Clin Oral Invest (2014) 18:471–478
DOI 10.1007/s00784-013-0992-5

ORIGINAL ARTICLE

Effects of enamel matrix proteins in combination with a bovine-derived natural bone mineral for the repair of bone defects

Richard J. Miron · Lingfei Wei · Dieter D. Bosshardt · Daniel Buser · Anton Sculean · Yufeng Zhang

Received: 6 November 2012 / Accepted: 23 April 2013 / Published online: 8 May 2013

Abstract
Objectives Previously, the use of enamel matrix derivative (EMD) in combination with a natural bone mineral (NBM) was able to stimulate periodontal ligament cell and osteoblast proliferation and differentiation. Despite widespread use of EMD for periodontal applications, the effects of EMD on bone regeneration are not well understood. The aim of the present study was to test the ability of EMD on bone regeneration in a rat femur defect model in combination with NBM.
Materials and methods Twenty-seven rats were treated with either NBM or NBM + EMD and assigned to histological analysis at 2, 4, and 8 weeks. Defect morphology and mineralized bone were assessed by μCT. For descriptive histology, hematoxylin and eosin staining and Safranin O staining were performed.
Results Significantly more newly formed trabecular bone was observed at 4 weeks around the NBM particles precoated with EMD when compared with NBM particles alone. The drilled control group, in contrast, achieved minimal bone regeneration at all three time points ($P<0.05$).
Conclusions The present results may suggest that EMD has the ability to enhance the speed of new bone formation when combined with NBM particles in rat osseous defects.
Clinical relevance These findings may provide additional clinical support for the combination of EMD with bone graft for the repair of osseous and periodontal intrabony defects.

Keywords Enamel matrix derivative · EMD · Emdogain · Natural bone mineral · Bio-Oss · Bone grafting materials

R. J. Miron · L. Wei · Y. Zhang
The State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) and Key Laboratory of Oral Biomedicine Ministry of Education, School and Hospital of Stomatology, Wuhan University, 237 Luoyu Road, Wuhan 430079, People's Republic of China

R. J. Miron · D. D. Bosshardt · A. Sculean
Department of Periodontology, School of Dental Medicine, University of Bern, Freiburgstrasse 7, 3010 Bern, Switzerland

D. Buser
Department of Oral Surgery and Stomatology, School of Dental Medicine, University of Bern, Freiburgstrasse 7, 3010 Bern, Switzerland

Y. Zhang (✉)
Department of Oral Implantology, School of Stomatology, Wuhan University, Wuhan 430079, China
e-mail: zyf@whu.edu.cn

Introduction

The treatment of bone defects or deficiencies still poses a great clinical challenge in periodontology and oral surgery. Although a variety of techniques exist to manage this problem, each technique presents its own advantages and disadvantages. One modality which has been shown to facilitate periodontal regeneration by stimulating new cementum, alveolar bone, and periodontal ligament (PDL) formation is the application of an enamel matrix derivative (EMD) [1–4]. The major component of EMD is amelogenins, a family of hydrophobic proteins that account for more than 95 % of the total protein content of the enamel matrix [5]. Other proteins found in the enamel matrix include enamelin, ameloblastin (also called amelin or sheathlin), amelotin, apin, and various proteinases [6, 7]. In order to improve the outcomes obtained with EMD, various combinations of EMD and different types of bone grafting materials have been used.

Findings from controlled clinical studies have indicated that a combination of EMD and some types of grafting materials may additionally improve the clinical outcomes compared to treatment with EMD alone or to grafting materials alone [8–14]. Despite the fact that periodontal regeneration has been histologically demonstrated and is accompanied by substantial clinical improvements following the use of EMD in intrabony defects [1], much less emphasis has been placed on determining the effects of EMD on pure bone defects.

One widely used and well-documented grafting material is the natural bone mineral (NBM), which is a highly purified anorganic bone matrix mineral from bovine origin. The material possesses an excellent biocompatibility and osteoconductivity and is routinely used in reconstructive bone surgery. Recently, we have demonstrated that EMD significantly increased cell attachment, proliferation and differentiation of human primary osteoblasts, and PDL cells on EMD-coated NBM particles when compared to control particles in vitro [15]. EMD also stimulated the release of growth factors, cytokines, and differentiation markers including bone morphogenetic protein 2, transforming growth factor beta 1, collagen1α1, alkaline phosphatase, and osteocalcin [15, 16]. To complement previous in vitro findings, the aim of the present study was to investigate the combination of EMD and NBM particles in a rat femur defect to determine the effects of EMD solely on bone regeneration. We hypothesized that precoating EMD on NBM particles would have a positive effect on bone regeneration in this model system due to the abundance of progenitor cells in the bone marrow cavity.

Materials and methods

Animals and surgical protocols

Twenty-seven male Wistar rats (mean body weight, 200 g) were used in this study with all handling and surgical procedures in accordance with the policies of the Ethics Committee for Animal Research, Wuhan University, China. All animals were kept at 20–25 °C under a 12-h light/dark cycle and allowed food and water ad libitum. All operations were carried out under sterile conditions with a gentle surgical technique. The surgeon (YF) was blinded to the treatment. A single intramuscular dose of penicillin 40,000 IU/ml was administered postoperatively. No significant preoperation or postoperation fractures were produced.

Femur defect drilling was performed under general anesthesia by intraperitoneal injection of sodium pentobarbital (40 mg/kg body weight). A linear skin incision of approximately 1 cm in the distal femoral epiphysis was made bilaterally, and blunt dissection of the muscles was performed to expose the femoral condyle [17, 18]. Then, a 2.2-mm-diameter anteroposterior bicortical channel was created perpendicular to the shaft axis to remove cancellous bone by using a trephine bur at a slow speed irrigated under saline solution to avoid thermal necrosis. The drilled holes were rinsed by injection with saline solution in order to remove bone fragments from the cavity. The morphology and position of the hole are presented in Fig. 1a. An equal amount of implant materials (0.1 g per hole) was then gently placed to fill the grilled defects according to group allocation: drilled control, NBM, and NBM + EMD (Emdogain® [i.e., EMD + propylene glycol alginate carrier], Institut Straumann, Basel, Switzerland), respectively (9 rats used at each time point (18 defects)). The three treatment groups were randomly divided into three groups of six defects. Each animal received two types of treatments such as control defects and NBM alone, or control defects and NBM + EMD, or NBM and NBM + EMD at each time point.

At each time point, 2.4 and 8 weeks after femur surgery, rats in these three groups were sacrificed accordingly. All femurs were removed and assigned to histological evaluation.

μCT analysis

The samples were fixed in 4 % formaldehyde for 24 h at room temperature. A μCT imaging system (μCT 50, Scanco Medical AG, Bassersdorf, Switzerland) was used to evaluate new bone formation within the defect region. All samples were placed in a custom-made holder to ensure that the long axis of the drilled channel was oriented perpendicular to the axis of X-ray beam. Scanning was performed at 55 kV and 114 μA with a thickness of 0.048 mm per slice in medium resolution mode, 1,024 reconstruction matrix, and 200 ms integration time.

Histological preparation and immunohistochemical studies

Femoral condyles were decalcified in 10 % ethylenediaminetetraacetic acid for 2 weeks, changed twice per week, and then dehydrated in a series of graded concentration of ethanol from 70 to 100 %. To get a distinct view of the defect, the orientation and alignment of femurs were carefully considered during paraffin embedding. A series of slices starting at a distance of 1 mm proximal from the end of the growth plate with a length of 2 mm were chosen for evaluation. For analysis of the bone regeneration process within the defect, the central region of the 2.5-mm-diameter defect was defined by analyzing a circular contour as area of measurement per slice, thus to obtain a consistent volume of interest and to avoid including the native bone margins.

Longitudinal serial sections, 4 μm thick, were cut and mounted on polylysine-coated microscope slides. For descriptive histology, hematoxylin and eosin (H&E) staining

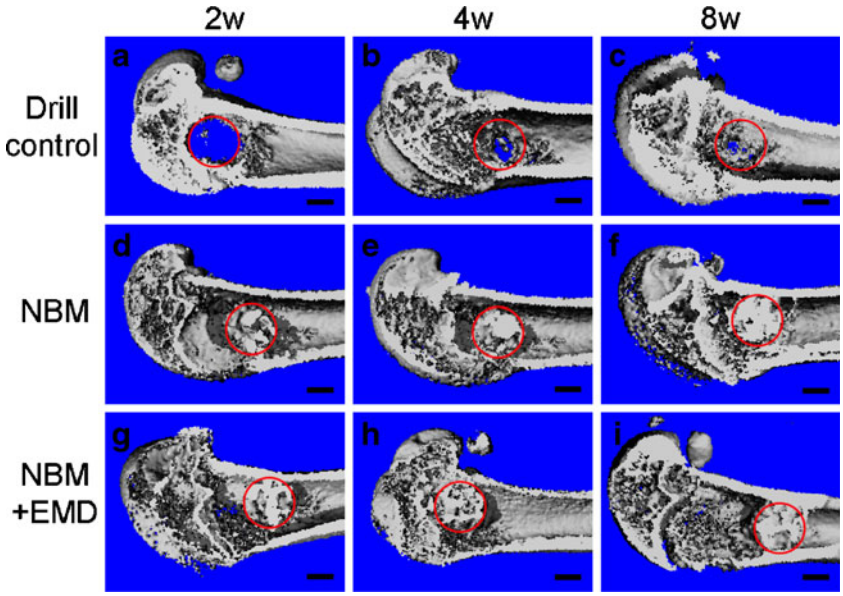

Fig. 1 μCT images demonstrating the morphology and defects either left unfilled (**a**–**c**), filled with NBM (**d**–**f**), or filled with NBM + EMD (**g**–**i**)

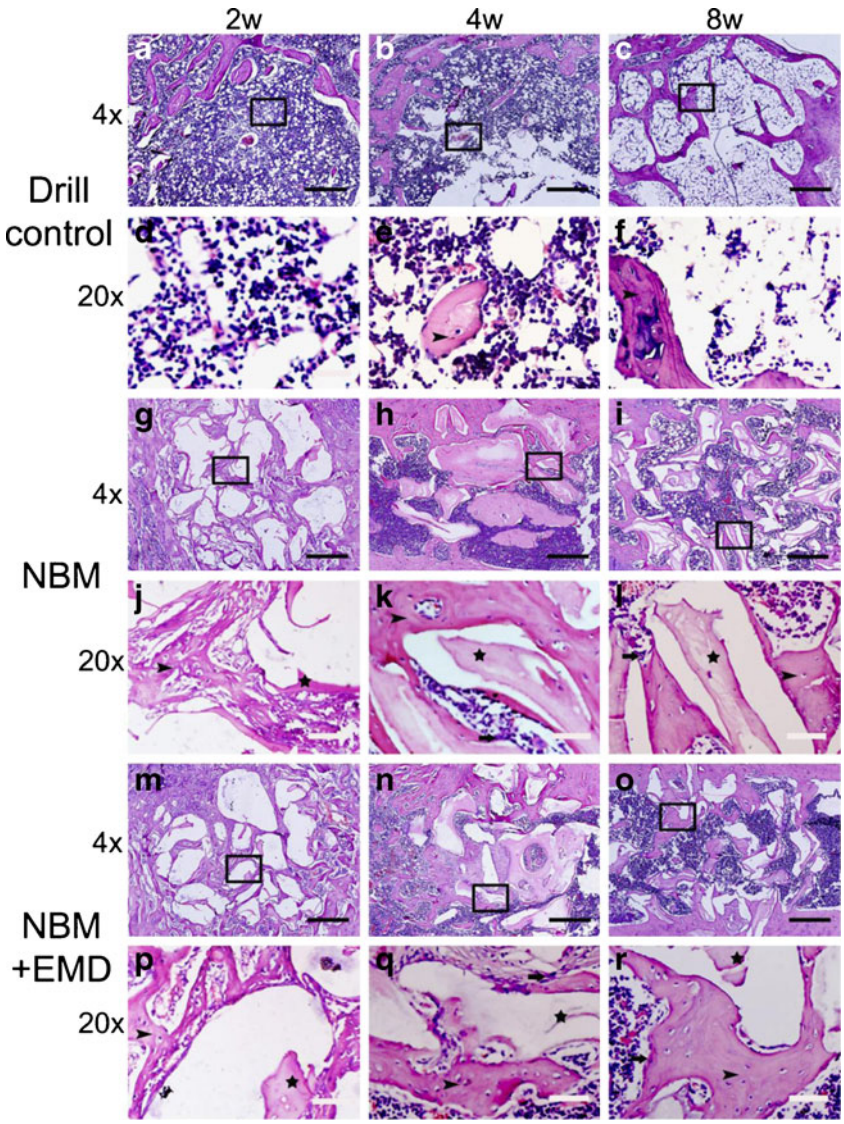

Fig. 2 Representative sections of H&E staining demonstrate the healing of defects left unfilled (**a**–**f**), filled with NBM (**g**–**l**), or filled with NBM + EMD (**m**–**r**) at 2, 4, and 8 weeks post-implantation. More newly formed bone can be observed in groups treated with NBM and NBM + EMD when compared to control drill defects. *Arrowhead*, newly formed bone; *black arrow*, osteoclasts; *asterisk*, filled graft; *black scale bar*, 500 μm; *white scale bar*, 50 μm

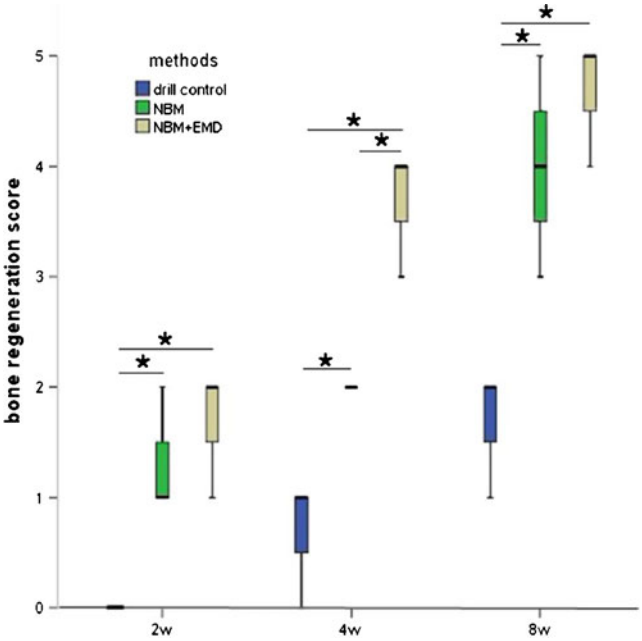

Fig. 3 Nonparametric analysis of the H&E staining images reveals that significantly higher new bone formation was observed in drilled defects filled with NBM + EMD when compared to control and NBM groups at 4 weeks (*P<0.05). At 2 and 8 weeks post-implantation, higher yet nonsignificant difference in new bone formation was detected between NBM + EMD and NBM alone treatment groups

and Safranin O staining (Sigma #S2255; Sigma-Aldrich, St. Louis, USA) were performed according to manufacturer's protocol. Specimens were examined under microscopic light by using Olympus DP72 microscope (Olympus Co., Japan). To validate the results, each experiment was repeated at least three times.

Evaluation of new bone formation

After digitizing the images deriving from H&E-stained sections, bone regeneration score was operated by three individual blinded observers to the identity of sections using a semiquantitative visual scoring method. Bone formation was evaluated using a bone score ranging from 0 to 5 as previously described (0, no bone formation; 1, minimal bone formation (only very small area in the defect); 2, low bone formation (less than one fourth of the defect); 3, moderate bone formation (less than one half and more than one fourth of the defect); 4, abundant bone formation (less than three fourths of the defect and more than one half of the defect); and 5, complete bone formation (more than three fourths of the defect).

Qualification of the regenerated bone was done according to Safranin O staining by using Image-Pro Plus 6.0 software (Media Cybernetics, USA). Areas of newly formed bone which acquired a bluish-green stain were delineated manually and then calculated as the percentage of new bone area in total cross-sectional area [(bone area/total area)×100 %] [19]. As previously mentioned, integrated optical density was representative parameters to assess the immunostaining intensity [20]. Nine randomly selected representative fields (2,048×1536 pixels) from each section were identified (original magnification ×10) and averaged.

Statistical analysis

All statistical analysis was performed by using Statistical Package for the Social Sciences (SPSS) 17.0 software (SPSS, Chicago, IL). Data (percentage of new bone formation and COL-1 staining intensity) were expressed as mean±standard deviation and analyzed using one-way ANOVA and post hoc t test. For the bone regeneration score, the Kruskal–Wallis H test was used followed by Mann–Whitney U test if statistically significant. A 5 % (P<0.05) level of significance was adopted.

Results

μCT analysis

μCT reconstruction was used to visualize the ability of unfilled, NBM-filled, and NBM + EMD-filled groups to influence new bone formation (Fig. 1). As can be seen from the unfilled control groups, little to no bone formation was observed at 2 weeks post-implantation (Fig. 1a) with little new bone formation occurring at the cortex of defects at 4 and 8 weeks post-implantation (Figs. 1b and 2c). Defects filled with NBM material initially demonstrate a large filled area of mineralized tissue since NBM grafting particles are mineralized (Fig. 1d). Increases in new bone formation are observed at 4 and 8 weeks post-implantation (Fig. 1e, f). A similar trend is observed for defects filled with NBM + EMD when compared to NBM alone (Fig. 1g–i). Due to the inability to quantify solely mineralized tissues produced from new bone (as opposed to the mineralized tissue contained in NBM particles), a conventional histological approach was utilized to quantify new bone formation using morphohistometric analysis (Figs. 2, 3, 4, and 5).

Histological observation and immunohistochemical assessment

During all experiments, the implantation's foreign body reaction was weak and there was no sign of inflammation. At each time point of 2, 4, and 8 weeks, representative sections of H&E staining and Safranin O staining are presented in Figs. 3 and 5, respectively, showing the interface between the tissue and the implanted graft.

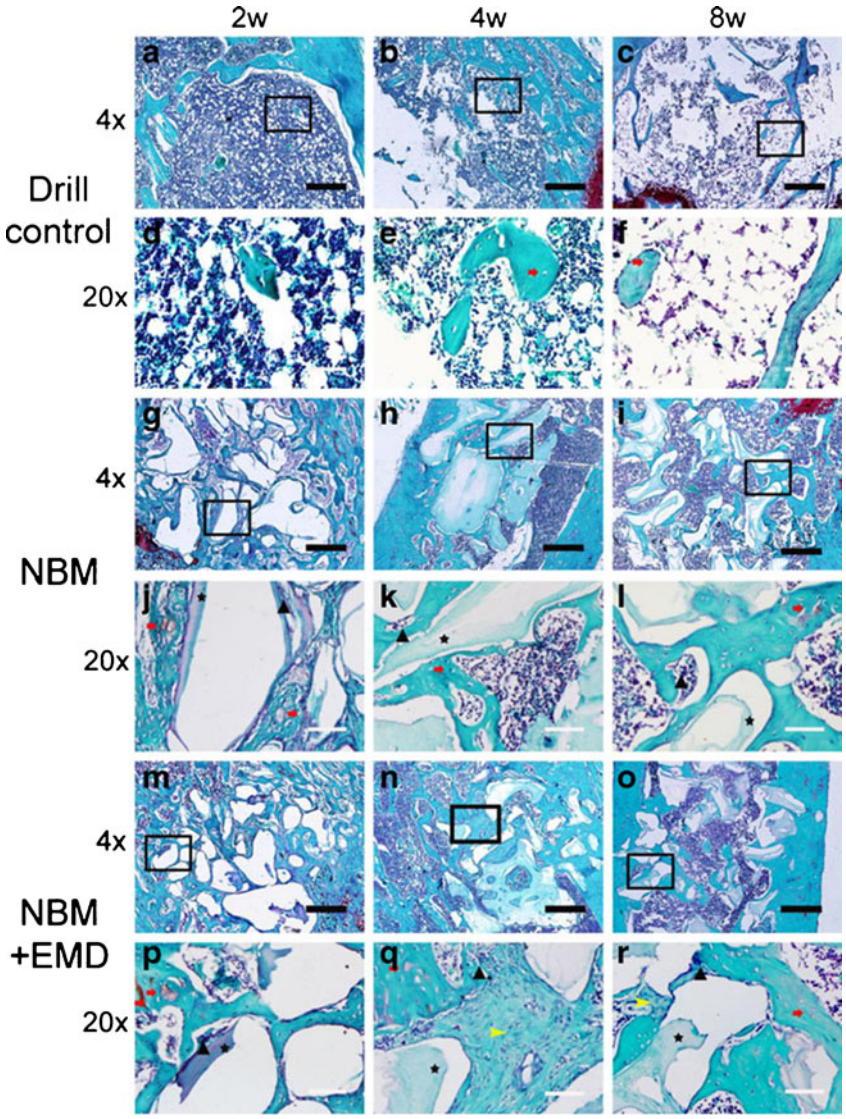

Fig. 4 **a**–**r** Representative sections of Safranin O staining demonstrating the healing of treatment at 2, 4, and 8 weeks post-implantation. *Red arrow,* osteoclast; *arrowhead*, osteoblast; *asterisk*, NBM particle; *solid triangle*, fibrous ossification; *black scale bar*, 500 μm; *white scale bar*, 50 μm

After 2 weeks, regenerated new bone and fibrous tissue were observed in treatment groups filled with both NBM and NBM + EMD (Fig. 2g, j, m, p), while the mesenchymal cells derived from marrow cavity occupied the whole drilled cavity of the drilled control group (Fig. 2a, d). No visible difference in new bone formation was observed between NBM and NBM + EMD groups at 2 weeks. At 4 weeks post-operation, minimal bone formation in the control samples could be observed with large voids still present (Fig. 2b, e). In contrast, by 4 weeks post-implantation, new bone formation in both the NBM precoated with EMD and NBM only filled defect (Fig. 2h, n) increased dramatically. In morphology, mature bone lacuna with the presence of osteocytes in the NBM + EMD-filled defect was more analogous when compared to NBM alone. In both experimental groups, multinuclear osteoclasts were visible between the interface of new bone and NBM particles. At 8 weeks post-operation, the continual progression of new bone formation around NBM particles was observed around NBM particles either precoated with/without EMD (Fig. 2i, l, o, r). Defects that were left unfilled were still void of any substantial amount of regenerated new bone formation (Fig. 2c, f).

The bone regeneration score (Fig. 3) was graded by the H&E staining images by using a semiquantitative analysis. Significantly much more newly formed bone was observed around both NBM + EMD and NBM alone when compared to the drilled control group at all time points ($P<0.05$). Statistical analysis revealed new bone formation was significantly higher in the NBM + EMD group at 4 weeks when compared to NBM alone ($P<0.05$). At 8 weeks, no

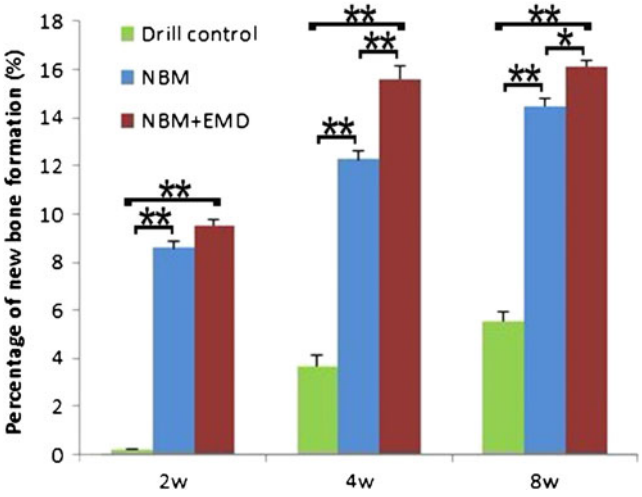

Fig. 5 Percentage of new bone formation measured by analysis of the Safranin O staining images. Significantly higher new bone formation was observed in NBM + EMD group when compared to control unfilled and NBM alone groups at 4 and 8 weeks post-implantation. *$P<0.05$, **$P<0.01$

significant difference between NBM + EMD and NBM could be observed although results still demonstrate increased bone formation in the defects treated with NBM + EMD.

Safranin O staining was utilized to quantify newly formed bone by delineating manually and calculating the percentage of new bone area in total cross-sectional area (Figs. 4 and 5). Consistent with the bone regeneration score, the fraction of new bone formation (Fig. 5) in defects filled with EMD + NBM was significantly higher among the three treatments at 4 and 8 weeks post-implantation ($P<0.05$). Although no significant difference between the NBM + EMD group and NBM only group could be observed at 2 weeks, the percentage of new bone formation increased dramatically from 9.517 ±0.404 % at 2 weeks to 15.618±0.353 % at 4 weeks and maintained stability of 16.135±0.273 % at 8 weeks, which were both significantly higher when compared to NBM alone and control unfilled defects ($P<0.05$).

Discussion

EMD is a widely used biologic agent capable of enhancing periodontal wound healing/regeneration [1]. In vitro studies on EMD have been well documented in both osteoblasts and PDL cells [15, 21–24]. EMD has a significant influence on cell behavior of many cell types by mediating cell attachment, spreading, proliferation, and survival as well as expression of transcription factors, growth factors, cytokines, extracellular matrix constituents, and other molecules involved in the regulation of bone remodeling [21, 25].

Although extensive literature provides strong evidence that EMD enhances periodontal regeneration, the effects on bone formation have primarily been limited to intrabony defects [1]. The specific stimulatory effects of EMD on bone formation in osseous defects have received much less attention. Although EMD has shown some osteopromotive effect on the early healing phases in rat femur defects [26], EMD failed to stimulate significant new bone formation in calvaria defects [27–29], rabbit tibia defects [30], and peri-implant defects in dogs when combined with various other grafting materials [31].

In this study, we sought to characterize the effects of EMD on pure bone defects in a rat femur model. This model was chosen because it contains an area with a high number of mesenchymal progenitor cells in the bone marrow cavity. Since EMD has been demonstrated to have a significant effect on PDL regeneration (an area that also contains a high number of progenitor cells), the major focus of this study was to determine if EMD could also enhance bone regeneration where a large pool of osteoblast progenitor cells are present.

In the presented study, we first sought to characterize new bone formation by μCT analysis; however, the inability to accurately quantify new bone formation as opposed to mineralized tissue contained in NBM particles led us to utilize a conventional histological approach to quantify new bone formation. The results from histological quantification of new bone formation demonstrated that EMD significantly increased new bone formation at 4 and 8 weeks post-implantation when compared to NBM particles alone and drilled control defects (Fig. 5). The results from this study are consistent with a previous study demonstrating that EMD has the potential to rapidly stimulate osteoblast proliferation at early time points and later induces osteoblast differentiation as assessed by real-time PCR and Alizarin Red staining [15].

Recently, the effects of EMD on the multilineage differentiation of human PDL cells in vitro have been tested [32]. Although PDL cells treated with EMD under nonselective growth conditions did not show any evidence of osteogenic, adipogenic, chondrogenic, neovasculogenic, neurogenic, and gliogenic differentiation, when supported with selective growth conditions, EMD upregulated osteogenic, chondrogenic, and neovasculogenic differentiation, but suppressed adipogenesis, neurogenesis, and gliogenesis. The results from Amin et al. demonstrate that under the right conditions, EMD is able to stimulate osteoblast differentiation, thus making it likely that EMD can enhance bone formatting in osseous defects in vivo.

Although EMD has previously been shown not to possess osteo-inductive potential, it possesses osteopromotive benefits since the combination with active DFDBA resulted in enhanced bone induction [33]. Since EMD is capable of

enhancing bone formation but may lack the ability to recruit progenitor cells, it is possible that the additional benefits of EMD observed in the present study may be caused by the femur defect model chosen. As femur defects in young rats are a prominent source of bone marrow, the additional benefits as seen in the present study when compared to other calvarial defect studies testing the efficacy of EMD may be caused by the availability for the defect site to contain a higher number of mesenchymal progenitor cells. These findings are in accordance with an in vitro study by Dean et al. who previously suggested that the effects of EMD are more beneficial on cells early in their differentiation process when compared to a more differentiated phenotype [34]. This rationale also provides support for the additional benefits of EMD for the repair of intrabony defects. The periodontal ligament is a highly regenerative tissue which contains a high number of mesenchymal progenitor cells capable of differentiating into many cell types. As enamel matrix proteins are of critical importance for cell differentiation during embryonic development, it is likely that historically, EMPs act upon a cell type early in their differentiation process. The results from the present study also seem to hint that EMD may target cells early in their differentiation process and offers rationale for the varied results when EMD is used to treat osseous defects.

Conclusions

Within the limits of the present study, these findings indicate that both experimental groups, NBM alone and NBM + EMD, are viable treatment options for filling osseous defects. Furthermore, the results from this study may suggest that additional benefits may exist for NBM particles precoated with EMD as this combination improved new bone formation both 4 and 8 weeks post-implantation. These results also support the clinical evidence that EMD may not only be confined to cementum and PDL regeneration but also that of bone.

Acknowledgments This project was supported by Program for New Century Excellent Talents in University (NCET-11-0414) and Excellent Youth Foundation of Hubei.

Conflict of interest The authors report no conflict of interest for this study.

References

1. Sculean A, Alessandri R, Miron R, Salvi G, Bosshard DD (2011) Enamel matrix proteins and periodontal wound healing and regeneration. Clin Adv Periodontics 1:101–117
2. Aspriello SD, Ferrante L, Rubini C, Piemontese M (2011) Comparative study of DFDBA in combination with enamel matrix derivative versus DFDBA alone for treatment of periodontal intrabony defects at 12 months post-surgery. Clin Oral Investig 15:225–232
3. Pietruska M, Pietruski J, Nagy K, Brecx M, Arweiler NB et al (2012) Four-year results following treatment of intrabony periodontal defects with an enamel matrix derivative alone or combined with a biphasic calcium phosphate. Clin Oral Investig 16:1191–1197
4. Oortgiesen DA, Meijer GJ, Bronckers AL, Walboomers XF, Jansen JA (2013) Regeneration of the periodontium using enamel matrix derivative in combination with an injectable bone cement. Clin Oral Investig 17:411–421
5. Lyngstadaas SP, Wohlfahrt JC, Brookes SJ, Paine ML, Snead ML et al (2009) Enamel matrix proteins; old molecules for new applications. Orthod Craniofacial Res 12:243–253
6. Margolis HC, Beniash E, Fowler CE (2006) Role of macromolecular assembly of enamel matrix proteins in enamel formation. J Dent Res 85:775–793
7. Bartlett JD, Ganss B, Goldberg M, Moradian-Oldak J, Paine ML et al (2006) Protein–protein interactions of the developing enamel matrix. Curr Top Dev Biol 74:57–115, 3
8. Guida L, Annunziata M, Belardo S, Farina R, Scabbia A et al (2007) Effect of autogenous cortical bone particulate in conjunction with enamel matrix derivative in the treatment of periodontal intraosseous defects. J Periodontol 78:231–238
9. Kuru B, Yilmaz S, Argin K, Noyan U (2006) Enamel matrix derivative alone or in combination with a bioactive glass in wide intrabony defects. Clin Oral Investig 10:227–234
10. Velasquez-Plata D, Scheyer ET, Mellonig JT (2002) 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. J Periodontol 73:433–440
11. Lekovic V, Camargo PM, Weinlaender M, Nedic M, Aleksic Z et al (2000) 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. J Periodontol 71:1110–1116
12. Zucchelli G, Amore C, Montebugnoli L, De Sanctis M (2003) Enamel matrix proteins and bovine porous bone mineral in the treatment of intrabony defects: a comparative controlled clinical trial. J Periodontol 74:1725–1735
13. Gurinsky BS, Mills MP, Mellonig JT (2004) Clinical evaluation of demineralized freeze-dried bone allograft and enamel matrix derivative versus enamel matrix derivative alone for the treatment of periodontal osseous defects in humans. J Periodontol 75:1309–1318
14. Trombelli L, Farina R (2008) Clinical outcomes with bioactive agents alone or in combination with grafting or guided tissue regeneration. J Clin Periodontol 35:117–135
15. Miron RJ, Bosshardt DD, Hedbom E, Zhang Y, Haenni B et al (2012) Adsorption of enamel matrix proteins to a bovine-derived bone grafting material and its regulation of cell adhesion, proliferation, and differentiation. J Periodontol 83:936–947
16. Miron RJ, Bosshardt DD, Zhang Y, Buser D, Sculean A (2013) Gene array of primary human osteoblasts exposed to enamel matrix derivative in combination with a natural bone mineral. Clin Oral Investig 17:405–410
17. Zhang Y, Cheng N, Miron R, Shi B, Cheng X (2012) Delivery of PDGF-B and BMP-7 by mesoporous bioglass/silk fibrin scaffolds for the repair of osteoporotic defects. Biomaterials 33:6698–6708
18. Zhang Y, Wu C, Luo T, Li S, Cheng X et al (2012) Synthesis and inflammatory response of a novel silk fibroin scaffold containing BMP7 adenovirus for bone regeneration. Bone 51:704–713
19. Wang L, Fan H, Zhang ZY, Lou AJ, Pei GX et al (2010) Osteogenesis and angiogenesis of tissue-engineered bone constructed by

prevascularized β-tricalcium phosphate scaffold and mesenchymal stem cells. Biomaterials 31:9452–9461
20. Wang CJ, Zhou ZG, Holmqvist A, Zhang H, Li Y et al (2009) Survivin expression quantified by Image Pro-Plus compared with visual assessment. Appl Immunohistochem Mol Morphol 17:530–535
21. Bosshardt DD (2008) Biological mediators and periodontal regeneration: a review of enamel matrix proteins at the cellular and molecular levels. J Clin Periodontol 35:87–105
22. Kasaj A, Willershausen B, Junker R, Stratul SI, Schmidt M (2012) Human periodontal ligament fibroblasts stimulated by nanocrystalline hydroxyapatite paste or enamel matrix derivative. An in vitro assessment of PDL attachment, migration, and proliferation. Clin Oral Investig 16:745–754
23. Mrozik KM, Gronthos S, Menicanin D, Marino V, Bartold PM (2012) Effect of coating Straumann bone ceramic with Emdogain on mesenchymal stromal cell hard tissue formation. Clin Oral Investig 16:867–878
24. Nokhbehsaim M, Deschner B, Winter J, Bourauel C, Jager A et al (2012) Anti-inflammatory effects of EMD in the presence of biomechanical loading and interleukin-1β in vitro. Clin Oral Investig 16:275–283
25. Miron RJ, Hedbom E, Ruggiero S, Bosshardt DD, Zhang Y et al (2011) Premature osteoblast clustering by enamel matrix proteins induces osteoblast differentiation through up-regulation of connexin 43 and N-cadherin. PLoS One 6:e23375
26. Kawana F, Sawae Y, Sahara T, Tanaka S, Debari K et al (2001) Porcine enamel matrix derivative enhances trabecular bone regeneration during wound healing of injured rat femur. Anat Rec 264:438–446
27. Potijanyakul P, Sattayasansakul W, Pongpanich S, Leepong N, Kintarak S (2010) Effects of enamel matrix derivative on bioactive glass in rat calvarium defects. J Oral Implantol 36:195–204
28. Donos N, Lang NP, Karoussis IK, Bosshardt D, Tonetti M et al (2004) Effect of GBR in combination with deproteinized bovine bone mineral and/or enamel matrix proteins on the healing of critical-size defects. Clin Oral Implants Res 15:101–111
29. Intini G, Andreana S, Buhite RJ, Bobek LA (2008) A comparative analysis of bone formation induced by human demineralized freeze-dried bone and enamel matrix derivative in rat calvaria critical-size bone defects. J Periodontol 79:1217–1224
30. Cornelini R, Scarano A, Piattelli M, Andreana S, Covani U et al (2004) Effect of enamel matrix derivative (Emdogain) on bone defects in rabbit tibias. J Oral Implantol 30:69–73
31. Casati MZ, Sallum EA, Nociti FH Jr, Caffesse RG, Sallum AW (2002) Enamel matrix derivative and bone healing after guided bone regeneration in dehiscence-type defects around implants. A histomorphometric study in dogs. J Periodontol 73:789–796
32. Amin HD, Olsen I, Knowles JC, Dard M, Donos N (2013) Effects of enamel matrix proteins on multi-lineage differentiation of periodontal ligament cells in vitro. Acta Biomater 9:4796–4805
33. Boyan BD, Weesner TC, Lohmann CH, Andreacchio D, Carnes DL et al (2000) Porcine fetal enamel matrix derivative enhances bone formation induced by demineralized freeze dried bone allograft in vivo. J Periodontol 71:1278–1286
34. Dean DD, Lohmann CH, Sylvia VL, Cochran DL, Liu Y et al (2002) Effect of porcine fetal enamel matrix derivative on chondrocyte proliferation, differentiation, and local factor production is dependent on cell maturation state. Cells Tissues Organs 171:117–127