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Research

Enamel Matrix Derivate for Periodontal Regeneration in the Interproximal Periodontal Defect Model

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The aim of this study was to evaluate the effect of enamel matrix derivate (EMD) with combination of composite bovine-derivate xenograft on the periodontal regeneration in the interproximal periodontal defect model. The interproximal periodontal defects (IPDs) were surgically prepared between the first and second maxillary premolar, and the second and third maxillary premolar in four beagle dogs. EMD, collagenized bovine hydroxyapatite (CBHA), combination of two material, and sham surgery were allocated for each IPD. After eight weeks, the animals were sacrificed and the defects were analyzed by radiographic, histologic, and histometric methods. Regenerated woven bone was observed and cementoid was created along the adjacent root surfaces with proliferation of cementoblasts in every group. In the combination of EMD and CBHA group, Sharpey's fiber was observed beyond the crest of new bone and along the newly formed cementum, and apical migration of junctional epithelium appeared to be blocked by new cementum. In the BC and EMD+CBHA groups, the residual bovine hydroxyapatite particles were found in the periodontal defect. No direct contact was observed between residual particles and tooth surfaces. No remarkable difference was found between the histometric results among the groups. Within the limitation of this study, EMD, CBHA, and combination of two materials showed similar periodontal regeneration in the interproximal periodontal defect model. Further investigation on combination with barrier membrane may be required for improvement of the regenerative potential.

Key words: enamel matrix proteins, xenograft bioprosthesis, periodontal disease, regeneration

Introduction

For periodontal regeneration, guided tissue regeneration (GTR) has been widely used with the concept of selective repopulation of periodontal ligament cells.[1,2] Histologically GTR has shown the formation of new cementum, periodontal ligament, and bone in animal studies in various defect types.[3-9] Human studies have also reported regeneration of cementum and periodontal ligament in histologic analysis.[1,2,10,11] Clinical attachment gain and probing depth reduction were observed following GTR in human clinical trials.[12-16] Therefore, GTR is considered as a reliable method for the regeneration of periodontal tissue.

However, alternative approaches for periodontal regeneration have been investigated for avoidance of complication and difficulty of GTR. Major complications of GTR include exposure of barrier membranes. When the membrane was exposed, cell occlusion is impaired and epithelial downgrowth is not blocked perfectly. In addition, GTR requires advanced flap management and suture techniques. The outcomes of GTR are thus often affected by technique sensitivity.

Enamel matrix derivate (EMD) has been introduced to facilitate the periodontal regeneration. Amelogenin is a protein that plays an important role in the development of enamel structure. EMD refers to purified hydrophobic amelogenins,[17] which promotes periodontal regeneration by mimicking the development of periodontium.[18] For the vehicle of EMD propylene glycol alginate (PGA) showed superior results to other alternatives such as hydroxyethyl cellulose.[19]

EMD showed formation of new cementum, periodontal ligament, and bone when applied in animal intrabony,[18] furcation,[20] and dehiscence type defects.[21,22] In human clinical studies Emdogain showed bone regeneration, clinical attachment gain, and probing depth reduction in intrabony defects[23-27] and class II furcation defects.[28,29]

Lack of space maintenance, however, prevents the periodontal regeneration by EMD in non-contained defects. The amount of radiographic and clinical gain followed by application of EMD is significantly less in the 1-wall intrabony defects than 2- or 3-wall defects.[30] In class III furcation defects EMD failed to achieve complete healing of the defect and there was no significant difference in clinical outcomes between EMD and GTR.[31]

Combination of bone material with EMD has been con-

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ducted for improvement of space maintenance and regeneration potentials. The effectiveness of combination of bone material with EMD shows inconsistent outcomes and needs more investigation. For space maintenance particulated bone material was used in combination with EMD. Bovine hydroxyapatite (BH) was widely used due to superior biocompatibility. In vitro EMD enhanced the cell activity on residual bovine bone particles.[32] However, the effectiveness of combination remained still controversial in human studies.[33-37] In animal intrabony defects EMD combined with BH enhanced the formation of new connective tissue and bone,[38] and similar results were shown in a human study.[39] However, other studies failed to show that combination of EMD with other agents can improve the final results.[37,40,41]

A collagenized bovine hydroxyapatite (CBHA) was also introduced in periodontal regeneration. CBHA consists of mainly deproteinized bovine cancellous bone granules (90%), which are embedded in highly purified collagen matrix (10%). In intrabony defects CBHA itself showed superior clinical gain in human clinical studies.[42-44] New bone, cementum, and periodontal ligament were observed in histologic analysis of human intrabony defects.[45] Grafted in extraction sockets, CBHA has exhibited less shrinkage of residual ridge,[46,47] which implies the potential of preservation of residual ridge. However, regenerative potential of combination therapy with CBHA and EMD has not been clarified.

The aim of this study was to evaluate the effect of EMD with application of CBHA on the periodontal regeneration in the interproximal periodontal defect model.

Materials and Method

Animals

Four 15-months-old beagle dogs with healthy periodontium, each weighing 10-15 kg, were used. Animal selection, management, preparation, and the surgical protocol followed the protocol that was approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea.

Surgical protocol

The surgical procedure was performed under general anesthesia with subcutaneous injection of atrophine 0.05 mg/kg and intravenous injection of compound of xylazine (Rompun®, Bayer Korea, Seoul, Korea) 2 mg/kg and ketamine hydrochloride (Ketalar®, Yuhan Co., Seoul, Korea) 10 mg/kg. After intubated, 2% enflurane was administered and the disinfection of surgical sites was performed. Routine dental infiltration anesthesia was performed with 2% lidocaine hydrochloride including epinephrine 1:100,000 (Kwangmyung Pharm., Seoul, Korea). Mucoperiosteal flap was reflected after a crevicular and vertical incision at the maxillary premolar area.

The interproximal periodontal defects (IPDs) were surgically prepared in each side between the first and second maxillary premolar (P1 and P2), and the second and third maxillary premolar (P2 and P3) with a fissure bur and a chisel with continuous saline irrigation for prevention of overheating. The width of the defect was 3 mm, measured from the center of the defect to buccal and palatal bone. The apical notch was created on the roots of the teeth adjacent to the defects to ensure the height of the defect to be 5 mm, measured from the apical reference notch to the cementoenamel junction (Figure 1).

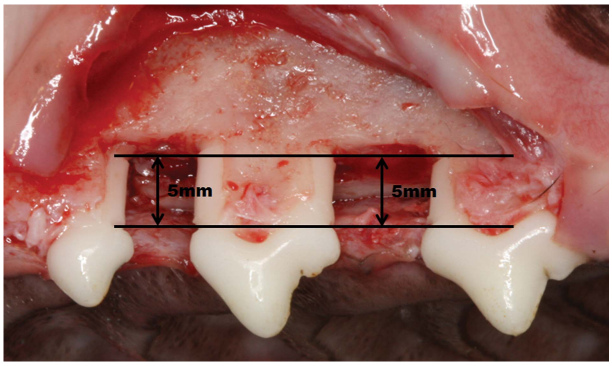

Figure 1. The interproximal periodontal defect (IPD) prepared between maxillary premolars.

The root planning of teeth were performed with root planning bur and Gracey curettes to remove old cementum. One of following material was randomly allocated for each IPD: Bio-oss Collagen® (Geistlich Sons Ltd., Wolhusen, Switzerland), Emdogain® (Straumann, Basel, Switzerland), combination of both materials, and sham surgery. After application of material, the flap was positioned coronally and primary tension-free wound closure was accomplished. The flap was sutured with 5-0 resorbable suture material (Polyglactin 910, braided absorbable suture, Ethicon, Johnson & Johnson Int., Edinburgh, U.K.) with interrupted suture.

Post-surgery management

Post-surgery management included intravenous injection of antibiotics (Cefazoline Sodium 20 mg/kg, Yuhan Co., Seoul, Korea) and topical application of chlorhexidine solution (Hexamedine 0.2%, Bukwang pharmaceutical Co., Seoul, Korea) daily for the prevention of infection. The sutures were removed at approximately 10 days post-surgery. The animals were euthanized after 8 weeks following the surgery by intravenous injection of anesthesia drug overdose.

Histologic processing and radiographic analysis

Block sections including segments of the defects at the surgical sites were dissected at sacrifice. The sections were fixed in 10% buffered formalin for ten days and rinsed with water. Then the blocks were scanned with a micro-CT (SkyScan 1072®, SkyScan, Aartselaar, Belgium) at a resolution of 35 lm (100 kV, 100 μA). The scanned data were converted into a

Digital Imaging and Communications in Medicine (DICOM) format and the surgical sites were three-dimensionally reconstructed with OnDemand 3D® software (Cybermed, Seoul, Korea). The residual material was selected in each axial cross section, and combined into red-tone in 3-D reconstruction view.

Histologic analysis

The sections were decalcified in 5% formic acid for 2 weeks after rinsed in sterile water, and they were dehydrated in a graded ethanol series and embedded in paraffin. Serial sections, 5 μm thick, were cut in a mesial-distal direction at intervals of 80 μm. Histologic specimens were observed under a light microscope (LEICA DM-LB, LEICA, WETZLAR, Germany) for evaluation of soft and hard tissue healing patterns, which include the degree of inflammation, bone and attachment gain.

Histometric analysis

Following parameters were analyzed by histometric analysis (Figure 2):

• Defect height (DH): distance from the cemento-enamel junction (CEJ) to the apical reference notch

• Long junctional epithelial attachment (LJE): distance from the CEJ to the apical extension of the junctional epithelium

• Connective tissue attachment (CTA): distance from the apical extension of the junctional epithelium to the coronal extension of new cementum

• Cementum regeneration (CR): distance from the coronal extension of new cementum or cementum-like substance to the apical reference notch

• Bone regeneration (BR): distance from the apical reference notch to the coronal extension of newly formed bone

Statistical analysis

The means and standard deviations of the measurements were analyzed descriptively for each group.

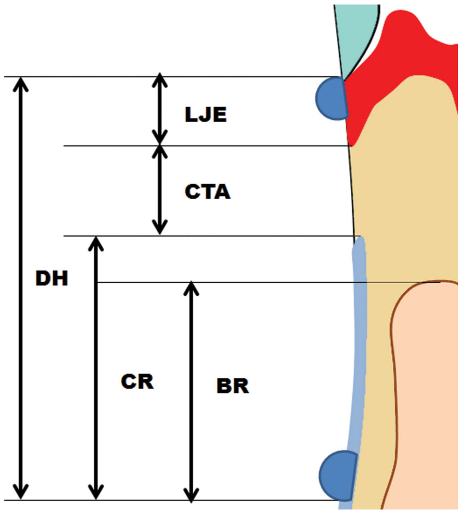

Figure 2. The schematic diagram of histometric measurement parameters. DH: Defect height; LJE: Long junctional epithelium; CTA: Connective tissue attachment; CR: Cementum regeneration; BR: Bone regeneration.

Results

Clinical observations and histologic analysis

The wound healing of defects was clinically uneventful except one defect. On the right side of dog #3, the sutures were loosened after the surgery and material was lost.

In common, regenerated woven bone was observed, and cementoid was created along the adjacent root surfaces with proliferation of cementoblasts. In some specimens, root resorption and associated osteoclasts were observed. In CBHA and EMD+CBHA groups, the residual bovine bone particles were found in the periodontal defect. No direct contact was

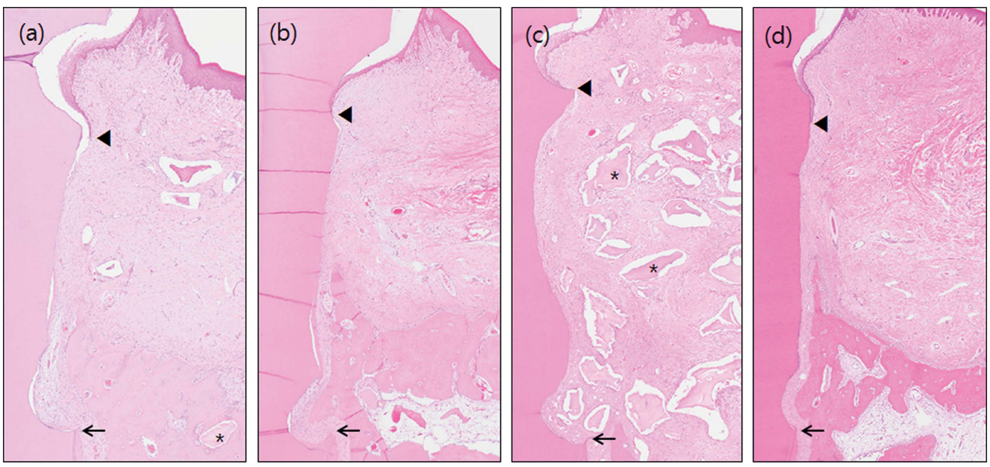

Figure 3. Histologic analysis (magnification × 40). New bone was formed beyond the bottom of the apical notch (arrows). Migration of junctional epithelium (arrowheads), connective tissue and vascularization was observed. Residual material (asterisks) was shown in CBHA and EMD+CBHA groups. (a) EMD+CBHA (b) EMD (c) CBHA (d) Control.

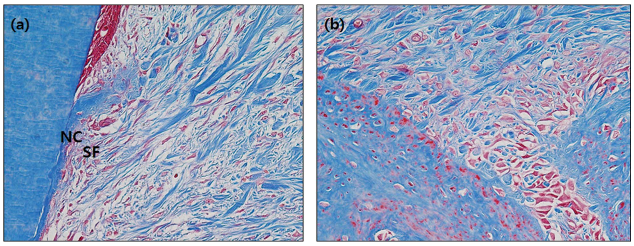

Figure 4. Histologic finding in EMD+CBHA group. Masson's trichrome stain (magnification × 400) (a) New cementum (NC) was formed along the root surface, and Sharpey's fiber (SF) was embedded into the newly formed cementum. Apical migration of junctional epithelium appeared to be blocked by NC. (b) New bone was formed, and inflammatory reaction was not observed.

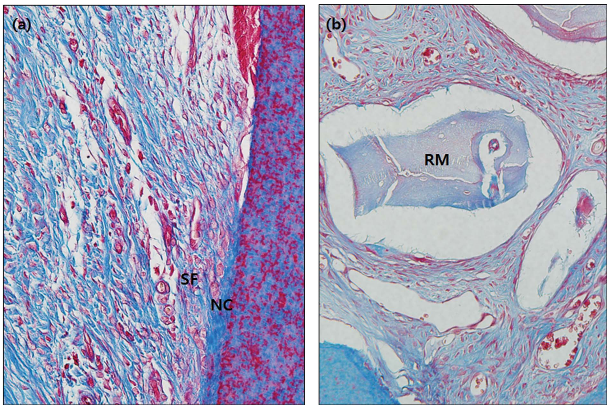

Figure 5. Histologic finding in CBHA group. Masson's trichrome stain (a) New cementum (NC) was formed along the root surface, and Sharpey's fiber (SF) was embedded into the newly formed cementum (magnification × 400). (b) Residual material (RM) was observed, without direct fibrous encapsulation or similar structures. Inflammatory reaction or other adverse effects were not observed (magnification × 200).

observed between residual particles and tooth surfaces. No remarkable difference was found between the occurrences of root resorption among the groups (Figure 3).

In Masson's trichrome stain, Sharpey's fiber was embedded into newly formed cementum. In EMD+CBHA groups, Sharpey's fiber was observed beyond the crest of new bone and along the newly formed cementum, and apical migration of junctional epithelium appeared to be blocked by new cementum (Figure 4). In CBHA groups, residual material was observed without direct fibrous encapsulation or similar structures. Sharpey's fiber was embedded into newly formed cementum, and inflammatory reaction or other adverse effects were not observed (Figure 5). Regenerated bone and cementum were observed in common including EMD group (Figure 6).

Histometric analysis

Table 1 shows the histometric results of DH, LJE, CTA, CR, and BR. Neither difference nor tendency was observed in any parameters among groups.

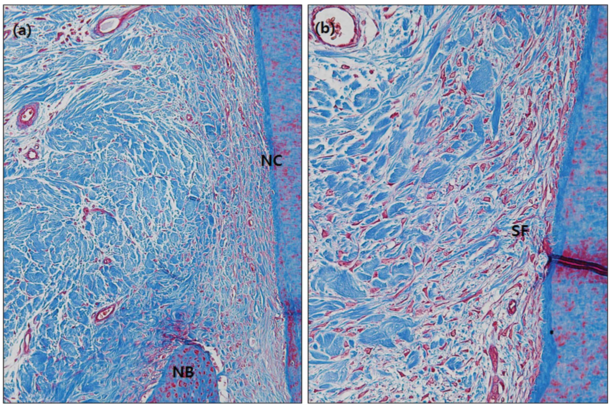

Figure 6. Histologic finding in EMD group. Masson's trichrome stain (a) New bone (NB) formation was observed, and new cementum (NC) was formed along the root surface (maginification × 40). (b) Sharpey's fiber (SF) was embedded into the newly formed cementum (magnification × 400).

Table 1. Histometric analysis

	EMD (n = 2)	EMD+Bio-oss (n = 2)	Bio-Oss (n = 5)	Control (n = 4)
LJE	1.22 ± 0.32	1.93 ± 0.37	1.82 ± 0.72	1.10 ± 0.24
CTA	0.39 ± 0.14	0.37 ± 0.35	0.23 ± 0.20	0.36 ± 0.30
CR	2.92 ± 0.23	2.26 ± 0.31	2.75 ± 0.74	2.92 ± 0.51
BR	1.75 ± 0.04	1.80 ± 0.09	2.06 ± 0.69	1.81 ± 0.15
DH	4.53 ± 0.25	4.55 ± 0.33	4.81 ± 0.18	4.38 ± 0.35

EMD, enamel matrix derivate; LJE, long junctional epithelial attachment; CTA, connective tissue attachment; CR, cementum regeneration; BR, bone regeneration; DH, defect height.

Radiographic analysis

Residual material was color-coded in three-dimensional reconstruction view. No root resorption was observed in any groups and new bone was formed beyond the bottom notch of the defect, which enhances the histologic findings. In every defect where CBHA was applied with our without EMD, residual material was observed in the interproximal defect (Figure 7).

Discussion

This study has shown the periodontal regeneration of combination therapy using EMD with CBHA in the periodontal interproximal defect model. All groups exhibited similar regeneration pattern and failed to reveal any differences when EMD and CBHA were combined.

The regenerative potential depends on the configuration of the defects. Previously, intrabony defects have been most investigated to assess the effect of EMD. The potential of regeneration depends on the number of bony walls because of wound stability and vascularization.[48,49] In contained defects

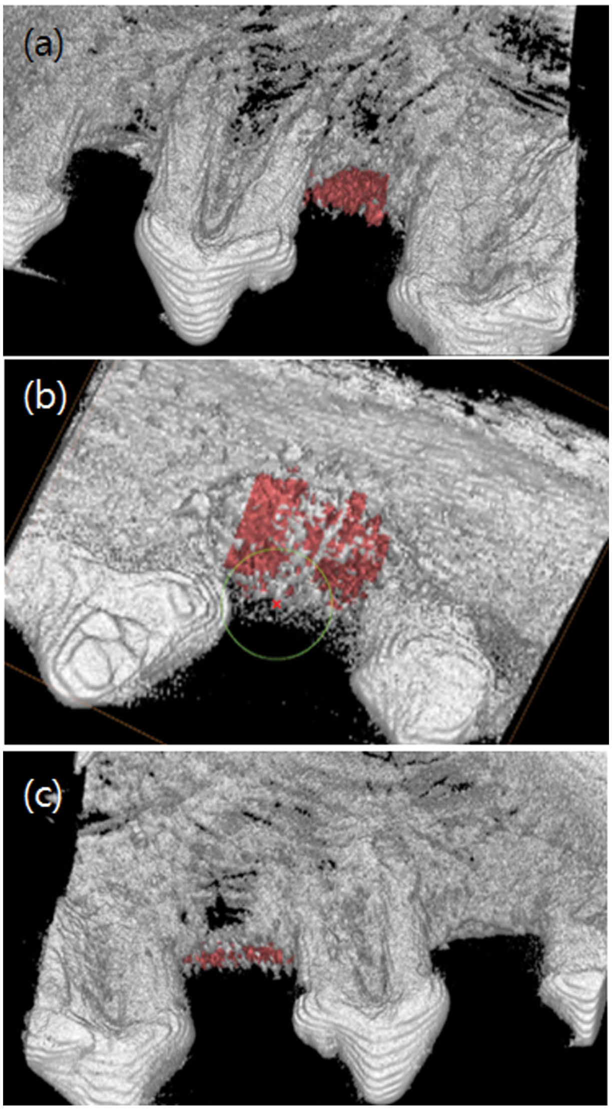

Figure 7. Residual material was color-coded into red-tone in (a, b) CBHA group (c) EMD+CBHA group.

such as 3-wall defects EMD alone showed significantly superior periodontal regeneration compared with control group and comparable regeneration with GTR procedure. However, non-contained defects have not shown consistent results. While in some studies EMD showed significantly higher periodontal regeneration than control in 1-wall and 2-wall defects,[24,50-52] other studies failed to show significant improvement of the probing depth and clinical attachment gain.[30,53] Histologic analysis in humans showed the evidence of periodontal regeneration in deep intrabony pockets but on an inconsistent basis.[54] Other types of non-contained defects have yielded much less favorable regeneration than in the intrabony defects. In the supraalveolar defects, the regeneration of new cementum was observed when EMD was applied in rats, but the authors failed to show the significant improvement over the control.[55] In the dehiscence defects of beagle dogs, new bone formation was limited in the dehiscence portion, even though the newly formed cementum and connective tissue regeneration was observed in the EMD group.

The periodontal interproximal defect used in this study can be considered as a more unfavorable defect than a dehiscence or supraalveolar defect. Neither buccal nor lingual wall is not present, and two opposing root surfaces lack vascularization. Therefore, the periodontal interproximal defect can be recognized as a defect without a bony wall.[56] Since the combination therapy in the supraalveolar and dehiscence defect failed to show superior results over control, the more unfavorable defect can hardly exhibit better periodontal regeneration.

The combination therapy of BH and EMD provided the promising results in several studies but on an inconsistent basis. In vitro the combination of EMD with BH enhanced the attachment, proliferation, and differentiation of periodontal ligament cells and osteoblasts on BH particles.[32] In the human intrabony defects EMD showed the formation of connective tissue attachment in human histologic analysis although the configuration of the defect was not exactly specified.[39] In addition, several studies have supported that BH helps to augment the effect of EMDs in clinical attachment gain and probing depth reduction.[33] On the contrary, other studies reported that combination of EMD with BH failed to provide significant differences compared with bovine bone material alone in terms of attachment gain, probing depth, and BR[37,40] in the intrabony defects.

This study reported the results using CBHA with EMD in the periodontal interproximal without membrane. To improve the result of combination therapy, further studies with barrier membrane may be required. Animal studies presented complete healing only when barrier membrane was applied regardless of application of EMD or BH.[57] In the non-contained defects, the combination of BH with a bilayered collagen barrier demonstrated greater bone and cementum regeneration in human radiographic and histologic analysis.[58] Similar findings have been reported by several studies.[59,60] In a human clinical study, EMD alone showed less probing depth reduction and clinical attachment gain than guide tissue regeneration using non-resorbable titanium-reinforced membrane in non-contained defects.[30] Therefore, application of barrier membrane combined with EMD, BH and/or CBHA in the interproximal periodontal defect model might result in better results in radiographic or histologic analysis.

Conclusion

Within the limitation of this study, EMD, CBHA, and combination of two materials showed similar periodontal regeneration in the interproximal periodontal defect model. Further investigation on combination with barrier membrane may be required for improvement of the regenerative potential.

Acknowledgement

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