The Effect of Enamel Matrix Derivative (Emdogain®) on Bone Formation: A Systematic Review

Florian Rathe, Dr. med. dent.,[1] Rüdiger Junker, Dr. med. dent., M.Sc.,[1] Betsy M. Chesnutt, M.Sc.,[1,2] and John A. Jansen, D.D.S., Ph.D.[1]

This systematic review focused on the question, if and to what extent enamel matrix derivative (Emdogain® [EMD]) promotes the regeneration of bone. The influence of combinations with other biomaterials was additionally evaluated. Twenty histomorphometric studies were included in this systematic review. Main results of the reviewed articles were (i) guide tissue regeneration (GTR) of infrabony defects seems to result in a higher degree of bone regeneration compared to treatment with EMD; (ii) combined therapy (GTR + EMD) of infrabony defects might not lead to better results than GTR therapy alone; (iii) there seems to be no additional benefit of combined therapy (GTR + EMD) in furcation defects over GTR therapy alone; (iv) EMD seems to lead to more bone regeneration of infrabony defects compared to open flap debridement; (v) however, EMD application might result in more bone formation when applied in supporting defects compared to nonsupporting defects; and (vi) EMD does not seem to promote external jaw/parietal bone formation in the titanium capsule model. The results of one study that suggest that EMD increases the initial growth of trabecular bone around endosseous implants by new bone induction need to be confirmed by additional research.

Introduction

PERIODONTAL REGENERATION is defined as the reproduction or reconstruction of lost or injured tissue so that the form and function of the lost structures are restored. In order to fulfill the criteria set by the American Academy of Periodontology,[1] periodontal regeneration has to include regeneration of cementum, functionally aligned periodontal ligament, alveolar bone, and gingiva.

Histological findings and clinical results are suggestive that regeneration of root cementum, periodontal ligament, and alveolar bone can in fact occur in human infrabony defects resulting from chronic periodontitis.[2,3]

Several studies have demonstrated that the use of enamel matrix derivative (Emdogain®[EMD]), a commercially available purified acidic extract from porcine enamel matrix containing the hydrophobic protein assembly of amelogenins, in periodontal regenerative surgery appears to favor the formation of new attachment characterized by the presence of new acellular and/or cellular cementum with inserting collagen fibers and new alveolar bone.[4–9] However, alveolar bone formation following the use of EMD has been reported to be minimal in spite of the presence of significant amounts of new cementum.[5,10,11] Up to now, the effect of EMD on bone formation is not well understood.

In vivo, EMD stimulates bone regeneration of rat femurs,[12] and it accelerates new bone formation in rat skull defects[13]; further, it has been shown that it contains both transforming growth factor– and bone morphogenetic protein–like growth factors that contribute to the induction of mineralization during periodontal regeneration.[14] However, EMD failed to show any significant benefit in promoting new bone formation around titanium implants in rabbits.[15] This systematic review focuses on the question, if and to what extent EMD promotes the regeneration of bone. The influence of combinations with other biomaterials was additionally evaluated.

Materials and Methods

Search strategy

A systematic literature search in electronic databases (PubMed and Cochrane Library) was conducted, using the following search term combination: "(emdogain OR EMD OR enamel matrix derivative OR enamel matrix proteins) AND (bone formation OR bone regeneration OR new bone formation OR osteogenesis)."

Further, a manual search in the references of the selected papers was performed, focusing on articles related to the effect of EMD on bone regeneration.

[1]Department of Periodontology and Biomaterials, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.
[2]Department of Biomedical Engineering, University of Memphis, Memphis, Tennessee.

TABLE 1. STUDIES EXCLUDED FROM SYSTEMATIC REVIEW

Study	Subjects	Sites investigated	Reason for exclusion
Bosshardt et al.[9]	Human	Periodontal infrabony defects	No histomorphometry performed
Gurinsky et al.[56]	Human	Periodontal infrabony defects	No histomorphometry performed
Mellonig[4]	Human	Periodontal infrabony defects	No histomorphometry performed
Sculean et al.[51]	Human	Periodontal infrabony defects	No histomorphometry performed
Yukna and Mellonig[8]	Human	Periodontal infrabony defects	No histomorphometry performed
Boyan et al.[59]	Animal	Mouse calf muscle	Ectopic bone formation investigated
Donos et al.[58]	Animal	Calvarial defects	No histomorphometry performed
Donos et al.[57]	Animal	Rat pectoralis profundi muscle	Ectopic bone formation investigated
Kanazashi et al.[55]	Animal	Periodontal buccal dehiscence defects	No bone formation investigated
Kawana et al.[12]	Animal	Rat femur	No histomorphometry performed
Koike et al.[54]	Animal	Rat rectus abdominis muscle	Ectopic bone formation investigated
Nemcovsky et al.[53]	Animal	Periodontal intra-suprabony defects	Mostly suprabony defects
Sculean et al.[52]	Animal	Fenestration-type defects	Defects healed spontaneously in all groups
Yoneda et al.[13]	Animal	Rat skull defect	No histomorphometry performed

Inclusion criteria

A literature search was performed to identify meta-analysis, systematic reviews as well as randomized-controlled clinical trials (RCTs), case reports, or case series.

Publications were considered for systematic review, if they were published until June 2007 in English language and listed in the electronic databases, PubMed or Cochrane Library, or were listed as reference in selected articles.

All articles had to provide histomorphometric data concerning the question if and to what extent EMD affects bone regeneration.

Selection of studies

Titles and abstracts of the publications identified by electronic databases were screened initially by two reviewers (F.R. and R.J.). Publications were included for full-text evaluation if the content of the abstracts met the inclusion criteria and matched to the focused question. Disagreement between the reviewers was resolved by evaluation of the full texts and discussion. Final authority for selection disagreements rested with R.J. Full-text assessment was performed by the reviewers without any disagreements. A manual search was performed among the references of the selected publications after full-text assessment.

Exclusion criteria

Publications were excluded if they did not meet the inclusion criteria (i.e., no histomorphometric analysis performed), or evaluated osteoinductive properties of EMD (i.e., ectopic bone formation), or did not provide relevant data for the focused question. Excluded studies and reasons for exclusion are listed in Table 1.

Results

Twenty studies were included for systematic review; studies were summarized in Tables 2 and 3. Of these 20 studies, 5 reported on human data. Three of these were case reports,[10,16,17] and two were RCTs.[5,11] The remaining 15 studies reported on data obtained from animal studies.

Results from human studies

The postoperative protocol was the same in three studies[5,10,11] and consisted of 1 g amoxicillin per day for 1 week postsurgery; patients were not allowed to perform oral hygiene at the surgical sites for 4 weeks and were instructed to rinse twice per day with 0.2% chlorhexidine mouth rinse during that time. Subsequently, professional prophylaxis was performed once every second week during the entire

TABLE 2. STUDIES REPORTING ON HUMAN DATA

Study	Type of defect	Sample size (number of defects)	Treatment groups	Results (mm of new bone formation/% of defect fill with new bone)	Histology performed (time allowed for healing)
Heijl[16]	Periodontal buccal dehiscence	n = 1	EMD	65%	4 months
Majzoub et al.[17]	Periodontal infrabony	n = 1	EMD	3.63 mm	9 months
Sculean et al.[5]	Periodontal infrabony	n = 14	G1: EMD	0.9 ± 1.0 mm	6 months
			G2: GTR	2.1 ± 1.0 mm	
Sculean et al.[10]	Periodontal infrabony	n = 2	EMD	0 and 1.7 mm	6 months
Windisch et al.[11]	Periodontal infrabony	n = 14	G1: EMD	0.78 ± 0.97 mm	6 months
			G2: GTR	1.93 ± 1.04 mm	

TABLE 3. STUDIES REPORTING ON ANIMAL DATA

Study	Type of defect	Sample size (number of defects)	Treatment groups	Results (mm of new bone formation/% of defect fill with new bone)	Histology performed (time allowed for healing)
Casati et al.[26]	Implant buccal dehiscence	n = 48	G1: EMD	55.55 ± 11.81% NBA	3 months
			G2: EMD + GBR	62.15 ± 18.47% NBA	
			G3: GBR	53.89 ± 16.35% NBA	
			G4: negative control	36.95 ± 25.10% NBA	
Cochran et al.[18]	Periodontal infrabony	n = 40	G1: EMD in 1-mm-wide defects	2.86 mm	5 months
			G2: OFD in 1-mm-wide defects	2.48 mm	
			G3: EMD in 2-mm-wide defects	3.14 mm	
			G4: OFD in 2-mm-wide defects	2.11 mm	
			G5: EMD in 4-mm-wide defects	1.22 mm	
			G6: OFD in 4-mm-wide defects	1.78 mm	
			G7: EMD in 6-mm-wide defects	2.01 mm	
			G8: OFD in 6-mm-wide defects	1.89 mm	
Donos et al.[23]	Class III furcation	n = 12	G1: EMD	71.95 ± 21.3%	5 months
			G2. EMD + GTR	73.3 ± 11.8%	
			G3: GTR	59.8 ± 32.5%	
			G4: CAF	43.9 ± 6.1%	
Donos et al.[28]	PTFE capsule	n = 16	G1: capsule + EMD	17.5%	120 days
			G2: capsule + DBBM	15.1%	
			G3: capsule + EMD + DBBM	12.0%	
			G4: capsule	39.7%	
Fernandes et al.[24]	Class III furcation	n = 20	G1: EMD + GTR	0.1 ± 0.3%	90 days
			G2: EMD + GTR + BG	2.0 ± 2.9%	
			G3: GTR + BG	0.8 ± 1.7%	
Hammarström et al.[21]	Periodontal buccal dehiscence	n = 94	G1: homog. enamel matrix	54%	8 weeks
			G2: amelogenin	73%	
			G3: enamelin	0%	
			G4: EMD	67%	
			G5: OFD	2%	
Murai et al.[29]	Calvarial	n = 28	G1: cap + β-TCP + EMD	42.2 ± 13.1%	1 month
			G2: cap + β-TCP	36.8 ± 10.3%	
			G1: cap + β-TCP + EMD	43.3 ± 6.8%	3 months
			G2: cap + β-TCP	41.2 ± 10.6%	
Onodera et al.[19]	Periodontal infrabony	n = 24	G1: GTR	55.2 ± 0.1%	4 weeks
			G2: GTR + EMD	77.6 ± 0.2%	
			G1: GTR	90.1 ± 0.12%	8 weeks
			G2: GTR + EMD	98.3 ± 0.04%	
Regazzini et al.[25]	Class II furcation	n = 24	G1: EMD	67.36 ± 3.93%	8 weeks
			G2: EMD + GTR	28.49 ± 10.32%	
			G3: OFD	31.65 ± 6.06%	

(Continued)

TABLE 3. (CONTINUED)

Study	Type of defect	Sample size (number of defects)	Treatment groups	Results (mm of new bone formation/% of defect fill with new bone)	Histology performed (time allowed for healing)
Sallum et al.[22]	Periodontal buccal dehiscence	$n = 24$	G1: EMD	2.01 ± 0.82 mm	4 months
			G2: GTR	0.91 ± 1.08 mm	
			G3: EMD + GTR	1.40 ± 1.08 mm	
			G4: OFD	1.14 ± 0.39 mm	
Sawae et al.[30]	Parietal	$n = 10$	G1: EMD	$0.97 \pm 0.04\%$	60 days
			G2: PGA	$0.74 \pm 0.17\%$	
Sculean et al.[6]	Periodontal infrabony	$n = 24$	G1: EMD	1.4 ± 0.9 mm	5 months
			G2: GTR	2.0 ± 1.1 mm	
			G3: EMD + GTR	2.1 ± 1.1 mm	
			G4: CAF	0.7 ± 1.1 mm	
Shimizu et al.[27]	Bone-to-implant contact	$n = 10$	G1: EMD	$12.82 \pm 1.42\%$[a]	30 days
			G2: PGA	$9.88 \pm 2.31\%$[a]	
Shirakata et al.[20]	Periodontal infrabony	$n = 16$	G1: α-TCP	3.58 ± 0.79 mm	10 weeks
			G2: α-TCP + EMD	3.07 ± 0.62 mm	
			G3: EMD	2.33 ± 0.92 mm	
			G4: OFD	1.95 ± 0.8 mm	
Stenport and Johansson[15]	Bone-to-implant contact	$n = 36$	G1: EMD	$10 \pm 8\%$ BMC	6 weeks
			G2: PGA	$12 \pm 3\%$ BMC	
			G1: EMD	$53 \pm 16\%$ NBA	
			G2: PGA	$58 \pm 11\%$ NBA	
			G1: EMD	$4.5 \pm 2.0\%$ BL	
			G2: PGA	$6.0 \pm 1.8\%$ BL	

[a]% of newly formed trabecular bone/medullary cavity after 30 days.
BG, bioactive glass; CAF, coronal advanced flap; BMC, bone-to-metal contact; BL, bone length.

study period. One study did not provide information about performed postoperative care,[16] and one study reported the use of 200 mg doxycycline per day for 10 days postsurgery.[17] The patient of this case report was instructed to rinse with 0.2% chlorhexidine three times daily for 6 weeks following surgery; the patient was recalled monthly for professional supragingival plaque control during the first 6 months postoperatively and every 3 months thereafter.

One buccal dehiscence defect in one case report[16] was surgically created. All other defects resulted from chronic periodontitis, and patients received full mouth scaling and root planing under local anesthesia 3 months prior to surgical procedures.

In two case reports, 37% orthophosphoric acid was applied for 15 s for root conditioning prior to application of EMD,[16,17] whereas 24% ethylenediaminetetraacetic acid (EDTA) was administrated for 2 min in the other three studies.[5,10,11]

Clinical healing was uneventful in all defects of all studies throughout the study periods, and no subjective adverse experiences were recorded.

New bone formation after treatment and the time point of histomorphometry are presented in Table 2.

In one case report,[16] a buccal dehiscence defect was created surgically at tooth 31 of a 49-year-old nonsmoking male and extended from the mesial to the distal line angle (at the line angle, the flat tooth surface converts into the curvature)

and was almost reaching the apex (exact defect size is not mentioned).

Majzoub et al.[17] reported on treatment with EMD of a mandible first molar of a 46-year-old woman presenting with a deep infrabony defect. The intraoperative morphology of the defect at the distal root demonstrated a deep infrabony circumferential defect involving all four surfaces of the root with an infrabony component of 8.5 mm; further, the tooth presented a trough and through furcation involvement. Two distinct healing patterns were evident 9 months after EMD treatment. At the distal surface of the distal root, new bone extended 3.63 mm coronal to the reference notch, which seemed to parallel cementum deposition throughout most of the defect, whereas an ankylosis was present at the furcal surface.

Two combined one- and two-walled advanced infrabony defects (maxillary left central incisor) had been treated with EMD in two patients aged 50 and 55 years in the third case report reviewed.[5]

Both RCTs[5,11] evaluated periodontal regeneration of advanced infrabony defects following the treatment with EMD or guided tissue regeneration (GTR) using bioabsorbable membranes (Resolut®). Fourteen infrabony defects were treated in each study, which were equally distributed to each group in the study from Sculean et al.[5] In the study of Windisch et al.,[11] eight defects were treated with GTR pro-

cedure and six with the use of EMD. Further, all patients were nonsmokers in the study of Sculean et al.,[5] whereas one smoker was treated with GTR procedure and two smokers were treated with EMD in the investigation of Windisch et al.[11] No membrane exposure was noted in either study. Both studies found a significant difference in new bone formation favoring the GTR treatment (Table 2). In 5 out of 13 specimens, no new bone formation could be evaluated histologically after EMD treatment; further, bone regeneration was minimal (0.2–0.5 mm) in four further defects. In the remaining four defects of the EMD group, bone regeneration could be evaluated with a range of 1.8–2.2 mm. Infrabony defects treated with GTR showed at least minimal bone formation (0.1 and 0.2 mm) in 2 defects, whereas the 12 remaining defects showed bone regrowth within a range of 1–3 mm.

Results from animal studies

Infrabony and dehiscence defects. All studies mentioned here investigated standardized periodontal infrabony[6,18–20] or dehiscence-type[21,22] defects, either in monkeys[6,18,21] or dogs.[19,20,22] In four studies,[6,18,19,22] ligature-induced inflammation was provoked after surgical defect creation to prevent spontaneous healing. In all studies, the postoperative protocol comprised the administration of antibiotics, whereas plaque control was only performed in four studies after regenerative therapy.[6,19,20,22] In most studies, root conditioning was only performed when EMD was applied.[6,18–20,22] Three studies[19–21] used phosphoric acid (concentration varied between 35% and 37% for 15 s), and all other studies[6,18,22] used EDTA-gel (concentration 24% for 2 min).

The sample size, type of defect, treatment modality, results, and time allowed for healing are presented in Table 3 for all studies reporting on animal data.

In the studies of Sculean et al.[6] and Onodera et al.,[19] infrabony defects of approximately 6- to 8-mm depth were created, but no information was given about the amount of bony walls. While reading the surgical procedure, one can assume that one- or two-wall defects were created. For the GTR procedure, either bioresorbable barrier membrane (Resolute[®6]) or nonresorbable extended-polytetrafluorethylene (e-PTFE) membrane (Gore-Tex[®19]) was used.

In the study of Sculean et al.,[6] a great amount of sites experienced membrane exposure; two sites (one of the GTR group and one of the GTR + EMD group) had to be excluded from histological evaluation because of early membrane exposure during the second postoperative week. Another site from the GTR group had to be excluded due to failure during histological processing. There were four additional sites showing membrane exposure in the third postoperative week (two of the GTR group and two of the GTR + EMD group). Those four sites were not excluded from histological evaluation, so that only two out of the four evaluated sites in the GTR group and three out of five sites of the GTR + EMD group experienced no membrane exposure. Even so, the most new bone formation was detected after the use of a barrier membrane.

No postoperative membrane exposure is mentioned in the study by Onodera et al.[19] In this study, no bone formation was evident 1 week after regenerative surgery in either group, but 2 (results not shown) and 4 weeks after surgery, significantly more bone formation ($p < 0.05$) could be de-

termined in the combined group (GTR + EMD, Onodera et al.[19]). However, 8 weeks postsurgery, no significant differences could be found between the two treatment groups.

The healing of standardized one-wall periodontal defects of 5×5×4 mm[20] and defects of standardized depth with varying width in the mesiodistal direction[18] was investigated in two further studies. Shirakata et al.[20] found no significant differences between EMD (G3) and open flap debridement (OFD) (G4) groups or between the EMD + α-tricalcium phosphate (TCP) (G2) and α-TCP (G1) groups (Table 3). However, if G3 and G4 are compared with G1 and G2 (G4 vs. G1 or G2, $p < 0.0001$; G3 vs. G1, $p < 0.0001$; G3 vs. G2, $p = 0.0057$), significantly more bone formation was determined in the EMD + α-TCP (G2) and α-TCP (G1) groups. The study of Cochran et al.[18] showed that EMD applied in narrow defects (1- and 2-mm-wide defects) resulted in 3.00-mm new bone compared to 2.29 mm of new bone for the control treatment, a 31% increase in bone height. In wider lesions (4- and 6-mm-wide defects), the amount of new bone was similar between EMD-treated sites and controls (1.67 and 1.84 mm).

Buccal dehiscence defects in both studies were approximately 6-mm deep,[21,22] whereas the width of the defect (4 mm) was only mentioned in the article by Sallum et al.[22] In the study by Hammarström et al.,[21] which was performed to explore the effect of locally applied enamel matrix and different protein fractions of the matrix on periodontal regeneration, it was shown that it is possible to obtain regeneration of 54–73% of bone tissues by applying the whole enamel matrix, the acid extract (amelogenin), or EMD in the buccal dehiscence model. Sallum et al.[22] utilized nonresorbable barrier membranes (Resolute) for the GTR procedure, and only one membrane of the EMD + GTR group experienced exposure in the first postoperative week. Regarding new bone formation, no statistically significant differences were observed among the groups (Table 3).

Furcation defects. Different regenerative procedures (Table 3) were evaluated in standardized furcation defects in monkeys[23] and dogs.[24,25] Two studies investigated class III furcation involvement with a vertical dimension of 3–4 mm[23] and 5 mm.[24] Regazzini et al.[25] created class II furcation defects with a vertical dimension of 5 mm and a horizontal component of 2 mm, which equals a class II furcation in dogs. Plaque formation with subsequent inflammation of the defect was provoked in all studies after surgical defect creation. Following regenerative surgery antibiotics were administered in all investigations, as well as plaque control regiments. Root conditioning was performed only in combination with EMD treatment in two studies,[23,24] whereas root surfaces of the control defects also received root conditioning in the trial of Regazzini et al.[25] EDTA-gel (24%) applied for 2 min was used in all studies for root conditioning. Resorbable membranes (Resolute[23] and Resolute XT[®24]) were utilized in two studies, and nonresorbable e-PTFE barrier membranes (Gore-Tex) were used in one study.[25]

Membranes were exposed 15 days after surgery in the study of Regazzini et al.,[25] but no information about the amount of exposed membranes was provided. No significant difference in terms of new bone formation was evident between the EMD + GTR group and the control treatment, but EMD treatment alone resulted in significant more bone

formation ($p < 0.05$) compared to groups EMD + GTR and OFD (for results see Table 3). In the experimental trial of Donos et al.,[23] exposure of the membrane occurred in two sites treated with GTR alone during the second postoperative week. In the third postoperative week, a defect treated with the combination of GTR and EMD presented exposure of the membrane. Additionally, one defect treated with EMD experienced recession of the flap to the level of the fornix of the furcation (for results see Table 3). No postoperative membrane exposure was mentioned in the study by Fernandes et al.[24] Statistical analysis of the treatment outcome did not show significant differences among the different groups for linear and area measurements; however, it must be pointed out that no negative control group (e.g., OFD) was evaluated (for results see Table 3).

Periimplant defects and bone-to-implant contact. Casati et al.[26] created buccal dehiscence defects (3.5×5.0 mm) in dogs before and 2 months after implant (3.75×8.5 mm; screw-shaped pure titanium) placement. The buccal dehiscence defects were treated with various regenerative procedures (Table 3; Resolute XT was used for guided bone regeneration [GBR]), and the postoperative protocol consisted of administration of antibiotics and daily plaque control until the animals were sacrificed. The percentage of bone-to-implant contact and new bone area (NBA) within the limits of the previously exposed threads of each implant were determined. After 3 months, no statistically significant differences were observed among the groups in terms of bone-to-implant contact (results not shown). However, the EMD + GBR group presented a greater ($p < 0.05$) area of new bone when compared to the control group. The groups treated by EMD or GBR alone showed no statistically significant differences in NBA when compared to controls or to the EMD + GBR group (Table 3). Thirty-six screw-shaped pure titanium implants (3.75×8.0 mm) were placed in rabbit femurs in the study by Stenport and Johansson.[15] Immediately prior to implant insertion, either EMD or its vehicle gel (propylene glycol alginate [PGA]) alone was placed into the surgically created implant site. The percentage of bone-to-metal contact and bone area were measured in all threads as well as the entire bone length along the implant surface, but the differences were not statistically significant. Shimizu et al.[27] inserted cylinder-shaped mini titanium implants (1.6×3.5 mm) with filling of medullary cavities with either EMD or its carrier. Mean percentage of newly formed trabecular bone per medullary cavity was assessed. In morphometric analysis, newly formed trabecular bone area within medullary cavities was significantly ($p < 0.05$) greater in EMD-treated femurs than in PGA-treated femurs 30 days postimplantation.

Bone formation in experimental capsule and parietal model. Donos et al.[28] placed hemispherical PTFE capsules (internal diameter: 5 mm and wall thickness: 0.5 mm) with openings facing the lateral aspect of the mandible ramus of Wistar rats. The animals were randomly allocated to four treatment groups (Table 3). Percentage of newly formed bone of the maximal possible space created by the capsule was determined by planimetric measurements. The PTFE capsule had been slightly displaced during healing in three out of five specimens of group 1, in one out of three of group 2, in

three out of four of group 3, and in two out of five in group 4 (Table 3), which resulted in a less-favorable treatment outcome compared to those capsules that had not been displaced. Statistically significant differences could be evaluated between capsule-alone group and capsule + deproteinized bovine bone mineral (DBBM) ($p = 0.034$) and capsule + DBBM + EMD ($p = 0.021$) groups, favoring the capsule-alone group in terms of new bone formation. In other words, there was no significant difference between capsule-alone group and capsule + EMD, whereas adding DBBM led to less-favorable results. The PTFE capsule model has also been utilized in rabbit calvarium by Murai et al.[29] The capsules were filled either with β-TCP + EMD or with β-TCP alone (Table 3). After both 1 ($p = 0.075$) and 3 months of healing (Table 3; $p = 0.92$), no significant differences in the amount of newly formed bone were found between test and control capsules. Sawae et al.[30] perforated the parietal bones of Wistar rats with a sterile round bur (0.8-mm diameter). The injured bone areas were immediately filled with EMD (test) or its PGA-carrier (control) and allowed to heal for 4, 7, 14, 30, and 60 days. The results were expressed as the mean percentages of newly formed bone areas per perforated space. Morphometric analysis showed that at only 60 days post-surgery, new bone formation in the EMD-treated parietal bones was significantly ($p < 0.05$) greater than that seen in PGA-treated controls (Table 3).

Discussion

The aim of this systematic review was to evaluate the osteopromotive properties of EMD *in vivo*, since it has not been done before. Twenty studies were included for systematic review. Five reported on human data with three of these being case reports and two being RCTs. The remaining 15 studies reported on data obtained from animal studies.

Unfortunately, none of the histomorphometric studies reporting on human data compared the amount of bone regeneration in periodontal defects after the use of EMD with a control surgery (i.e., OFD and coronal advanced flap). If bone regrowth was evaluated after the use of EMD and compared with results obtained after utilizing the GTR therapy with resorbable membranes, GTR therapy resulted in a higher degree of bone regeneration than treatment with EMD (Table 2[5,10,11]).

The amount of bone regrowth after the use of EMD in the two cases reported by Sculean et al.[10] is in accordance with the minimal bone regrowth reported by Sculean et al.[5] and Windisch et al.[11] Heijl[16] published outstanding results with respect to bone regeneration. He achieved a bone regrowth of 65% of the presurgical bone height of a buccal dehiscence defect reaching almost the apex. The discrepancy between the results reported by Sculean et al.[5,10] and Windisch et al.[11] and the case of Heijl[16] can be explained, at least in part, by spontaneous healing, since the buccal dehiscence defect treated by Heijl[16] was of an acute type (i.e., defect was surgically created and immediately treated without previous plaque exposure period). Histological studies in monkeys have shown that in acute defect models approximately 50–70% spontaneous regeneration can be expected, which may lead to difficulties in interpreting the results.[31] Majzoub et al.[17] achieved a bone regrowth of 3.63 mm in his case report. This result is comparable to the outstanding result

achieved by Heijl[16] with the difference that the defect treated by Majzoub et al.[17] was not created surgically but resulted from chronic periodontitis. Majzoub et al.[17] reported that bone regrowth appeared to parallel new cementum deposition, which is in contrast to the other publications[5,10,11] reporting new bone formation to be minimal compared to cementum deposition (results not shown).

Another interesting result reported by Majzoub et al.[17] is the ankylosis of the experimental tooth in the furcation area. It can be speculated that the necrotizing effect of low-pH phosphoric acid might have impaired the vitality and subsequently the healing potential of precursor cells involved in cementum and connective tissue formation, which resulted in ankylosis.[32–34] Root surface conditioning has been conventionally performed with 37% phosphoric acid with a pH value of one[16,35,36] or with EDTA 24% at a neutral pH.[37–41] Although it has been shown that long-time etching at low pH jeopardizes periodontal healing,[32] the low pH of phosphoric acid does not seem to impair bone regeneration when used according to the published protocols.[16,17]

Regarding the distal infrabony defect, the flap supporting the circumferential structure leading to wound stability could be a possible explanation for the high amount of bone regrowth achieved by Majzoub et al.,[17] since results from animal studies suggest that EMD results in less regeneration in non-supporting defects.[18,20]

Due to its gel-like consistence, EMD has no space-providing properties in order to avoid collapsing of the flap into the defect in cases where anatomical structures do not support the flap. However, this space provision seems to be a prerequisite for bone regeneration.[42]

The results of Polimeni et al.[42] seem to be supported by the finding that EMD gives rise to more bone regrowth in smaller-sized one-wall defects compared to larger-sized one-wall defects.[18] However, it should also be taken into account that the bone crest was narrower and, hence, more resorbed adjacent to the larger defects. As a result, the amount of regeneration was likely influenced by the position of the bone crest. In standardized nonsupporting one-wall defects, no difference between treatment with EMD and OFD could be determined. However, a statistically significant difference of OFD was achieved after adding a space-providing α-TCP cement to the EMD.[20] Sculean et al.[6] and Sallum et al.[22] also achieved more favorable results in terms of bone regrowth when EMD was combined with a space-providing GTR barrier membrane compared to EMD treatment alone. Differences in the study of Sculean et al.[6] were not statistically significant, and combined treatment resulted in new bone formation comparable to GTR therapy alone. Membrane exposure and subsequent bacterial colonization and infection were frequently occurring complications in this study, which might explain why Sallum et al.[22] found significantly better results for the combined treatment compared to EMD alone (only one exposed membrane). Surprisingly, Sallum et al.[22] reported no difference between GTR alone and OFD. Interestingly, Onodera et al.[19] determined that in the combined treatment procedure (GTR + EMD), EMD influenced new bone formation positively during the first 4 weeks of healing compared with GTR therapy alone, whereas no difference was apparent after week 8 of healing.

When treatment of infrabony defects with EMD alone is compared with OFD, most studies report significantly better results for the EMD therapy.[6,21,22] Unfortunately, supportive or nonsupportive structure (i.e., amount of bony walls) as well as the exact defect dimensions (width at the base of the pocket in orobuccal dimension, width in mesiodistal dimension, and height of the defect) are not reported in these articles, which makes it difficult to draw conclusions on the influence of anatomical factors.

Membrane exposure seemed to occur more frequently in monkey[6,23] than in dog models.[19,22,24,26] A possible explanation is that monkeys play with the sutures at the surgical site, which jeopardizes primary healing. It has been shown that membrane exposure with subsequent bacterial colonization and infection leads to a less-favorable treatment outcome.[43–47]

Regazzini et al.[25] reported significantly more new bone formation when EMD was applied in class II furcation defects, compared to OFD and the combined therapy (GTR + EMD). The authors of this study did not find a significant difference between the combined treatment and OFD. An explanation can be that membrane exposure is responsible for the treatment outcome of the combined group; unfortunately, the amount of sites that became exposed is not mentioned and could be minimal since the study was performed in dogs. The good treatment outcome for the EMD group could indicate that space-providing barrier membranes are not of major importance in class II furcation defects, since the tooth roots prevent collapse of the flap. Donos et al.[23] reported most favorable results for the combined therapy although differences to the EMD-alone group were negligible in the treatment of class III furcation defects. However, one out of three sites treated with the combined therapy (GTR + EMD) experienced membrane exposure that led to less-favorable results. The amount of new bone formation of the GTR-alone group was located between the other two test groups (GTR + EMD and EMD alone) and the OFD group, but it has to be taken into account that two out of three sites treated with GTR alone experienced membrane exposure. In the GTR-treated defect where membrane was not exposed, the defect had healed almost completely with bone. Whereas in those sites where the membrane became exposed, the amount of newly formed bone was minimal. On the other hand, only one of the three furcation defects treated with EMD alone had healed almost completely, which might suggest that treatment of class III furcation defects with EMD is unpredictable. In the assessment of bone fill in furcation class III defects in dogs, minimal formation was observed, limited to the basis of the defect in the three test groups studied (Table 3) without any statistical difference between the groups.[24] However, the number of animals is a weak point to be considered and a limitation to finding differences among groups.

Stenport and Johansson[15] concluded in their study that EMD treatment does not contribute to bone formation around titanium implants in a rabbit model. The authors suggested that the model used was not ideal to study the effects of bone-stimulating proteins because of the large cortical area that had to be resorbed before osseointegration could occur. This theory is supported by the findings of Tonetti et al.,[41] who reported in a clinical study that highly cortical and highly cancellous bone types negatively impacted the outcome of EMD treatment in infrabony defects. In contrast, the results of Shimizu et al.[27] suggest that EMD

increases the initial growth of trabecular bone around endosseous implants by new bone induction in the medullary cavities and maintains such bony support of implants by filling the surfaces with newly formed bone trabeculae. These newly formed bone trabeculae were immunoactive for bone sialoprotein (BSP), which is a noncollagenous bone matrix protein, and were localized at the mineralization site of developing bone.[48] BSP-positive trabecular bone formed after implantation is thought to be bone matrix produced by newly differentiated osteoblasts,[49] indicating that EMD promotes osteogenic differentiation of pluripotent mesenchymal cells. The results of Casati et al.[26] also indicate that EMD positively influences bone healing after GBR around titanium implants. EMD alone, however, had no statistically significant effect. The membrane could have provided a protected coagulum and also helped to avoid collapse of the flap into the dehiscence-type defect, supporting the results of Polimeni et al.[42]

In contrast to the findings from Shimizu et al.,[27] BSP was only weakly observed in the newly formed trabecular bone of parietal bone defects; however, 60 days postoperation, new bone formation in EMD-applied defects was significantly greater than that seen in PGA-applied controls.[30] Using a Teflon capsule model, the study by Donos et al.[28] failed to support the hypothesis that the adjunctive application of EMD to GBR would positively influence the formation of new jaw bone outside the skeletal envelope. This finding is in accordance with the study by Murai et al.[29] who found no significant difference in promotion of new bone formation between β-TCP alone, and the combination of EMD and β-TCP within a titanium capsule, but in contrast to observations in which a positive influence on bone formation was seen following the application of EMD under a barrier membrane.[22,26]

Conclusion

The findings of the studies should be considered with caution due to the small sample sizes. Small numbers of animals are frequently observed in the literature and are a consequence of the limitations when testing on larger animals, such as dogs and monkeys. In addition, direct extrapolation of data obtained from animal studies to humans should be interpreted cautiously. Further, small sample sizes and a lack of matching defects, due to obvious reasons, are the limitations in human histological trials.

Another very important point that needs to be mentioned is that the maximum time allowed for healing in the reviewed articles was 9 months, but bone regeneration seems to continue to increase over 36 months after EMD treatment.[50] Thus, bone regrowth might not be completed at the time of histological preparation.

The reviewed articles allow for the following conclusions:

Human studies have shown that GTR therapy of infrabony defects seems to result in a higher degree of bone regeneration than treatment with EMD. Animal studies suggest that EMD seems to lead to more bone regeneration of infrabony defects compared to OFD; however, EMD application might result in more bone formation when applied in supporting defects compared to nonsupporting defects. Further, most animal studies suggest that combined therapy

(GTR + EMD) of infrabony defects might not lead to better results than GTR therapy alone.

In animals, EMD seems to result in more bone formation compared to OFD in both furcation class II and III defects, but treatment of class III furcation defects with EMD is unpredictable. Additionally, there seems to be no additional benefit of combined therapy (GTR + EMD) in furcation defects.

EMD does not seem to promote external jaw/parietal bone formation in the titanium capsule model.

EMD might increase growth of trabecular bone around implants by new bone induction in the medullary cavities. However, when bony defects adjacent to dental implants have to be treated, combination with GBR procedures seems to lead to better results compared to application of EMD alone and GBR alone.

The cautious use of phosphoric acid for root conditioning prior to EMD application does not seem to influence bone regeneration negatively.

It might be suggested that EMD promotes osteogenic differentiation of pluripotent mesenchymal cells in vivo. Thus, EMD seems to promote bone formation, but the lack of space-providing properties might be a limiting factor. Further, an additional benefit of EMD in combination with a space-providing GTR technique seems to be limited.

For future studies, it would be desirable to mention the exact size of the infrabony defects because the width of the defect base seems to be especially crucial for determining the extent of bone regeneration.[42] The amount of bony walls should also be mentioned in order to allow for further conclusions and easier comparison between studies.

Conflict of Interest and Source of Funding Statement

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Address correspondence to:
Florian Rathe, Dr. med. dent.
Department of Periodontology and Biomaterials
Radboud University Nijmegen Medical Centre
Philips van Leydenlaan 25
6525 EX Nijmegen
The Netherlands

E-mail: f.rathe@dent.umcn.nl