

Original Article

Effect of the combination of enamel matrix derivatives and deproteinized bovine bone materials on bone formation in rabbits' calvarial defects

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ABSTRACT

Background: Various types of materials are used in bone regeneration procedures. The aim of this study was to investigate the use of the enamel matrix derivative (EMD), deproteinized bovine bone mineral (Bio-Oss), and a combination of Bio-Oss plus EMD in the treatment of bone defects created in the rabbits' calvaria.

Materials and Methods: Twenty New Zealand white rabbits were included in this experimental randomized single blind study. Four equal cranial bone defects ($3 \times 6 \times 0.5 \text{ mm}^3$) were created in frontal and parietal bone and randomly grafted with Bio-Oss (Group 1), EMD (Group 2), EMD + Bio-Oss (Group 3) and one of them was left unfilled to serve as a control group (Group 4). After 2, 4, 8, and 12 weeks the defects were evaluated by using histological and histomorphometric analysis. Data were analyzed by the Bonferroni test using SPSS 13 statistical software. P value < 0.05 considered as statistically significant level.

Results: Bone formation in the EMD + Bio-Oss group after 2 weeks was diminished when statistically compared to the other groups ($P < 0.05$). Bone augmentation after 4 weeks from the lowest to the highest were found in groups 1, 3, 2, and 4, respectively, and these differences were statistically significant ($P < 0.05$). Using EMD with Bio-Oss increased bone formation in the non-critical defects in the rabbit calvaria during 8 and 12 weeks ($P < 0.05$).

Conclusions: Boosting of EMD plus Bio-Oss seems to have synergic effect on bone regeneration in bone defects.

Key Words: Bone defect, bone grafting, deproteinized bovine bone mineral, enamel matrix derivative

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INTRODUCTION

Bone deficiency is the major concern in all branches of dentistry. Bone loss in the oral cavity is occurring due to pathogens or iatrogenically.^[1] In order to verify true periodontal regeneration such as new alveolar

bone formation, periodontal ligament regeneration, and cementogenesis, it must be determined histologically.^[2]

Various types of the graft materials are used for treatment of the bone defects in the craniofacial region, e.g., auto grafts or allograft.^[2] According to Block *et al.*'s theory, an ideal bone grafting materials must have characteristics such as tissue osteoinductivity, osteoconductivity, compatibility, degradation capacity, lack of foreign body reaction, feasible application, and a low risk for infection.^[3]

Autogenous bone grafts from intra- or extraoral cavity are a gold standard and are used to regenerate

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bone defects in the craniofacial region. However, the disadvantages, such as donor morbidity, additional trauma, increasing costs, and special skills, can be avoided for using bone autographs, so most of the clinicians prefer to use other graft materials.^[4] Replacement of the bone grafts provides regeneration through conductive or inductive processes and produces growth factors by inductive or stimulation cell mechanisms.^[2]

The osteoconductive graft such as Bio-Oss acts as a scaffold to support new tissue growth and is eventually replaced by the host tissue.^[2] The inductive processes involve the grafts or growth factor stimulating of host tissue to regenerate bone defects.^[5]

Bio-Oss is derived from cancellous bovine bone, and all organic components and pathogens agents are removed by chemical extraction and have the ability to act as a barrier for epithelial exclusion and prevent soft tissue collapse into the defect.^[6] This material presents morphological and structural properties very similar to the human bone tissue.^[7] Furthermore, Bio-Oss rough topography favors ectoblastic anchorage, proliferation, and synthesis of bone matrix on its surfaces^[8] and it is the most frequently used biomaterial in the bone regeneration procedures.^[9]

Using of enamel matrix proteins, available commercially as enamel matrix derivative (Emdogain) represents a novel approach to stimulating periodontal regeneration.^[10] Enamel matrix proteins that are produced from Hertwig's epithelial sheath during tooth development play a critical role in cementogenesis, bone formation, and periodontal regeneration.^[11]

Enamel matrix derivative (EMD) contains of 90% amelogenins and 10% non-amelogenin including: tuftelin, tuft protein, serum, ameloblastin, enamelin, and salivary proteins.^[12,13] The Emdogain gel was believed to mimic the role of these proteins during cementogenesis and alveolar bone formation,^[14] and also increasing of collagen synthesis and mineralization have been seen, but these proteins do not enhance epithelial cell proliferation.^[15]

Clinical studies have shown that the EMD stimulates the regeneration of periodontal tissue including acellular cementum, periodontal ligament (PDL), and alveolar bone.^[12,15,16] Scheyer *et al.* showed that EMD stimulates the expression of alkaline phosphatase and the release of TGF- β 1 forms both human periodontal ligament fibroblasts (HPLF) and human gingival fibroblast (HGF).^[2] Hoang *et al.* demonstrated that

enamel matrix proteins are novel attachment proteins for both PDL and bone cells.^[17] The EMD have disadvantages such as different effects on variable types of osteoblast implicated and concentration rate,^[18] high degradation rate,^[19] and lack of growth factors like TGF- β 1.^[20] At present, it is not clear whether combination of the Bio-Oss plus EMD offers synergetic effect or not.

The aim of this study was to determine the bone formation by using combination of Bio-Oss and EMD in bone defects on the rabbit calvaria.

MATERIALS AND METHODS

This study was submitted to and approved by the Ethical Committee for Research in Hamadan University of Medical Sciences, Hamdan, Iran, and had no conflict with the declaration of Helsinki. Twenty New Zealand white male rabbits weighed between 2.5 and 3 kg were included in this randomized single blind study. The animals were accommodated in the official stable at 22–24°C with 55–70% humidity, light cycle of 12 h, air renewal 15 times/h and they have the same diet. Each rabbit was anesthetized with an intramuscular injection of ketamine 10% (Imalgen 1000, Rhone, Merieux, France) (40 mg/kg) and 5 mg/kg of xylazine 2% (Alafason, Woerden, Holland). The head was shaved, and the skin surface was disinfected with povidone iodine solution before the operation. The calvarias bone was exposed through a skin incision approximately 5 cm in length on the mid-line. Following a coronal–sagittal approach, the periosteum was dissected by a periosteal elevator and four identical full thickness bony defects (terminated over the dura mater) were created on the calvaria. Two circular slits (3 mm inner diameter and 0.5 mm deep) were adjusted together by trephine drill and direct longitudinally and their intermediate septum removed by the round bur surgery [Figure 1a].

Defects created in frontal and parietal bones with a distance of approximately 1 mm from the sagittal and coronal sutures. The defects were randomly filled with demineralized bovine bone matrix (Bio-Oss; Geistlich and Sons, Wolhusen, Switzerland) for Group 1, EMD (Emdogain 0.7 ml, stratum, USA) for Group 2, and Bio-Oss plus EMD for Group 3 [Figure 1b]. One of the defects was left unfilled to serve as a control group (Group 4); there were five animals in each group. The periosteum was closed with resorbable 4/0 suture (Vicryl, Johnson Somerville, NJ) and nonresorbable

4/0 suture (SURGIPRO Polypropylene Monofilament, Richmond, VA, USA) for the calvarial skin.

Rabbits recovered from anesthesia without complications. They were given postoperative narcotic pain medication (tramadol 2 mg/day) for 3 days and antibiotics (Enrofloxacin 20 mg/day) for 1 week, intramuscularly. Rabbits were sacrificed by using overdose of sodium pentobarbital (Dolethal; Vetoquinol, Lure, France), 100 mg/kg intravenously at 2, 4, 8, and 12 weeks. The entire calvaria were removed with a reciprocating saw, without encroaching on the grafted areas. Samples were fixed in formaldehyde, 10% buffer solution at pH 7.0. Specimens were treated with 10% formic acid decalcifying solution for 2 weeks. Samples were dehydrated with alcohols and embedded in paraffin. Histological sections were prepared perpendicular to the long axis of each defect in an anterior to posterior direction with a thickness of 5 mm. Frothy sections were provided from each defect. The specimens were routinely stained with hematoxylin and eosin. Histomorphometric analysis of the bone formation carried out using a digital camera (Nikon fujix HC-300Zi; Nikon, Japan) with a magnification of 40 \times and histomorphometric software (Image-pro pulse 4.5; Media Cybernetics, Milan, Italy). Histological evaluation was performed using a 400 \times magnified optical microscope (Olympus BX 51-Olympus co, Tokyo, Japan). Pathologists were blinded to the graft material and the time period for each sample.

Intergroup comparison was performed using Bonferroni test by SPSS 13 statistical software and $P < 0.05$ considered statistically significance.

RESULTS

Histological results showed neither foreign body reaction nor severe inflammation in each of the specimens. Inflammations in all groups were chronic. Also bone tissue type in the EMD + Bio-Oss was

similar to the control group [Table 1]. New bone formation was observed in both the centers and margins of defects [Figures 1c-f].

The mean percent of the osteogenesis in 2 weeks for the control group was significantly higher in comparison with the remaining groups. There was a higher bone formation rate in the Bio-Oss group in the 2 weeks in comparison to EMD group ($P = 0.067$) and EMD + Bio-Oss group ($P = 0.001$), which were statically significant. Regenerated bone in the Bio-Oss group was statistically lower in 4 weeks than other groups ($P = 0.000$).

The amount of the regenerated bone was significantly higher in the EMD + Bio-Oss group in 8 (87%) and 12 weeks (96.6%), according to the histomorphometric evaluation bone regeneration of the EMD + Bio-Oss group in 8 and 12 weeks was significantly higher than the remaining groups ($P = 0.000$) [Figure 2] [Table 2].

DISCUSSION

Significant histologic improvements were observed in both EMD + Bio-Oss groups compared to the control group. This comparison revealed that more new bones were produced in the EMD + Bio-Oss group, indicating that the combined treatment displays a stronger synergistic effect.

Histomorphological findings are necessary to determine bone regeneration in the alveolar bone defects. Therefore, we selected the animal study.^[12] According to Yoneda *et al.* we used the rabbit's skull to evaluate the osteogenesis rate due to wide area of bone and feasibility.^[15] Since, in other studies, the time intervals for evaluation of bone formation were 2, 4, 6, and 12 months, there were chances to miss the effect of biomaterials on bone conductivity and inductivity.^[13,21-23] We designed our time intervals to 2, 4, 8, and 12 weeks, so we would be able to evaluate the osteogenesis rate without missing any variation.

Table 1: Type of regenerated bone in each treatment groups at 2-, 4-, 8-, and 12-week intervals

Treatment groups	Lamellar bone	Woven bone	Mixed bone	Total
Control	10	5	5	20
Bio-Oss	6	5	9	20
EMD	4	1	15	20
Bio-Oss + EMD	10	5	5	20
Total	30	16	34	80

Table 2: Histomorphometric parameters (means and standard deviations in each treatment groups at 2-, 4-, 8-, and 12-week intervals

Type of materials	2 weeks	4 weeks	8 weeks	12 weeks
Control	14.1 \pm 1	49.48 \pm 1.2	60.74 \pm 0.7	74.4 \pm 0.5
Bio-Oss	9.8 \pm 0.7	25.08 \pm 1.1	40.12 \pm 0.7	70 \pm 0.8
EMD	8.9 \pm 0.8	40.14 \pm 0.9	78.1 \pm 1.0	83.3 \pm 0.9
Bio-Oss + EMD	6.6 \pm 0.5	40.22 \pm 0.9	87 \pm 0.8	96.6 \pm 1.3

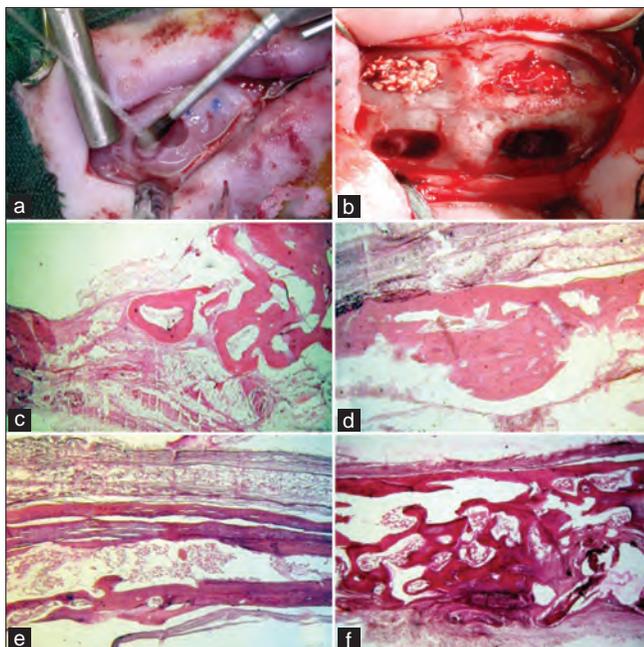


Figure 1: Surgical procedure: (a) creating bone defect, (b) four different treatment groups. Histologic results of defects repaired after 12 weeks: (c) immature bone in control, (d) bone formation with EMD, (e) mature bone formation, and (f) irregular bone formation with Bio-Oss

We used tramadol (2 mg/kg) as an analgesic drug because the ketoprofen that has been used in other study can create an effect on prostaglandin secretion, and prostaglandins may interact with the bone formation rate, so tramadol does not interact on the bone formation.^[24] Soleymani Shayesteh *et al.* created more than two intrabony defects by using surgical round bur in their surgical procedure; this procedure has disadvantages such as a more inflammatory reaction and a lack of unique defects.^[25] To solve this problem, we used trephine drill to create the defects on the rabbit calvaria.

Bone formations at 2 weeks in the control group were found to be more than the EMD group. In the 2-weeks period, the osteogenesis rate in the Bio-Oss group was higher than other experimental groups. This indicated that the Bio-Oss is an exceptional biomaterial carrying characteristic of a scaffold, especially in early time of bone formation. The osteogenesis rate during the 4-week period in the control group was significantly higher compared to experimental groups which are similar to Cornellini *et al.* study.^[19]

The regeneration rate in 8 weeks in the EMD + Bio-Oss group was higher compared to other groups, and this rate remains until 12 weeks. Since penetrated proteins exist in the Emdogen gel inside, Bio-Oss

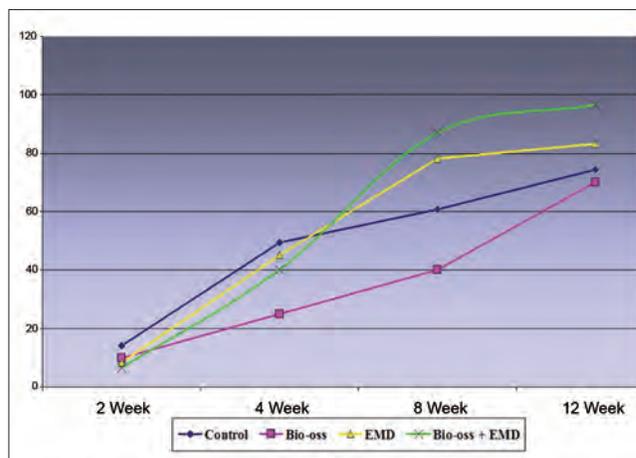


Figure 2: Bone regeneration of study groups in different weeks

particles make combined biomaterial as a sustainable release material that releases proteins and activates certain biological factors in the long term for increasing the osteogenesis rate. Bone augmentation in 8 and 12 weeks for the combined group was significantly higher than the Bio-Oss group which is similar to Yamamoto *et al.*'s study.^[26] In contrast with our study, Scheyer *et al.* reported equal effects of combined biomaterials with the Bio-Oss on the bone formation rate.^[2] Their result can be interpreted to long term evaluation implantation; in this long period, bone formation took place completely in both groups. Donos *et al.* reported that the bone formation rate of EMD + Bio-Oss was not more than guided tissue regeneration (GTR), which showed equal effects of both materials on involved cells in the bone formation.^[10] However, the augmentation rate of the control group in 2- and 4-week periods due to noncritical and nonpathogenic defects was higher than other groups. However, in the following weeks, this deficit compensates due to the effect of enamel matrix protein exist on the EMD and its effect on the osteoblast activity via growth factor production. Although EMD does not have enough osteoconductive effects due to the lack of the growth factor such as TGFb1 but in combination with Bio-Oss and by passing enough time can accelerate degradation of Bio-Oss particles and increase the osteogenesis rate and this delayed effect has been reported by Schwartz.^[18]

CONCLUSION

In conclusion using combined EMD + Bio-Oss had a positive effect on the osteogenesis rate in 8 and 12 weeks. Increased bone formation was due to sustained release of proteins exist in the EMD and

synergistic effect of both materials. Boosting of EMD plus Bio-Oss seems to have synergic effects on bone regeneration in bone defects. To our knowledge, there is no systematic review on Emdogain and it is necessary to know the advantage and disadvantage of this material for the application of it in clinical treatments.

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