently the only procedure to determine the extent of actual periodontal regeneration. In addition to surgical re-entry and bone sounding, radiographs also provide a relatively noninvasive assessment of bone fill; however, this method is not as reliable as surgical re-entry⁷¹.

Multiple studies have demonstrated that application of EMD in treating intrabony defects results in significant gains in CAL and reductions in PD compared to open flap-debrided defects^{73, 74}. They reported no statistically significant difference between these treatment modalities when recession was evaluated.^{25, 59, 73-75} Some studies also reported notable heterogeneity in clinical outcomes associated with EMD treatment.⁷⁶ EMD plus bone

graft was associated with a lower coefficient of variation in mean defect fill than observed for EMD only (21 versus 163, respectively). In other words, treatment of intrabony defects with EMD alone was associated with a greater variability in defect fill. This variability in outcome was also reported for GTR therapy with only a membrane in intrabony defects⁷⁵.

Clinically, EMD and GTR therapy result in comparable improvements in clinical measures in the treatment of intrabony defects.^{29, 76-78} Multiple studies report significant improvements in clinical and radiographic parameters when GTR is combined with a bone graft^{77, 79}. Rosenberg (1998) reported that an average bone fill of 3 mm, or about 60% defect fill, regardless of type of graft material⁸⁰. Furthermore, superior histological healing has also been reported when GTR is combined with a bone graft²⁵.

Clearly, in any regenerative procedure, whether EMD or GTR or a combination of these surgical techniques with bone graft, predictability in obtaining improved clinical improvements is important. Independent of the extent of clinical improvement, bone grafts and GTR therapy have been shown to reduce variability in clinical outcome, when compared to open flap debridement^{75, 81}. As we look for different bone grafts and membrane materials in treating intrabony defects, it is unclear what effects these may have on variability in treatment outcomes⁷⁵. However, based on clinical evidence, combination therapy offers improved relative predictability and superior clinical outcomes⁷⁵.

In this systematic review, EMD alone and EMD plus bone graft supported significant improvements in CAL and PD. No significant differences between EMD alone or EMD

plus bone graft were observed for PD reduction, CAL gain, REC, and radiographic bone fill. However, a statistically significant difference in bone fill between groups was found, favoring EMD plus bone graft. This finding is in agreement with majority of the studies that report comparable clinical outcomes for both treatments.

Factors that can affect the treatment outcome and influence the predictability of periodontal regeneration include the anatomic and biological characteristics of the defect, properties of the graft material, use of biologically active mediators, environmental factors such as smoking, the clinician's experience and surgical skill, and the patient's compliance with the post-operative instructions for oral hygiene^{82, 83}. The anatomical characteristics of the defect can positively or negatively affect the clinical outcomes. For example, flap position is well maintained in narrow 3-wall defects, whereas flap support is generally not provided in 1-wall defects without a graft material^{15, 83}. With increasing loss of defect walls, clotting and wound stabilization become less predictable. Presurgical PD directly affects the CAL gain and amount of bone formation^{83, 84}. Deeper defects generally exhibit more regenerative potential than shallower defects.

Of course, not all grafts function similarly in defect regeneration. In a case series by Rosen and Reynolds, treatment of intrabony defects with EMD plus FDBA resulted in superior results in CAL in comparison to EMD plus DFDBA¹⁴. This difference was attributed to the better physical properties of FDBA. In comparison of the changes in clinical outcomes of mammalian bone grafts and synthetic bone graft, it is suggested

that the mammalian bone graft material enhances all changes in clinical parameters in comparison to synthetic bone graft material.

Synthetic graft materials, when used alone, do not support periodontal regeneration.

Clinical studies document the potential for synthetic grafts to support reductions in PD and gains in CAL; however, histologic studies reveal the formation only of a long junctional epithelium and fibrous encapsulation of synthetic particles¹³. Interestingly, a histologic study reported that EMD plus bioactive glass group was predominantly characterized by formation of cementum and an associated PDL, whereas treatment with bioactive glass alone resulted mostly in a down growth of epithelium and no or only limited regeneration of cementum and PDL.⁶⁷

Smoking is the most important contributing factor for periodontal disease⁸⁵. The deleterious effects of smoking on regenerative procedures also been well documented in many studies^{86, 87, 88}. Smokers exhibit compromised healing and regenerative potential. In the present review, 5 studies excluded smokers, regardless of their smoking habits^{60, 65-67, 69}. One study allowed 2 smokers in each group, with the rationale that equal numbers in the treatment groups balanced the potential negative effects of smoking equally across groups⁶⁸. One study allowed non-smokers and occasional smokers⁶³. Two studies allowed the inclusion of smokers into their studies, but did not discuss the influence of smoking on their outcomes^{53, 68}. The remaining studies in this review did not exclude smokers or provide information on their inclusion in the study^{59, 62, 64}. Overall, the impact of smoking was not reported or summarized for the study outcomes.

Another factor that influences the outcome of regenerative surgery is surgical protocol. Flap management and accurate graft and biologic material handling can enhance or negatively affect the therapy outcome. Flap management in the reviewed clinical studies could affect the final results. Conservative surgery with interproximal tissue preservation that allows for primary coverage of the regenerating tissues and early oral hygiene procedures can achieve superior clinical results in comparison with intrasulcular incisions^{82, 89}. Only 5 studies utilized simplified or modified papilla preservation technique. The remaining studies used intrasulcular incision with full thickness flap reflection. Use of microscopes can greatly result in clinically significant amounts of CAL gains and minimal recession⁹⁰. Application of microscopes and high magnification loupes will allow the operator to be more accurate in incision design and use smaller sutures to obtain primary closure. Only one study in this review used a microscope help achieve primary closure.⁶⁰

Root conditioning is another factor that may not enhance clinical outcomes but may improve the predictability of the regenerative outcome. In this review, 11 of 13 studies used 24% EDTA to remove the smear layer^{53, 55, 59-64, 67-69}. Ogihara et al. used minocycline (10mg/ml) for 3 minutes prior to application of EMD⁶⁶. One study did not mention if they used EDTA or any other root conditioning agent⁶⁵. The question arises whether application of any specific conditioning agent is necessary. Recent research has shown that the type and concentration of conditioning agent (EDTA, citric acid, tetracycline or minocycline) affects the ability of EMD to bind effectively to the root surface. The application of 24% EDTA (pH neutral) has been shown to selectively

remove the smear layer, retain collagen fibers, and permit cellular colonization in comparison to citric acid or phosphoric acid, which cause tissue necrosis adjacent to the denuded dentin surfaces and remove collagenous matrix^{44-46, 91}. In an in vitro study, Miron et al. showed that EDTA was able to generate an even layer of demineralized acellular extrinsic fiber cementum matrix, which allowed a more even labeling for EMD at the root surface⁹²; the non-EDTA treated surfaces lacked this even absorption, which may clinically affect the PDL cell attachment⁹².

Larger clinical trials are necessary incorporating similar surgical protocols, comparable intrabony defects with similar morphology, and clinical re-entry or radiographic assessment of bone fill to determine the effectiveness of EMD in combination with bone grafts in the treatment of intrabony defects.

VI. Conclusion

In conclusion, EMD or EMD in combination with a bone graft supports significant improvement in clinical parameters in the treatment of intrabony defects. Direct measurement at surgical reentry revealed greater defect fill following treatment with EMD and graft, when compared to EMD alone. Moreover, EMD combined with mammalian bone grafts resulted in greater defect that EMD combined with synthetic grafts.

References

1. Eke PI, Dye BA, Wei L, et al. Update on Prevalence of Periodontitis in Adults in the United States: NHANES 2009 - 2012. *Journal of Periodontology* 2015;1-18.
2. Schluger S. Osseous resection; a basic principle in periodontal surgery. *Oral Surg Oral Med Oral Pathol* 1949;2:316-325.
3. Ochsenein C. A Primer for Osseous Surgery. *The International Journal of Periodontics & Restorative Dentistry* 1968;6:8-47.
4. Diem CR, Bowers GM, Moffitt WC. Bone blending: a technique for osseous implants. *Journal of Periodontology* 1972;43:295-297.
5. Robinson E. Osseous coagulum for bone induction. *Journal of Periodontology* 1969;40:503-510.
6. Schallhorn RGH, W.H.; Boyce, W. Iliac transplants in periodontal therapy. *Journal of Periodontology* 1970;41:566-580.
7. Nabers CL. Long-term results of autogenous bone grafts. *The International Journal of Periodontics & Restorative Dentistry* 1984;4:50-67.
8. Nabers CL, O'Leary TJ. Autogenous Bone Transplants in the Treatment of Osseous Defects. *Journal of Periodontology* 1965;36:5-14.
9. Drago MR, Sullivan HC. A clinical and histological evaluation of autogenous iliac bone grafts in humans. I. Wound healing 2 to 8 months. *Journal of Periodontology* 1973;44:599-613.
10. Drago MR, Sullivan HC. A clinical and histological evaluation of autogenous iliac bone grafts in humans. II. External root resorption. *Journal of Periodontology* 1973;44:614-625.
11. Hiatt WH, Schallhorn RG. Intraoral transplants of cancellous bone and marrow in periodontal lesions. *Journal of Periodontology* 1973;44:194-208.
12. Froum SJ, Kushner L, Scopp IW, Stahl SS. Human clinical and histologic responses to Durapatite implants in intraosseous lesions. Case reports. *Journal of Periodontology* 1982;53:719-725.
13. Meffert RMT, J. R.; Caudill, R. F. Hydroxyapatite implantation--clinical and histologic analysis of a treated lesion and speculations regarding healing phenomena. *The International Journal of Periodontics & Restorative Dentistry* 1986;6:60-65.
14. Rosen PSR, M.A. A Retrospective Case Series Comparing the Use of Demineralized Freeze-Dried Bone Allograft and Freeze-Dried Bone Allograft Combined With Enamel Matrix Derivative for the Treatment of Advanced Osseous Lesions. *Journal of Periodontology* 2002;73:942-949.
15. Bowers GM, Chadroff B, Carnevale R, et al. Histologic evaluation of new attachment apparatus formation in humans. Part II. *Journal of Periodontology* 1989;60:675-682.
16. Bowers GM, Chadroff B, Carnevale R, et al. Histologic evaluation of new attachment apparatus formation in humans. Part III. *Journal of Periodontology* 1989;60:683-693.

17. Bowers GMC, B.; Carnevale, R.; Mellonig, J.; Corio, R.; Emerson, J.; Stevens, M.; Romberg, E. Histologic evaluation of new attachment apparatus formation in humans. Part I. *Journal of Periodontology* 1989;60:664-674.
18. Becker W, Becker BE, Berg L, Samsam C. Clinical and volumetric analysis of three-wall intrabony defects following open flap debridement. *Journal of Periodontology* 1986;57:277-285.
19. Melcher AH. On the repair potential of periodontal tissues. *Journal of Periodontology* 1976;47:256-260.
20. Nyman S, Gottlow J, Karring T, Lindhe J. The regenerative potential of the periodontal ligament. An experimental study in the monkey. *Journal of Clinical Periodontology* 1982;9:257-265.
21. Gottlow J, Nyman S, Lindhe J, Karring T, Wennstrom J. New attachment formation in the human periodontium by guided tissue regeneration. Case reports. *Journal of Clinical Periodontology* 1986;13:604-616.
22. Gottlow J, Laurell L, Lundgren D, et al. Periodontal tissue response to a new bioresorbable guided tissue regeneration device: a longitudinal study in monkeys. *The International Journal of Periodontics & Restorative Dentistry* 1994;14:436-449.
23. Cortellini P, Pini Prato G, Tonetti MS. Periodontal regeneration of human intrabony defects with bioresorbable membranes. A controlled clinical trial. *Journal of Periodontology* 1996;67:217-223.
24. Reynolds MA, Bowers GM. Periodontal regeneration following surgical treatment. *Curr Opin Periodontol* 1996;3:126-139.
25. Sculean A, Nikolidakis D, Schwarz F. Regeneration of periodontal tissues: combinations of barrier membranes and grafting materials - biological foundation and preclinical evidence: a systematic review. *Journal of Clinical Periodontology* 2008;35:106-116.
26. Luepke PGM, J. T.; Brunsvold, M. A. A clinical evaluation of a bioresorbable barrier with and without decalcified freeze-dried bone allograft in the treatment of molar furcations. *Journal of Clinical Periodontology* 1997;24:440-446.
27. McClain PK, Schallhorn RG. Long-term assessment of combined osseous composite grafting, root conditioning, and guided tissue regeneration. *The International Journal of Periodontics & Restorative Dentistry* 1993;13:9-27.
28. Khoshkam V, Chan HL, Lin GH, et al. Outcomes of regenerative treatment with rhPDGF-BB and rhFGF-2 for periodontal intra-bony defects: a systematic review and meta-analysis. *Journal of Clinical Periodontology* 2015;42:272-280.
29. Kao RT, Nares S, Reynolds MA. Periodontal regeneration - intrabony defects: a systematic review from the AAP Regeneration Workshop. *Journal of Periodontology* 2015;86:S77-104.
30. Lekovic V. C, P.M., Weinlaender, M., Nedic, M., Aleksic, Z., Kenny E.B. A Comparison Between Enamel Matrix Proteins Used Alone or in Combination With Bovine Porous Bone Mineral in the Treatment of Intrabony Periodontal Defects in Humans. *Journal of Periodontology* 2000;71:1110-1116.
31. Mellonig J. Enamel Matrix Derivative for Periodontal Reconstructive Surgery: Technique and Clinical and Histological Case Report. 1999.

32. Sculean A, Barbe G, Chiantella GC, Arweiler NB, Berakdar M, Brex M. Clinical evaluation of an enamel matrix protein derivative combined with a bioactive glass for the treatment of intrabony periodontal defects in humans. *Journal of Periodontology* 2002;73:401-408.
33. Zucchelli G, Amore C, Montebugnoli L, De Sanctis M. Enamel matrix proteins and bovine porous bone mineral in the treatment of intrabony defects: a comparative controlled clinical trial. *Journal of Periodontology* 2003;74:1725-1735.
34. Brookes SJ, Robinson C, Kirkham J, Bonass WA. Biochemistry and molecular biology of amelogenin proteins of developing dental enamel. *Arch Oral Biol* 1995;40:1-14.
35. Slavkin HC. Towards a cellular and molecular understanding of periodontics. Cementogenesis revisited. *Journal of Periodontology* 1976;47:249-255.
36. Owens PD. Ultrastructure of Hertwig's epithelial root sheath during early root development in premolar teeth in dogs. *Arch Oral Biol* 1978;23:91-104.
37. Hammarstrom L. Enamel matrix, cementum development and regeneration. *Journal of Clinical Periodontology* 1997;24:658-668.
38. Maycock J, Wood SR, Brookes SJ, Shore RC, Robinson C, Kirkham J. Characterization of a Procine Amelogenin Preparation, EMADOGAIN, a Biological Treatment for Periodontal Disease. *Connective Tissue Research* 2002;43:472-476.
39. Newman SAC, S.A.; Jotwani, R.; Iacono, V.J.; Cutler, C.W. Effects of Enamel Matrix Derivative on Porphyromonas gingivalis. *Journal of Periodontology* 2003;74:1191-1195.
40. Spahr AL, S.P.; Boeckh, C.; Andersson, C.; Podbielski, A.; Haller, B. Effect of the enamel matrix derivative EmdogainA on the growth of periodontal pathogens in vitro. *Journal of Clinical Periodontology* 2002;29:62-72.
41. Miron RJ, Dard M, Weinreb M. Enamel matrix derivative, inflammation and soft tissue wound healing. *Journal of periodontal research* 2014.
42. Yan XZ, Rathe F, Gilissen C, et al. The effect of enamel matrix derivative (Emdogain(R)) on gene expression profiles of human primary alveolar bone cells. *Journal of Tissue Engineering and Regenerative Medicine* 2014;8:463-472.
43. Heng NH, Zahlten J, Cordes V, et al. Effects of Enamel Matrix Derivative and Transforming Growth Factor-beta1 on Connective Tissue Growth Factor in Human Periodontal Ligament Fibroblasts. *Journal of Periodontology* 2015;86:569-577.
44. Wu SC, H.; Chin, Y.; Lin, H.; Chiang, C.; Tu, H.; Fu, M.M.J.; Fu, E. Effects of enamel matrix derivative on the proliferation and osteogenic differentiation of human gingival mesenchymal stem cells. *Stem Cell Research & Therapy* 2014;5.
45. Amin HD, Olsen I, Knowles JC, Dard M, Donos N. Effects of enamel matrix proteins on multi-lineage differentiation of periodontal ligament cells in vitro. *Acta Biomaterialia* 2013;9:4796-4805.
46. Kawase TO, K.; Momose, M.; Kato, Y.; Yoshi, H.; Burns, D.M. Enamel matrix derivative (EMDOGAIN) rapidly stimulates phosphorylation of the MAP kinase family and nuclear accumulation of smad2 in both oral epithelial and fibroblastic human cells. *Journal of Periodontal Research* 2001;36:367-376.

47. Sculean AA, T.M.; Donos, N.; Brex, M.; Arweiler, N.B. Effect of an enamel matrix protein derivative (EmdogainA) on ex vivo dental plaque vitality. *Journal of Clinical Periodontology* 2001;28:1074-1078.
48. Arweiler NB, Auschill TM, Donos N, Sculean A. Antibacterial effect of an enamel matrix protein derivative on in vivo dental biofilm vitality. *Clinical Oral Investigations* 2002;6:205-209.
49. Blomlof JP, Blomlof LB, Lindskog SF. Smear layer formed by different root planing modalities and its removal by an ethylenediaminetetraacetic acid gel preparation. *The International Journal of Periodontics & Restorative Dentistry* 1997;17:242-249.
50. Blomlof J, Blomlof L, Lindskog S. Effect of different concentrations of EDTA on smear removal and collagen exposure in periodontitis-affected root surfaces. *Journal of Clinical Periodontology* 1997;24:534-537.
51. Blomlof J, Lindskog S. Root surface texture and early cell and tissue colonization after different etching modalities. *Eur J Oral Sci* 1995;103:17-24.
52. Heijl L, Heden G, Svardstrom G, Ostgren A. Enamel matrix derivative (EMDOGAIN) in the treatment of intrabony periodontal defects. *Journal of Clinical Periodontology* 1997;24:705-714.
53. Bhutda G, Deo V. Five years clinical results following treatment of human intrabony defects with an enamel matrix derivative: a randomized controlled trial. *Acta Odontol Scand* 2013;71:764-770.
54. Parodi RL, G.; Patrucco, P.; Brunel, G.; Santarelli, G.A.E.; Birardi, V.; Gasparetto, B. Use of Emdogain in the treatment of Deep Intrabony Defects: 12-Month Clinical Results. Histologic and Radiographic Evaluation. *The International Journal of Periodontics & Restorative Dentistry* 2000;20:585-595.
55. Yukna R.; Mellonig JT. Histologic Evaluation of Periodontal Healing in Humans Following Regenerative Therapy With Enamel Matrix Derivative. A 10-Case Series. *Journal of Periodontology* 2000;7:752-759.
56. Zetterstrom O, Andersson C, Eriksson L, et al. Clinical safety of enamel matrix derivative (EMDOGAIN) in the treatment of periodontal defects. *Journal of Clinical Periodontology* 1997;24:697-704.
57. Wang HLG, H.; Fiorellini, J.; Giannobile, W.; Offenbacher, S.; Salkin, L.; Townsend, C.; Sheridan, P.; Genco, R. J. Periodontal regeneration. *Journal of Periodontology* 2005;76:1601-1622.
58. Ebell MHS, J.; Weiss, B.D.; Woolf, S.H.; Susman, J.; Ewigman, B. Strength of Recommendation Taxonomy (SORT): a patient-centered approach to grading evidence in the medical literature. *Am Fam Physician* 2004;69:549-557.
59. Bokan I, Bill JS, Schlagenhauf U. Primary flap closure combined with Emdogain alone or Emdogain and Cerasorb in the treatment of intra-bony defects. *Journal of Clinical Periodontology* 2006;33:885-893.
60. De Leonardis D, Paolantonio M. Enamel matrix derivative, alone or associated with a synthetic bone substitute, in the treatment of 1- to 2-wall periodontal defects. *Journal of Periodontology* 2013;84:444-455.
61. Guida L, Annunziata M, Belardo S, Farina R, Scabbia A, Trombelli L. Effect of autogenous cortical bone particulate in conjunction with enamel matrix derivative

- in the treatment of periodontal intraosseous defects. *Journal of Periodontology* 2007;78:231-238.
62. Gurinsky BS, Mills, M.P., Mellonig, J.T. Clinical Evaluation of Dimenralized Freeze-dried Bone Allograft and Enamel Matrix Derivative Versus Enamel Matrix Derivative Alone for the Treatment of periodontal Osseous defects in humans. *Journal of Periodontology* 2004;75:1309-1318.
 63. Jepsen S, Topoll H, Rengers H, et al. Clinical outcomes after treatment of intra-bony defects with an EMD/synthetic bone graft or EMD alone: a multicentre randomized-controlled clinical trial. *Journal of Clinical Periodontology* 2008;35:420-428.
 64. Kuru B, Yilmaz S, Argin K, Noyan U. Enamel matrix derivative alone or in combination with a bioactive glass in wide intrabony defects. *Clinical Oral Investigations* 2006;10:227-234.
 65. Meyle J, Hoffmann T, Topoll H, et al. A multi-centre randomized controlled clinical trial on the treatment of intra-bony defects with enamel matrix derivatives/synthetic bone graft or enamel matrix derivatives alone: results after 12 months. *Journal of Clinical Periodontology* 2011;38:652-660.
 66. Ogihara S, Tarnow DP. Efficacy of enamel matrix derivative with freeze-dried bone allograft or demineralized freeze-dried bone allograft in intrabony defects: a randomized trial. *Journal of Periodontology* 2014;85:1351-1360.
 67. Sculean A, Windisch P, Keglevich T, Gera I. Clinical and histologic evaluation of an enamel matrix protein derivative combined with a bioactive glass for the treatment of intrabony periodontal defects in humans. *The International Journal of Periodontics & Restorative Dentistry* 2005;25:139-147.
 68. Velasquez-Plata DS, E.T.; Mellonig, J.T. Clinical Comparison of an Enamel Matrix Derivative Used Alone or in Combination With a Bovine-Derived Xenograft for the Treatment of Periodontal Osseous Defects in Humans. *Journal of Periodontology* 2002;73:433-440.
 69. Yilmaz S, Cakar G, Yildirim B, Sculean A. Healing of two and three wall intrabony periodontal defects following treatment with an enamel matrix derivative combined with autogenous bone. *Journal of Clinical Periodontology* 2010;37:544-550.
 70. Newman MG, Weyant R, Hujoel P. JEBDP improves grading system and adopts strength of recommendation taxonomy grading (SORT) for guidelines and systematic reviews. *J Evid Based Dent Pract* 2007;7:147-150.
 71. Alpiste Illueca FM, Buitrago Vera P, de Grado Cabanilles P, Fuenmayor Fernandez V, Gil Loscos FJ. Periodontal regeneration in clinical practice. *Med Oral Patol Oral Cir Bucal* 2006;11:E382-392.
 72. Listgarten MA, Rosenberg MM. Histological study of repair following new attachment procedures in human periodontal lesions. *Journal of Periodontology* 1979;50:333-344.
 73. Yilmaz S, Kuru B, Altuna-Kirac E. Enamel matrix proteins in the treatment of periodontal sites with horizontal type of bone loss. *Journal of Clinical Periodontology* 2003;30:197-206.

74. Jentsch HP, R. A clinical study evaluating the treatment of supra-alveolar-type defects with access flap surgery with and without an enamel matrix protein derivative: a pilot study. *Journal of Clinical Periodontology* 2008;35:713-718.
75. Aichelmann-Reidy ME, Reynolds MA. Predictability of clinical outcomes following regenerative therapy in intrabony defects. *Journal of Periodontology* 2008;79:387-393.
76. Esposito M, Coulthard P, Worthington HV. Enamel matrix derivative (Emdogain) for periodontal tissue regeneration in intrabony defects. *Cochrane Database Syst Rev* 2003;2:CD003875.
77. Keles GC, Sumer M, Cetinkaya BO, Tutkun F, Simsek SB. Effect of autogenous cortical bone grafting in conjunction with guided tissue regeneration in the treatment of intraosseous periodontal defects. *Eur J Dent* 2010;4:403-411.
78. Sculean A, Windisch P, Chiantella GC, Donos N, Brex M, Reich E. Treatment of intrabony defects with enamel matrix proteins and guided tissue regeneration. A prospective controlled clinical study. *Journal of Clinical Periodontology* 2001;28:397-403.
79. Paolantonio M. Combined periodontal regenerative technique in human intrabony defects by collagen membranes and anorganic bovine bone. A controlled clinical study. *Journal of Periodontology* 2002;73:158-166.
80. Rosenberg E, Rose LF. Biologic and clinical considerations for autografts and allografts in periodontal regeneration therapy. *Dent Clin North Am* 1998;42:467-490.
81. Reynolds MA, Aichelmann-Reidy ME. The era of biologics and reparative medicine: a pivotal clinical trial of platelet-derived growth factor for periodontal regeneration. *Journal of Periodontology* 2005;76:2330-2332.
82. Graziani F, Gennai S, Cei S, et al. Does enamel matrix derivative application provide additional clinical benefits in residual periodontal pockets associated with suprabony defects? A systematic review and meta-analysis of randomized clinical trials. *Journal of Clinical Periodontology* 2014;41:377-386.
83. Trombelli L, Kim CK, Zimmerman GJ, Wikesjo UM. Retrospective analysis of factors related to clinical outcome of guided tissue regeneration procedures in intrabony defects. *Journal of Clinical Periodontology* 1997;24:366-371.
84. Lindhe J, Westfelt E, Nyman S, Socransky SS, Heijl L, Bratthall G. Healing following surgical/non-surgical treatment of periodontal disease. A clinical study. *Journal of Clinical Periodontology* 1982;9:115-128.
85. Bergstrom JP, H. Tobacco use as a risk factor. *Journal of Periodontology* 1994;65:545-550.
86. Rosen PS, Marks MH, Reynolds MA. Influence of smoking on long-term clinical results of intrabony defects treated with regenerative therapy. *Journal of Periodontology* 1996;67:1159-1163.
87. Tonetti MS, Pini-Prato G, Cortellini P. Effect of cigarette smoking on periodontal healing following GTR in intrabony defects. A preliminary retrospective study. *Journal of Clinical Periodontology* 1995;22:229-234.
88. Reynolds MA, Kao RT, Camargo PM, et al. Periodontal regeneration - intrabony defects: a consensus report from the AAP Regeneration Workshop. *Journal of Periodontology* 2015;86:S105-107.

89. Murphy K. Interproximal tissue maintenance in GTR procedures: description of a surgical technique and 1-year reentry results. *The International Journal of Periodontics & Restorative Dentistry* 1996;16:463-477.
90. Cortellini PT, M.S. Microsurgical approach to periodontal regeneration. Initial evaluation in a case cohort. *Journal of Periodontology* 2001;72:559-569.
91. Blomlof JJ, L.; Blomlof, L.; Lindskog, S. Root surface etching at neutral pH promotes periodontal healing. *Journal of Clinical Periodontology* 1996;23:50-55.
92. Miron RJ, Bosshardt DD, Laugisch O, Katsaros C, Buser D, Sculean A. Enamel matrix protein adsorption to root surfaces in the presence or absence of human blood. *Journal of Periodontology* 2012;83:885-892.