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A Comparable Study of Combinational Regenerative Therapies Comprising Enamel Matrix Derivative plus Deproteinized Bovine Bone Mineral with or without Collagen Membrane in Periodontitis Patients with Intrabony Defects

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Abstract

Aim: The aim of the present study was to examine the effectiveness of collagen membrane (CM) in regenerative therapy with deproteinized bovine bone mineral (DBBM) and enamel matrix derivative (EMD) for periodontal intrabony defects. **Methods:** Eighteen periodontal intrabony defects of nine chronic periodontitis patients were evaluated. Two defects per patient with probing pocket depth (PPD) ≥ 6 mm were assigned to two different types of treatments: EMD + DBBM + CM or EMD + DBBM. Clinical parameters including Gingival Index (GI), PPD, clinical attachment level (CAL), gingival recession (GR), bleeding on probing (BOP), tooth mobility (MOB), and the filled bone volume/rate (FBV/FBR), which was measured by cone beam computed tomography, were compared at baseline and 12 months post-treatment. Differences between groups were determined by the chi-square test, McNemar's test, and Wilcoxon signed-rank test. **Results:** Clinically, PPD, CAL, and FBR significantly improved in both groups ($p < 0.05$). The between-group comparison showed that the EMD + DBBM + CM group resulted in slightly greater PPD reduction, CAL gain, and FBR; however, these differences were not statistically significant. **Conclusion:** Periodontal regenerative therapies comprising EMD and DBBM with and without CM resulted in positive clinical outcomes. The use of CM may result in better outcomes in MOB decrease; however, long-term prognosis must be further studied.

Keywords

Periodontitis, Regenerative Therapy, Membrane, Enamel Matrix Derivative, Bone Substitutes, Computed Tomography

1. Introduction

Regenerative periodontal therapy aims to restore periodontal tissues lost due to periodontitis. Evidence of periodontal regeneration using bone graft (BG) substrates, guided tissue regeneration (GTR) membranes, and/or enamel matrix derivatives (EMD) have been accumulated [1] [2] [3] [4]. Systematic reviews of clinical trials have shown that multiple surgical approaches using BG, EMD, or GTR in combination with flap surgery could result in superior clinical results for periodontal intrabony defects in terms of probing pocket depth (PPD) reduction, clinical attachment level (CAL) gain, and hard tissue fill or filled bone rate (FBR) [2] [3] [4].

Three primary factors are involved in biological regeneration: cells, signaling molecules, and scaffolds. The effectiveness of BG and collagen membrane (CM) as a scaffold for regenerative therapy has been well demonstrated and this combinational therapy has been used in clinical practice. Combinational therapy comprising CM and BG, such as deproteinized bovine bone mineral (DBBM), is superior to CM use only to maintain space for periodontal tissue regeneration [5]. Additionally, EMD was originally reported not only to enhance the potential of soft tissue healing and new cementum formation around decontaminated root surfaces but has also recently been shown to contain signaling molecules, which stimulate cell growth and differentiation of host cells including periodontal ligament cells, fibroblasts, and osteoblasts [6] [7]. Thus, combinational therapy comprising EMD and BG is often used in clinical practice for periodontal regenerative operation, sometimes in conjunction with CM. We recently reported excellent outcomes for the treatment of intrabony defects of 40 patients with periodontitis by using both triple (EMD + DBBM + CM) and double (EMD + DBBM) combination therapies [1]. Our clinical trial found that regenerative therapy using EMD + DBBM showed comparable effects to EMD + DBBM + CM therapy [1]. In our previous study, to avoid the influence of factors such as operator skill and defect morphology, the same types of periodontal defects were selected and treated by a single periodontal specialist; however, comparisons were made between different patients (one defect per patient). To confirm our findings and avoid the influence of patient characteristics including individual healing ability, oral hygiene, lifestyle, or immunological factors, we compared two periodontal intrabony defects in each individual patient.

In the present study, 18 periodontal intrabony defects of nine patients, who had two intrabony defects of the same tooth type, were treated by a single periodontist and analyzed to avoid patient-specific confounding factors.

2. Materials & Methods

The study protocol was approved by the Institutional Review Board of Niigata University (approval No. 25-R8-17-16). Informed consent was obtained from all subjects, and this study was performed according to ethical principles laid down in the Declaration of Helsinki for medical research involving human subjects. This clinical study was registered in the University Hospital Medical Information Network Clinical Trial Registry (UMIN-CTR ID: UMIN000011709). Sample size was calculated [1] and nine patients with chronic periodontitis were enrolled during Oct. 2013 to Mar. 2015, and their clinical characteristics are shown in **Table 1**. The following diagnostic criteria were used as the inclusion in the study: generalized chronic periodontitis patients diagnosed by the classification of American Academy of periodontology in 1999 [8], aged from 35 to 80 years, who had non-surgical mechanical curettage performed prior to the study. The following patients were excluded: 1) patients with severe systemic condition as a contraindication for surgery, 2) pregnant or breastfeeding women, and 3) untreated periodontal patients. Patients who had prescribed medicine, which

Table 1. Characteristics of patients.

	EMD + DBBM + CM (n = 9)	EMD + DBBM (n = 9)	*p*-value
Age (years; mean ± SD)	57.7 ± 6.3	57.7 ± 6.3	
No Male/Female (light smoker)	5 (1)/4 (1)	5 (1)/4 (1)	
Osseous walls			
1-walled defect/non-contained	5	4	NS
2- or 3-walled defect/Contained	4	5	
Treated teeth			
Maxillary incisors/canines	2	1	
Maxillary premolars	1	2	
Maxillary molars	3 (2)	2 (1)	
Mandibular incisors/canines	1	1	
Mandibular premolars	0	0	
Mandibular molars	2	3 (1)	
Furcation involvement			
Peridontal Biotype Thick/Thin	6/3	7/2	NS

Statistically significant was calculated using non-paired t test. EMD: enamel matrix derivative; DBBM: deproteinized bovine bone mineral; CM: collagen membrane; NS: Not significant.

could influence periodontal status and/or bone metabolism before and during the study period were also excluded. Very light smokers (<1/2 packs per day) were included among the subjects [1]. Defect morphology was classified based on the component that was the most dominant in the defect as previously described [1] [5]. Defects that extended to bifurcation were included for stratified analyses (**Table 1**).

All clinical parameters, Löe's Gingival Index (GI) [9], PPD, CAL, and gingival recession (GR), were measured at baseline and 12 months post-treatment. The sites of deepest PPD in the intrabony defect of each tooth were used for clinical evaluations. A custom-made stent composed of acrylic resin was used for measurements [1]. Cone beam computed tomography (CBCT) (Finecube®, Yoshida, Tokyo, Japan) was used to analyze the volume of the bone defect at baseline and 12 months post-treatment, and then the FBR was calculated as described previously [1] (**Figure 1**).

All measurements and surgical procedures were performed by a calibrated single operator as previously described [1]. In brief, a mucoperiosteal flap was raised and granulation tissue was removed from the intrabony defect. Debridement of root surface was performed with metal curettes, rotary, and ultrasonic

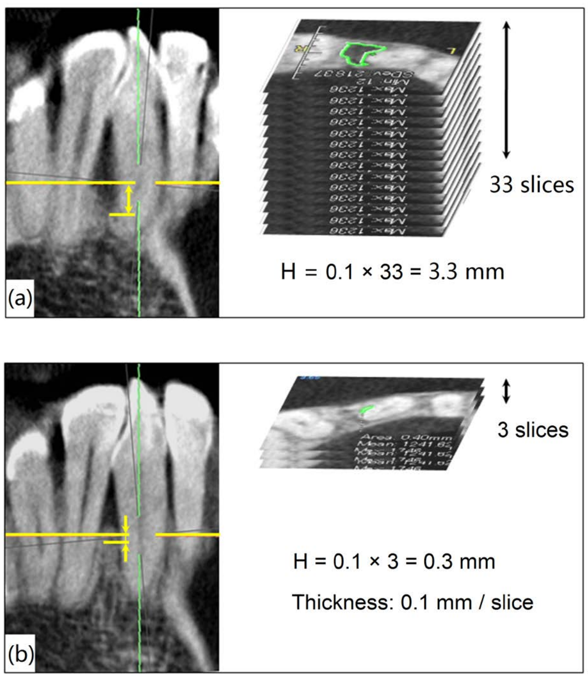

Figure 1. Analyses of the volume of the bone defect at baseline and 12 months post-treatment, and calculation for the filled bone rate (FBR). (a) Reference plane (RP) on cone beam computed tomography (CBCT) image at baseline and cross sections parallel to RP. The area is automatically calculated by tracing the outline of the bone defect on the CT software. Bone defect volume (DVB) is calculated by summation of the area of bone defect in each slice multiplied by a slice width of 0.1 mm (B_x mm^2) for all slices. $B_1 + B_2 + \dots B_{33}$ = DVB (mm^3). H: height of bone defect. (b) RP on CBCT image at 12 months post-treatment. DVB was calculated as described in **Figure 1(a)**. $P_1 + P_2 + P_3$ = DVP (mm^3), FBR (%) = (DVB − DVP)/DBP.

instruments. After the root surface was cleaned, EMD (Emdogain®, Straumann, Basel, Switzerland) was applied, and then DBBM (0.25 mm diameter, Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland) was filled inside the defect. In the test group, CM (Bio-Gide®, Geistlich Pharma AG) was trimmed and placed to cover the defect and bone substitute. The flap was prepositionally placed back and sutured with a 5-0 monofilament. Recall visits were once a month thereafter. Each measurement was made at follow-up visits at 6 and 12 months post-surgery. A representative case is shown in Figure 2 and Figure 3.

Figure 2. Images of a 53-year-old man at baseline and 12 months post-treatment. (a) Clinical picture at baseline. (b) Dental roentgenograph at baseline. (c) Clinical picture at 12 months post-treatment. (d) Dental roentgenograph at 12 months post-treatment.

Figure 3. Surgical procedures. (a) Debridement of intrabony defect. (b) Application of EMD and filling with DBBM. (c) Control group (without CM; left green arrow), Test group (with CM; right white arrow). (d) Suturing.

Comparisons of the proportion of defect morphology and periodontal biotypes were made using the chi-square test. For between-group comparisons, McNemar's test for bleeding on probing (BOP) and the Wilcoxon signed-rank test for gingival index (GI), tooth mobility (MOB), PPD, CAL, GR, and FBR were applied. The significance level was set at 5% for all analyses, and data were analyzed using SPSS 21.0 for Windows (IBM, Tokyo, Japan).

3. Results

There was no statistically significant difference in characteristics of defects between the EMD + DBBM + CM and EMD + DBBM groups (Table 1). All patients showed no infection or complications after surgery and completed the maintenance program. BOP in the EMD + DBBM + CM group significantly improved at 12 months post-treatment ($p = 0.031$). MOB significantly improved in the EMD + DBBM + CM group, while there was no statistically significant difference in the EMD + DBBM group ($p = 0.046$) (Table 2).

Table 3 shows mean values and the changes in select clinical parameters post-treatment. At 12 months post-treatment, the mean PPD and CAL decreased by 4.3 mm and 4.3 mm, respectively, in the EMD + DBBM + CM group, while it decreased by 3.3 mm and 3.3 mm, respectively, in the EMD + DBBM group. Thus, both groups showed statistically significant improvements post-treatment ($p = 0.007$).

Table 2. Changes in clinical parameters I.

	Baseline	12 months	*p*-value
BOP (positive/negative)			
EMD + DBBM + CM	7/2	0/9	**0.031***
EMD + DBBM	9/0	3/6	ND
p-value$	ND	**0.375***	
GI			
EMD + DBBM + CM	0.00 ± 0.00 [0, 0, 0]	0.00 ± 0.00 [0, 0, 0]	1$
EMD + DBBM	0.56 ± 0.73 [0, 0, 2]	0.00 ± 0.00 [0, 0, 0]	0.059$
p-value$	0.059$	1$	
MOB			
EMD + DBBM + CM	0.56 ± 053 [0, 1, 1]	0.11 ± 0.33 [0, 0, 1]	**0.046$**
EMD + DBBM	0.33 ± 0.50 [0, 0, 1]	0.21 ± 0.10 [0, 0, 1]	0.564$
p-value$	0.22$	0.44$	

Upper values are given as mean ± SE, lower values at brackets are shown as minimum, median and maximum. The boldface shows significant changes. Statistical significance of variation was verified by McNemar's test* and Wilcoxon signed-rank test$. BOP: Bleeding on probing; GI: Gingival index; MOB: Mobility.

Table 3. Changes in clinical parameters II.

	Baseline	12 months	*p*-value	Change	Rate of change (%)
PPD (mm)					
EMD + DBBM + CM	8.00 ± 0.53	3.67± 0.33	**0.007$**	4.33 ± 0.29	54.38 ± 1.89
EMD + DBBM	8.11 ± 0.48	4.78 ± 0.32	**0.007$**	3.33 ± 0.47	40.23 ± 4.18
p-value	0.931	**0.015$**		0.098	**0.021$**
CAL (mm)					
EMD + DBBM + CM	9.22 ± 0.62	4.89 ± 0.46	**0.007$**	4.33 ± 0.33	47.43 ± 2.72
EMD + DBBM	8.67 ± 0.47	5.33 ± 1.58	**0.007$**	3.33 ± 029	39.15 ± 3.85
p-value	0.401	0.551		0.071	0.11
GR (mm)					
EMD + DBBM + CM	1.22 ± 0.52	1.22 ± 0.49	1	0.00 ± 0.24	ND
EMD + DBBM	0.56 ± 0.29	0.56 ± 0.38	1	0.00 ± 0.44	ND
p-value	0.285	0.336		1	
FBR (%)					
EMD + DBBM + CM	0	77.58 ± 6.11	ND	77.58 ± 6.11	ND
EMD + DBBM	0	63.06 ± 8.81	ND	63.06 ± 8.81	ND
p-value	ND	0.066		0.066	

Values are given as mean ± SE. ND means no data. The boldface shows significant changes. Statistical significance of variation was verified by Wilcoxon signed-rank test. PPD: probing pocket depth; EMD: enamel matrix derivative; DBBM: deproteinized bovine bone mineral; CM: collagen membrane; CAL: Clinical attachment level; GR: Gingival recession; FBR: filled bone volume.

The change rate in mean PPD in the EMD + DBBM + CM group was significantly different from that in the EMD + DBBM group (54.4 vs. 40.2%, p = 0.021). However, there were no statistically significant differences in the change of mean CAL and GR between the two groups at 12 months post-treatment. Both groups showed increased bone fill at 12 months post-treatment; however, the difference in the FBR (77.6 vs. 63.1%) between groups was not statistically significant (**Table 3**).

4. Discussion

In the present study, clinical parameters including PPD, CAL, and the FBR were significantly improved with both combinational regenerative therapies by using either EMD + DBBM or EMD + DBBM + CM. The rate of PPD decrease was significantly greater in the CM-containing group than in the control group. Moreover, CAL gain and the FBR in the EMD + DBBM + CM group tended to be superior to those in the EMD + DBBM group, although these differences were not statistically significant. This lack of significance may be due to the slightly greater GR in the EMD + DBBM + CM group, which may reflect a significant difference in PPD decrease. A greater degree of GR was reported in GTR using resorbable membrane compared to EMD [10], while membrane use was believed to prevent epithelial downgrowth [11] and fibrous encapsulation of the bone substitute material [12] leading to bone substitute stability [13]. Some difficulties using membranes include increased GR [10], extended operation time attributed to membrane trimming and placement, gingival thinning and release incision, as well as higher cost. A report suggested that it is better not to isolate the site of bone regeneration from the periosteum, considering the possible influence of growth factors from the periosteum [14]. In the present study, EMD could act as a chemical barrier of epithelial downgrowth and/or EMD-derived growth factors might be activated by contact with the periosteum [7] [10]. In the literature, a comparison study of CM vs. CM + BG vs. open flap debridement (OFD) indicated CM and CM + BG showed a significantly higher CAL gain than OFD, while CM and CM + BG showed similar results [15], which suggests the efficacy of CM in regenerative therapy. Another study of combinational therapies comprising CM + DBBM and EMD + DBBM determined that use of EMD in place of CM could achieve a similar result [5]. The findings of our present study support the results of these previous studies, as we did not observe statistically significant differences in periodontal parameters between EMD + DBBM and EMD + DBBM + CM groups. Both surgical procedures were similarly as effective for even light smokers as non-smokers, which was consistent with our previous result [1].

Another finding of the present study was that three-dimensional (3D) CBCT analyses may be useful for filled bone evaluation. Few reports have reported the use of CBCT for radiographic measurements [1] [16]. CBCT was confirmed to be a useful method for morphological assessment of complicated forms of defects, such as bifurcation on curved root surfaces [1]. Further development of 3D analysis software using artificial intelligence is anticipated.

In summary, combinational regenerative therapy with triple materials (EMD, DBBM, and CM) for periodontal intrabony defects demonstrated that the rate of PPD reduction12 months post-treatment was significantly greater than that with double material combinations (EMD and DBBM). However, there were no significant differences between treatment groups in terms of CAL, FBR, BOP, and other parameters, although both combinational therapies resulted in better outcomes than those previously reported using single regenerative procedures.

5. Conclusion

The present study demonstrated that the use of EMD and DBBM is effective with or without CM for regeneration of periodontal tissues. The added use of CM seems to be effective for reducing degree of BOP, PPD and MOB; however, further studies on longer-term outcomes and a greater accumulation of cases are necessary to confirm our findings.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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