ORIGINAL ARTICLE

Evaluation of gingival crevicular fluid transforming growth factor-β1 level after treatment of intrabony periodontal defects with enamel matrix derivatives and autogenous bone graft: A randomized controlled clinical trial

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Abstract

Aim: The present study aimed to evaluate the effects of enamel matrix derivatives (EMD) either alone or combined with autogenous bone graft (ABG) applied to intrabony defects in chronic periodontitis patients on clinical/radiographic parameters and gingival crevicular fluid (GCF) transforming growth factor-β1 (TGF-β1) level and to compare with open flap debridement (OFD).
Materials and Methods: A total of 30 deep intrabony defects in 12 patients were randomly treated with EMD + ABG (combination group), EMD alone (EMD group), or OFD (control group). Clinical parameters, including plaque index, gingival index, bleeding on probing, probing depth, relative attachment level, and recession were recorded at baseline and 6 months postsurgery. Intrabony defect fill percentage was calculated on the standardized radiographs. TGF-β1 level was evaluated in GCF just before surgery and 7, 14, 30, 90, 180 days after surgery using enzyme-linked immunosorbent assay.
Results: All treatment procedures led to significant improvements at 6 months ($P < 0.01$). Gain in attachment level ($P < 0.01$) and radiographic defect fill ($P < 0.05$) of the combination and EMD groups were found to be significantly higher than those of the control group, while the use of EMD either with ABG or alone was observed to produce significantly less recession than the OFD ($P < 0.05$).
Conclusion: The findings suggest no clinical and radiographic differences between the combination and EMD groups whereas GCF TGF-β1 level demonstrates an increase during the healing phase and is positively affected from EMD.

Key words: Autogenous bone graft, chronic periodontitis, enamel matrix derivatives gingival crevicular fluid, periodontal bone loss, periodontal flap surgery, transforming growth factor

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Introduction

The ultimate goal of regenerative periodontal therapy is to prevent further attachment loss and restore the supporting

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structures (i.e., alveolar bone, periodontal ligament, and root cementum) that were lost due to trauma or disease in such a way that the architecture and function can be re-established.[1] Periodontal regeneration can be attained by the use of a commercially available enamel matrix derivative (EMD) product (Emdogain® Gel, Straumann, Switzerland) which contains mainly amelogenin and related proteins derived from porcine tooth germs.[2-5] When the regenerative treatment is targeted to intrabony defects, combining this product with a graft has been recommended in order to overcome the soft tissue collapse and maintain space because of its gel-like consistency.[6] Among different available graft materials, autogenous bone graft (ABG) has several ideal characteristics, including osteogenic, osteoinductive, and osteoconductive properties.[7,8] By the use of ABG together with EMD, two different wound healing types can be achieved in the intrabony defect as a result of a synergistic effect between these biomaterials.[8] While the ABG provides osteoinductive and/or osteoconductive effect, avoids flap collapse by maintaining space together with stabilizing the biomaterial, the EMD can exert biological potential by stimulating the development of new periodontal ligament and cementum.[8] The data from several clinical studies evaluating the use of EMD + ABG combination in intrabony defects suggest comparable outcomes in attachment gain (AG) and bone fill parameters.[8-10]

Although the underlying mechanism of EMD during periodontal wound healing process is not clear, some evidence emerged from *in vitro* studies. Besides expression of a number of molecules, that is, extracellular matrix molecules, cytokines, and growth factors, effects on the attachment, proliferation, chemotaxis, spreading, and survival properties of different types of periodontal cells have been demonstrated by the use of EMD.[11] There are limited data concerning the action mechanism of EMD on periodontal wound healing process following nonsurgical or surgical therapy by assessing the protein gingival crevicular fluid (GCF) level of any biomarker.[12-14] GCF could be considered to reflect the ongoing activities around the periodontium such as tissue formation, remodeling, tissue inflammation, and destruction.[15-19] Transforming growth factor-β1 (TGF-β1) is a multifunctional peptide which is produced by both activated macrophages and neutrophils that are existing during the initial phases of wound healing. It shows a chief role in wound healing process, including tissue remodeling and regeneration by stimulating differentiation, proliferation and expression of cells.[16,20-23] TGF-β1 alone or in combination with other growth factors accelerates several stages of wound healing.[24-26] It stimulates the development of new granulation tissue through angiogenesis and collagen production by fibroblasts.[24,26,27] By this way, TGF-β1 exerts regulation responsibility in collagen metabolism in some kind of pathological conditions, including periodontitis.[16,22,28,29] It is shown that TGF-β1 has some degree of clinical effectiveness in stimulating periodontal regeneration *in vivo*.[16,30,31] The present study aimed to evaluate the effects of EMD either alone or combined with ABG applied to intrabony defects in chronic periodontitis patients on clinical, radiographic parameters and GCF TGF-β1 level and to compare with open flap debridement (OFD). Hypothesis of the current study is to test whether the use of ABG and EMD combination in the treatment of intrabony periodontal defects of chronic periodontitis patients enhance the clinical, radiographic, and biochemical parameters in comparison with the OFD alone.

Materials and Methods

Experimental design

Three different treatment modalities to treat deep intrabony defects were compared in this randomized, prospective, controlled, parallel, clinical study. The control group defects were treated with OFD only while the test group defects were treated with EMD alone (EMD group) and EMD combined with ABG (combination group). The same periodontal flap procedure was accomplished in three groups, except EMD application on the root surfaces in both test groups. Additional graft usage was the only difference between two test groups. Clinical and radiographic parameters were measured at baseline and 6 months after surgery.

Sample size calculation

A sample size calculation was performed. An α error of 5% with 80% power and standard deviation of 1.35 mm with a difference of 1.85 mm AG score between the groups was considered clinically significant which is evident from a recent clinical study.[32] It was indicated that a sample of 9 defects per group would be needed. Bearing in mind that some defects could be lost during the follow-up period, 10 defects per group were included.

Patient and site selection

A total of 30 defects in 12 chronic periodontitis patients (6 female and 6 male) with a mean age of 44.17 ± 7.80 years were included (i.e., 10 defects in each group) from September 2010 to September 2012. Prior to the study, informed consent was signed by all patients. The design and flow chart of the study are shown in Figure 1. Treatments of the patients were performed at the Clinics of Department of Periodontology, Faculty of Dentistry, Marmara University, Istanbul, Turkey. The inclusion criteria were: (a) No systemic diseases that contraindicated periodontal surgery and could affect the consequences of the therapy; (b) no smoking (c) no medications (d) no pregnancy or lactation; (e) a good oral hygiene level (plaque index [PI] <1)[33] and full mouth bleeding on probing (BOP) score <20% after initial periodontal treatment (IPT), (f) compliance with the maintenance program and

(g) minimum one intrabony defect existence with a probing depth (PD) ≥6 mm, radiographic depth ≥3 mm as detected on the radiographs.[10,34,35]

Ethical approval

The study was completed compatible with the Helsinki Declaration presented in 1975 which is revised in 2000. Ethical approval of the study protocol was obtained from the Research Ethical Board of Marmara University Faculty of Medicine, Istanbul, Turkey (number: MAR-YÇ-2009–0048).

Randomization and allocation concealment

All defects included in this study were distributed to three treatment groups according to a randomization table created in software, which can be reached through internet access (http://www.randomization.com). If a patient had two or more intrabony defects, they were distributed to the treatment groups in a clockwise direction.

Clinical parameters

The PI,[33] gingival index (GI),[33] BOP, PD, relative attachment level (RAL), recession scores were noted for the deepest site of the defect before and 6 months after the surgery using the same type of periodontal probe (UNC 15, Hu-Friedy, Chicago, IL, USA). Probing assessments were recorded at the nearest millimeter mark, or if the obtained measurement was positioned between two marks, it would be considered the increment of 0.5 mm during the evaluation. The clinical assessment were done at 6 sites of the tooth, vestibulary (mesial, mid, distal) and oral (mesial, mid, and distal) using an adapted acrylic stent with reference holes by one calibrated examiner (OBA), who was not blinded to the surgical procedures.

Radiographic assessment

Standardized periapical radiographs of all intrabony defects were taken at baseline and after 6 months with long cone paralleling technique using an appropriate screening device (RWT Roentgenographic-System, Kentzler-Kaschner Dental GmbH, Germany). Radiographs were digitized using a digital camera (Canon Powershot G10, Japan) and edited at a resolution of 8-bits 300 dpi grayscale images by photo editor software (ACD Photo Editor 3.1, ACD Systems Ltd., USA). Before evaluation, two radiographs taken from the same defect were brought to the same size by using cut and resizing tools of the software. Then, obtained images were analyzed in image processing software (Image J 1.43u, Wayne Rasband, National Institute of Health, USA). The image measurements were used in a formula $(1 - [A2/A1 \times (L1/L2)^2] \times 100)$ in order to calculate the radiographic bone fill percentage where A1 represents the baseline area specified by borders of the defect; A2 the postoperative area specified by borders of the defect; L1 linear length between mesial and distal cementoenamel junctions on baseline radiograph and L2 linear length between mesial and distal cementoenamel junctions on the radiograph taken at 6 months [Figure 2].

Intrasurgical measurements

The intrabony defect depth (IDD) was measured as the distance from the maximum coronal part of the alveolar bone to the bottom of the defect. Besides, defect configuration (i.e., number of walls) was recorded during surgery.

Determination of gingival crevicular fluid transforming growth factor-β1 levels

GCF samples were collected with paper strips (PerioPaper® Oraflow Inc., New York, USA) just before surgery and 7, 14, 30, 90, 180 days after surgery and evaluated by enzyme-linked immunosorbent assay using a commercially available kit for TGF-β1 (Quantikine Human TGF-β1, R and D Systems, Inc., USA) according to the manufacturers' instructions.[16] The volume of samples as determined by a standardized automated gingival fluid measuring device (Periotron 8000, Smithtown, New York, USA), and strips placed into sterile tubes were stored at −80°C.

Treatment of intrabony defects

All procedures were performed by the same operator (OBA). All patients received IPT including oral hygiene instructions, scaling and root planing using both hand (Gracey, SG 3/4, 5/6, 7/8, 11/12, 13/14, Minifive, SAS 3/4, Hu-Friedy, USA) and ultrasonic instruments (Cavitron® Bobcat Pro®, Dentsply International Inc., USA). After 8 weeks, each defect was randomly assigned to the groups. After local anesthesia (Ultracain® D-S forte, Hoechst Marion Roussel, Turkey), sulcular incisions were made and full-thickness flaps were raised buccally and lingually, granulation tissues removed, and the root surfaces gently scaled and planed. Special care was taken to create a surgical area free from blood and saliva. In the EMD and combination groups, the exposed root surfaces were conditioned with 24% EDTA gel (Prefgel®, Straumann, Switzerland) for 2 min.[36] Surgical area was then rinsed with saline. EMD gel was injected onto the intrabony defects and root surfaces. Then, in the combination group, the adequate amount of ABG obtained from adjacent bone surfaces by using hand instruments (Ochsenbein Periodontal Chisel CO_2, Rhodes Back Action Periodontal Chisel C36/37, Hu-Friedy Inst. Co. USA) was mixed with the gel and placed into the bone defects. Finally, a second layer of EMD gel was injected to cover the ABG. Then, the flaps were sutured interdentally with an absorbable polyglycolide-co-lactide suture (5–0 pegelak, Doğsan A. R. Trabzon, Turkey).

Postoperative care

Patients received amoxicillin + potassiumclavulanate (1000 mg tablet, GlaxoSmithKline, Istanbul, Turkey) twice a day for 7 days, naproxen sodium (550 mg tablet,

Bilim Ilac, Istanbul, Turkey) twice a day for 7 days and 0.12% chlorhexidine + benzydaminehydrocloride mouth rinse (Drogsan, Ankara, Turkey), twice a day for 4 weeks. Mechanical tooth cleaning was not allowed in the surgical area for the first 4 postoperative weeks. Sutures were removed at 14 days following surgery. Patients were recalled strictly for supra-gingival tooth cleaning and polishing procedures weekly in the first 2 months, once for 2 weeks during the 3rd month and monthly after 3 months.

Statistical analysis

Statistical software (SPSS® 15.0 for Windows, Chicago, IL, USA) was used for data analysis. Nonparametric tests were used because the data did not show normal distribution. The Friedman test was used for intragroup repeated-measures, Wilcoxon test was used for paired intragroup comparison. Kruskal–Wallis test was used to detect intergroup differences, followed by a *post-hoc* Bonferroni corrected Mann–Whitney U-test at $P < 0.017$ level. Statistical significance was evaluated at $P < 0.05$ level.

Results

A total of 30 intraosseous defects in 12 patients were randomly treated. No dropouts occurred until the end of the study. No adverse reactions were seen. Minor complications such as swelling and bleeding were present in the early healing phase after surgery.

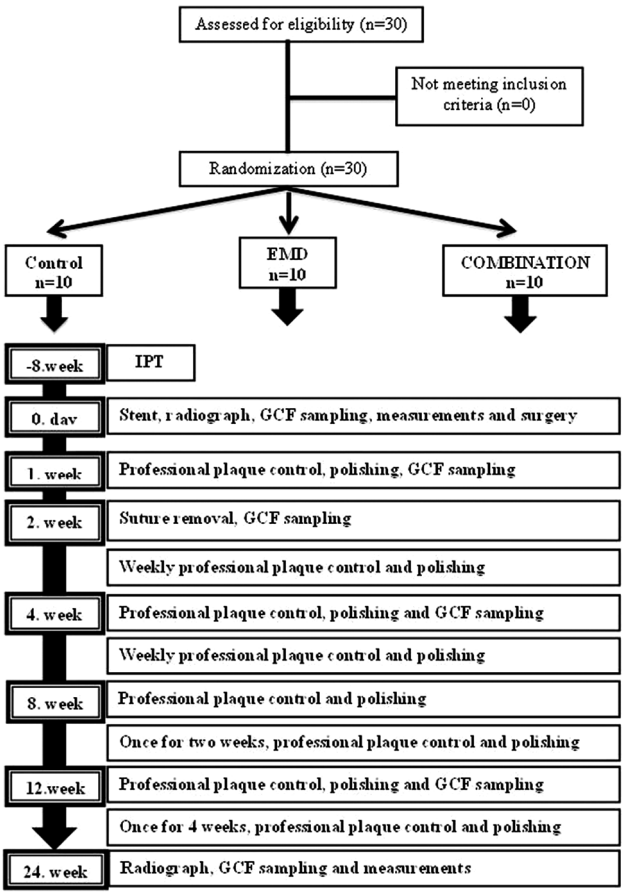

Figure 1: Flow chart of the study

Clinical and radiographic parameters

Table 1 demonstrates similar initial clinical parameters of the groups. There were no significant differences in any of the clinical measurements among the groups at baseline ($P > 0.05$). Although, the defect distribution and configuration showed a comparable outcome [Table 2], there was no statistically significant difference in the IDD measurements among the groups ($P > 0.05$) [Table 1]. Table 3 demonstrates the intragroup comparisons of the

Table 1: Initial clinical parameters

	Control	EMD	Combination	$P^{\dagger}$
PI				
Full mouth	0.36±0.17	0.23±0.06	0.32±0.14	0.121
Interproximal	0.75±0.26	0.65±0.24	0.55±0.16	0.105
GI				
Full mouth	0.31±0.15	0.24±0.09	0.28±0.12	0.321
Interproximal	0.90±0.21	0.90±0.21	0.78±0.24	0.305
BOP (%)				
Full mouth	8.50±2.03	8.29±2.33	8.98±2.29	0.598
Interproximal	62.50±17.68	55.00±10.54	60.33±17.29	0.525
PD (mm)	7.60±1.51	8.30±1.70	7.93±1.66	0.624
IDD (mm)	5.60±1.64	6.40±1.95	5.20±1.39	0.280

†Kruskal-Wallis test, $P<0.05$. PI=Plaque index; GI=Gingival index; BOP=Bleeding on probing; PD=Probing depth; EMD=Enamel matrix derivatives; IDD=Intrabony defect depth

Table 2: Characteristics of intrabony defects

	Number of defect walls			Defect localization		
	1 Walled	1-2 Walled	1-2-3 Walled	Incisor/ canine	Premolar	Molar
Control	1	9	-	3	2	5
EMD	4	5	1	2	6	2
Combination	1	6	3	5	2	3

EMD=Enamel matrix derivatives

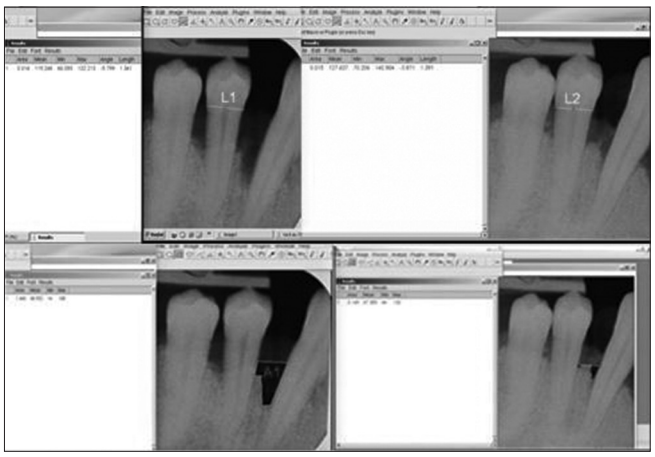

Figure 2: The measures for calculation of the radiographic bone fill percentage

Table 3: Intragroup, intergroup, and paired comparisons of clinical and radiographic parameters

	Control				EMD				Combination			
	Baseline	6 months	Change	P*	Baseline	6 months	Change	P*	Baseline	6 months	Change	P*	P¥
PI	0.75±0.26	0.35±0.24	0.40±0.21	0.005	0.65±0.24	0.15±0.24	0.50±0.00	0.002	0.55±0.16	0.05±0.16	0.50±0.00	0.002	0.126
GI	0.90±0.21	0.70±0.22	0.20±0.22[a]	0.038	0.90±0.21	0.25±0.26	0.65±0.33	0.006	0.78±0.24	0.27±0.34	0.51±0.33	0.010	0.011
BOP (%)	62.50±17.68	37.50±17.68	25.00±0.00[a,b]	0.002	55.00±10.54	12.50±13.18	42.50±12.08	0.004	60.33±17.29	12.62±13.05	47.71±18.49	0.005	0.002
PD (mm)	7.60±1.51	3.20±0.79	4.40±1.17	0.004	8.30±1.70	3.30±0.67	5.00±1.41	0.005	7.93±1.66	3.22±0.41	4.71±1.63	0.005	0.660
RAL (mm)	12.10±2.13	10.50±1.78	1.60±0.70[a,b]	0.004	13.70±2.58	9.20±2.66	4.50±3.24	0.005	13.06±1.77	9.51±0.84	3.55±1.46	0.005	0.001
Recession (mm)	4.70±1.70	7.30±1.49	−2.70±0.95[a,b]	0.004	5.40±1.96	5.90±2.28	−0.50±2.72	0.310	5.12±1.91	6.28±0.82	−1.16±1.62	0.064	0.001
Radiographic bone fill (%)			35.31±20.56[a,b]				65.98±14.76				64.56±24.23		0.003

*Wilcoxon signed-rank test, $P<0.05$; ¥Kruskal-Wallis test, $P<0.05$; [a]Different from EMD, Mann-Whitney test, Bonferroni *post-hoc* test, $P<0.17$; [b]Different from combination, Mann-Whitney test, Bonferroni *post-hoc* test, $P<0.17$. EMD=Enamel matrix derivatives; PI=Plaque index; GI=Gingival index; BOP=Bleeding on probing; PD=Probing depth; RAL=Relative attachment level

mean PI, GI, BOP, PD, RAL, recession scores. All measured parameters at 6 months with respect to their baseline value revealed statistically significant improvements in all groups ($P < 0.05$), except the control group which demonstrated statistically significant recession ($P < 0.01$) at 6 months. The intergroup comparisons of the changes in clinical and radiographic parameters displayed statistically significant differences among the groups ($P < 0.05$), except PI and PD scores ($P > 0.05$) [Table 3].

Further, paired comparisons revealed statistically significant differences between the control and EMD groups also between the control and combination groups ($P < 0.05$), whereas no significant differences, were present between the combination and EMD groups in any of the clinical and radiographic parameters ($P > 0.05$) [Table 3].

Biochemical parameters

Table 4 demonstrates the changes in GCF volume throughout the study period. In the control group, the GCF volume showed a slight increase on day 7 and diminished under the initial value during 180 days period. GCF volume in the control group decreased from a baseline value of 1.03 ± 0.59 μL to 0.51 ± 0.31 μL at 6 months. However, this decrease was not statistically significant ($P > 0.05$) [Table 4]. GCF volume measured in the EMD group decreased from 0.87 ± 0.52 μL to 0.45 ± 0.35 μL at 6 months ($P > 0.05$) [Table 4]. In the combination group, baseline GCF volume (1.01 ± 0.74 μL) exhibited increase in 7th and 14th day evaluation periods ($P < 0.05$) and decreased to 0.62 ± 0.53 μL at 6 months ($P < 0.05$) [Table 4]. Statistically significant differences were detected in GCF volume at 90th day with respect to baseline, 7th day and 14th with respect to 90th day, and 180th day with respect to 14th day in the combination group [Table 5].

TGF-β1 could not be detected in 25% of total GCF samples, 41% of the control group, 26% of the EMD group and 6% of the combination group [Table 6]. TGF-β1 concentration in the control group increased on day 7 remained high with respect to the baseline value until the end of the evaluation period. However, the intragroup statistical analysis could not be performed in the control group because of the excessive number of TGF-β1 undetected GCF samples. In the EMD group, TGF-β1 concentration showed a slight increase on day 7 and remained high until the 30th day and then decreased below the baseline concentration at day 90. These changes were not statistically significant throughout the whole evaluation period ($P > 0.05$). In the combination group, TGF-β1 concentration showed similar changes with the EMD group and decreased from baseline value of 4.39 ± 3.57 ng/mL to 3.63 ± 1.85 ng/mL at 6 months ($P > 0.05$). As shown in Table 4, baseline TGF-β1 concentrations did not show statistically significant

Table 4: Changes of GCF volume, TGF-β1 concentration and TGF-β1 amount

	Days						
	0	7	14	30	90	180	*P**
GCF volume (μL)							
Control	1.03±0.59	1.26±0.55	1.10±0.63	0.83±0.45	0.56±0.48	0.51±0.31	0.060
EMD	0.87±0.52	1.21±0.52	1.13±0.75	0.94±0.61	0.71±0.25	0.45±0.35	0.098
Combination	1.01±0.74	1.23±0.71	1.16±0.82	0.88±0.61	0.38±0.15	0.62±0.53	0.028
P†	0.803	0.904	0.996	0.964	0.041	0.724	
TGF-β1 concentration (ng/mL)							
Control	1.15±0.51	1.61±1.05	4.31±8.74	2.93±3.08	4.06±4.57	2.75±2.53	-
EMD	4.49±4.38	5.16±4.95	4.23±0.84	4.85±3.43	3.00±2.21	2.98±1.52	0.477
Combination	4.39±3.57	6.03±7.26	4.92±4.89	4.88±5.04	3.71±3.59	3.63±1.85	0.388
P†	0.130	0.019	0.096	0.549	0.953	0.564	
TGF-β1 amount (pg)							
Control	1.47±0.77	2.28±2.09	1.74±1.22	1.40±1.11	1.40±1.52	0.29±0.15	-
EMD	2.82±2.26	3.49±2.00	4.90±2.81	3.49±1.76	1.95±1.38	1.26±0.44	0.168
Combination	3.68±3.15	5.89±4.85	3.04±1.82	3.44±2.46	1.45±1.24	1.86±1.47	0.141
P†	0.222	0.184	0.025	0.232	0.713	0.050	

†Kruskal-Wallis test, $P<0.05$, *Friedman test, $P<0.05$. EMD=Enamel matrix derivatives; GCF=Gingival crevicular fluid; TGF-β1=Transforming growth factor-β1

Table 5: Comparison of the GCF volume in combination group at different evaluation periods

	Days														
	0/7	0/14	0/30	0/90	0/180	7/14	7/30	7/90	7/180	14/30	14/90	14/180	30/90	30/180	90/180
P†	0.307	0.475	0.575	0.028	0.123	0.333	0.050	0.013	0.161	0.123	0.013	0.036	0.068	0.173	0.237

†Wilcoxon signed rank test, $P<0.05$. GCF=Gingival crevicular fluid

Table 6: Number of GCF samples which TGF-β1 was not detected

Day	Control (*n*=60)	EMD (*n*=60)	Combination (*n*=60)
0	4	-	-
7	2	3	-
14	2	3	-
30	6	3	2
90	4	2	1
180	6	4	1
Total (%)	25 (41)	16 (26)	4 (6)

EMD=Enamel matrix derivatives; GCF=Gingival crevicular fluid; TGF-β1=Transforming growth factor-β1

difference among the groups ($P > 0.05$), except day 7 ($P < 0.05$) [Table 4].

In the control group, TGF-β1 amount demonstrated a slight increase on day 7 and 14 followed by a decrease under the baseline value at 6 months. However, this change could not be evaluated statistically because of the undetected TGF-β1 amount in GCF samples. In the EMD group, TGF-β1 amount decreased from a baseline value of 2.81 ± 3.15 pg to 1.26 ± 0.44 pg at 6 months ($P > 0.05$). In the combination group TGF-β1 amount displayed similar changes and decreased from 3.68 ± 3.15 pg baseline amount to 1.86 ± 1.47 pg at 6 months. These changes were not statistically significant ($P > 0.05$). Intergroup comparisons revealed that TGF-β1 amount in the EMD group was significantly higher than that in the combination group at 14th day only ($P < 0.05$) [Table 4].

Discussion

The primary aim of this study was to assess regenerative effects of EMD in intrabony periodontal defects combined with ABG. Results have shown that all treatment procedures led to statistically significant clinical and radiographic improvements. The present trial demonstrates that the application of EMD together with OFD promotes relevant advantages in the therapy of intrabony defects compared to OFD alone. However, combining EMD with ABG did not show any advantage compared to the use of EMD alone.

In this study, gingival health was evaluated by GI and BOP measurements. At 6 months, changes in the interproximal GI and BOP parameters were statistically significant among the groups ($P < 0.05$). Reductions of these parameters were significantly higher in the EMD and combination groups than the control group. However, there is no significant difference between the EMD and combination groups ($P > 0.05$). These findings are in line with the studies which had examined the possible advantage of EMD when used with OFD and shown that addition of EMD exhibited statistically significant soft tissue healing when compared with OFD alone.[2-5,37,38] Possibly by the decrease of matrix metalloproteinase levels in the EMD treated sites,[39] and antimicrobial[40,41] and anti-inflammatory effects[42] of PGA.

Clinical evaluations demonstrated similar and significant PD reductions in all groups at 6 months ($P < 0.05$) with insignificant differences among the groups ($P > 0.05$). The

PD reductions obtained from the studies evaluated the potential effect of the use EMD alone or its combinations with graft materials in the intrabony defects have reported to be between 1.85 mm and 5.40 mm,[8-10,43-47] while our results showing 5.0 mm and 4.71 mm reduction of the EMD and combination groups, were in accordance with these clinical trials. The PD reductions of the studies evaluating the effect of OFD in the intrabony defects with initial PD score >6 mm ranged from 1.4 mm to 4.5 mm.[45,47-52] The present study demonstrated similar PD reductions in the control group with 7.60 mm mean initial PD, which supports the results of these studies. Assessing the PD reductions together with the gingival recession scores may reflect the regenerative response of the periodontal treatment.[53]

Clinical evaluations demonstrated significant AG in all groups at 6 months ($P < 0.01$). AGs obtained in the control, EMD and combination groups were 1.60 ± 0.70 mm, 4.50 ± 3.24 mm, 3.55 ± 1.46 mm, respectively. The AG obtained in the EMD and combination groups was significantly higher than the control group ($P < 0.01$) but, there was no statistically significant difference between the EMD and combination groups ($P > 0.05$). The studies evaluating the clinical effect of OFD in intrabony defects demonstrated AG between 1.19 mm and 2.75 mm.[37,41,47,48,50,54-56] AG in our control group supported these evidences as1.60 ± 0.70 mm. In the present study, surgical use of EMD in intrabony defects displayed significant AG when compared with OFD. This finding was in agreement with a systematic review investigating the probable advantage of EMD when used in addition to OFD.[57] Another recently published systematic review showed that various combinations of EMD with different types of grafting materials have the potential to enhance AG compared to EMD alone.[10,43,44,58-60]

Although the combination of EMD with a number of bone grafts has led to significant AG results, a larger number of studies have failed to show any significant differences in terms AG.[8,61-68] The mean AG results revealed in the EMD and combination group were 4.50 ± 3.24 mm and 3.55 ± 1.46 mm, respectively. These AG results are consistent with the AG results revealed from the studies evaluating the regenerative response of the use of EMD alone or various combinations of graft materials with EMD in intrabony periodontal defects.[8,10,44,60,61,63,65,66]

The radiographic evaluation showed the amount of the radiographic bone fill percentage within the defect, was 35.31 ± 20.56, 65.98 ± 14.76 and 64.56 ± 24.23 for the control group, EMD group, and combination group, respectively. These obtained radiographic results were in agreement with the radiographic findings of other studies that present radiographically detected additional newly formed hard tissue.[8,54,69-72] Crea *et al*.[69] presented 50% bone fill 1-year after EMD application.

GCF is a vehicle which represents a noninvasive access to the periodontium. Monitoring the contents of GCF can provide detecting the tissue and cell-derived molecules not only originated from microbiota but also originated from the host response. Evaluation of varying molecular levels detected in the GCF might be of value as a prognostic marker of periodontal and systemic health, wound healing activity, and therapeutic progress following periodontal therapy.[16-18,73-76]

In the present study, TGF-β1 level present in the GCF during reconstruction process was evaluated after the treatment of intrabony defects with three different treatment approaches. At the early healing phase, GCF volume and TGF-β1 levels increased followed by reductions below respective baseline levels. Kuru *et al*.[16] suggested that TGF-β1 could be noticeable in GCF and the level of this growth factor rises transiently after regenerative periodontal surgery using nonresorbable membranes. In a recent study, Ribeiro *et al*.[14] applied OFD with a minimally invasive surgical technique with and without EMD application and analyzed the levels of mediators, including TGF-β1 involved in GCF after the periodontal surgery. According to this study, TGF-β1 levels increased after 15 days in both groups and reduced to baseline values after 3 months which are similarly changed in our study. In contradiction to our results, no differences in TGF-β1 levels were observed between the groups in that study. Studies have presented the existence of TGF-β1 or TGF-β-like molecules, bone morphogenic protein-like growth factor and bone sialoprotein-like molecules in EMD together with its stimulative effects on various types of periodontal cells.[11,77-81] Our biochemical results revealed by the evaluation of the GCF samples support these *in vitro* evidence biochemically. However, in our study the use of ABG together with EMD in the regenerative treatment of periodontal intrabony defects did not show any significant difference in terms of detected TGF-β1 levels in GCF.

A limitation of the current study may be the sample size. Although a power analysis was performed from the current literature, the number of defects included to the study groups may limit the generalizability of this study. Further clinical trials with larger sample size are needed for enlightening the clinical and biochemical benefits of EMD. Another limitation was that the measurements and interventions were made without blinding of the clinical examiners to the experimental groups, which has the potential for bias. However, the potential bias was minimized by randomly assigning the participants to the groups and following the standardized study protocol. It must be taken into account that the examiners were calibrated to confirm accuracy and reproducibility of measurements.

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Conflicts of interest

There are no conflicts of interest.

References

1. Karring T. Regenerative periodontal therapy. J Int Acad Periodontol 2000;2:101-9.
2. Hammarström L, Heijl L, Gestrelius S. Periodontal regeneration in a buccal dehiscence model in monkeys after application of enamel matrix proteins. J Clin Periodontol 1997;24(9 Pt 2):669-77.
3. Hammarström L. Enamel matrix, cementum development and regeneration. J Clin Periodontol 1997;24(9 Pt 2):658-68.
4. Heijl L. Periodontal regeneration with enamel matrix derivative in one human experimental defect. A case report. J Clin Periodontol 1997;24(9 Pt 2):693-6.
5. Mellonig JT. Enamel matrix derivative for periodontal reconstructive surgery: Technique and clinical and histologic case report. Int J Periodontics Restorative Dent 1999;19:8-19.
6. Froum S, Lemler J, Horowitz R, Davidson B. The use of enamel matrix derivative in the treatment of periodontal osseous defects: A clinical decision tree based on biologic principles of regeneration. Int J Periodontics Restorative Dent 2001;21:437-49.
7. AlGhamdi AS, Shibly O, Ciancio SG. Osseous grafting part I: Autografts and allografts for periodontal regeneration – A literature review. J Int Acad Periodontol 2010;12:34-8.
8. Guida L, Annunziata M, Belardo S, Farina R, Scabbia A, Trombelli L. Effect of autogenous cortical bone particulate in conjunction with enamel matrix derivative in the treatment of periodontal intraosseous defects. J Periodontol 2007;78:231-8.
9. Trombelli L, Annunziata M, Belardo S, Farina R, Scabbia A, Guida L. Autogenous bone graft in conjunction with enamel matrix derivative in the treatment of deep periodontal intra-osseous defects: A report of 13 consecutively treated patients. J Clin Periodontol 2006;33:69-75.
10. Yilmaz S, Cakar G, Yildirim B, Sculean A. Healing of two and three wall intrabony periodontal defects following treatment with an enamel matrix derivative combined with autogenous bone. J Clin Periodontol 2010;37:544-50.
11. Bosshardt DD. Biological mediators and periodontal regeneration: A review of enamel matrix proteins at the cellular and molecular levels. J Clin Periodontol 2008;35 8 Suppl: 87-105.
12. Giannopoulou C, Andersen E, Brochut P, Plagnat D, Mombelli A. Enamel matrix derivative and systemic antibiotics as adjuncts to non-surgical periodontal treatment: Biologic response. J Periodontol 2006;77:707-13.
13. Okuda K, Miyazaki A, Momose M, Murata M, Nomura T, Kubota T, *et al.* Levels of tissue inhibitor of metalloproteinases-1 and matrix metalloproteinases-1 and -8 in gingival crevicular fluid following treatment with enamel matrix derivative (EMDOGAIN). J Periodontal Res 2001;36:309-16.
14. Ribeiro FV, Casarin RC, Júnior FH, Sallum EA, Casati MZ. The role of enamel matrix derivative protein in minimally invasive surgery in treating intrabony defects in single-rooted teeth: A randomized clinical trial. J Periodontol 2011;82:522-32.
15. Embery G, Waddington R. Gingival crevicular fluid: Biomarkers of periodontal tissue activity. Adv Dent Res 1994;8:329-36.
16. Kuru L, Griffiths GS, Petrie A, Olsen I. Changes in transforming growth factor-beta1 in gingival crevicular fluid following periodontal surgery. J Clin Periodontol 2004;31:527-33.
17. Kuru L, Kirby AC, Griffiths GS, Petrie A, Olsen I. Changes in soluble adhesion molecules in gingival crevicular fluid following periodontal surgery. J Periodontol 2005;76:526-33.
18. Kuru L, Yilmaz S, Kuru B, Köse KN, Noyan U. Expression of growth factors in the gingival crevice fluid of patients with phenytoin-induced gingival enlargement. Arch Oral Biol 2004;49:945-50.
19. Offenbacher S, Collins JG, Arnold RR. New clinical diagnostic strategies based on pathogenesis of disease. J Periodontal Res 1993;28 (6 Pt 2):523-35.
20. Igarashi A, Okochi H, Bradham DM, Grotendorst GR. Regulation of connective tissue growth factor gene expression in human skin fibroblasts and during wound repair. Mol Biol Cell 1993;4:637-45.
21. Kingsley DM. The TGF-beta superfamily: New members, new receptors, and new genetic tests of function in different organisms. Genes Dev 1994;8:133-46.
22. Laiho M, Keski-Oja J. Growth factors in the regulation of pericellular proteolysis: A review. Cancer Res 1989;49:2533-53.
23. Parkar MH, Kuru L, Giouzeli M, Olsen I. Expression of growth-factor receptors in normal and regenerating human periodontal cells. Arch Oral Biol 2001;46:275-84.
24. Lynch SE, Colvin RB, Antoniades HN. Growth factors in wound healing. Single and synergistic effects on partial thickness porcine skin wounds. J Clin Invest 1989;84:640-6.
25. Canalis E. Clinical review 35: Growth factors and their potential clinical value. J Clin Endocrinol Metab 1992;75:1-4.
26. Rodrigues TL, Marchesan JT, Coletta RD, Novaes AB Jr, Grisi MF, Souza SL, *et al.* Effects of enamel matrix derivative and transforming growth factor-beta1 on human periodontal ligament fibroblasts. J Clin Periodontol 2007;34:514-22.
27. Dennison DK, Vallone DR, Pinero GJ, Rittman B, Caffesse RG. Differential effect of TGF-beta 1 and PDGF on proliferation of periodontal ligament cells and gingival fibroblasts. J Periodontol 1994;65:641-8.
28. Gürkan A, Emingil G, Cinarcik S, Berdeli A. Post-treatment effects of subantimicrobial dose doxycycline on clinical parameters and gingival crevicular fluid transforming growth factor-beta1 in severe, generalized chronic periodontitis. Int J Dent Hyg 2008;6:84-92.
29. Overall CM, Wrana JL, Sodek J. Independent regulation of collagenase, 72-kDa progelatinase, and metalloendoproteinase inhibitor expression in human fibroblasts by transforming growth factor-beta. J Biol Chem 1989;264:1860-9.
30. Selvig KA, Wikesjö UM, Bogle GC, Finkelman RD. Impaired early bone formation in periodontal fenestration defects in dogs following application of insulin-like growth factor (II). Basic fibroblast growth factor and transforming growth factor beta 1. J Clin Periodontol 1994;21:380-5.
31. Tatakis DN, Wikesjö UM, Razi SS, Sigurdsson TJ, Lee MB, Nguyen T, *et al.* Periodontal repair in dogs: Effect of transforming growth factor-beta 1 on alveolar bone and cementum regeneration. J Clin Periodontol 2000;27:698-704.
32. Francetti L, Del Fabbro M, Basso M, Testori T, Weinstein R. Enamel matrix proteins in the treatment of intra-bony defects. A prospective 24-month clinical trial. J Clin Periodontol 2004;31:52-9.
33. Löe H. The gingival index, the plaque index and the retention index systems. J Periodontol 1967;38 Suppl: 610-6.
34. Sculean A, Barbé G, Chiantella GC, Arweiler NB, Berakdar M, Brecx M. Clinical evaluation of an enamel matrix protein derivative combined with a bioactive glass for the treatment of intrabony periodontal defects in humans. J Periodontol 2002;73:401-8.
35. Sculean A, Pietruska M, Schwarz F, Willershausen B, Arweiler NB, Auschill TM. Healing of human intrabony defects following regenerative periodontal therapy with an enamel matrix protein derivative alone or combined with a bioactive glass. A controlled clinical study. J Clin Periodontol 2005;32:111-7.
36. Blomlöf JP, Blomlöf LB, Lindskog SF. Smear removal and collagen exposure after non-surgical root planing followed by etching with an EDTA gel preparation. J Periodontol 1996;67:841-5.
37. Rasperini G, Ricci G, Silvestri M. Surgical technique for treatment of infrabony defects with enamel matrix derivative (Emdogain): 3 case reports. Int J Periodontics Restorative Dent 1999;19:578-87.
38. Yilmaz S, Kuru B, Altuna-Kiraç E. Enamel matrix proteins in the treatment of periodontal sites with horizontal type of bone loss. J Clin Periodontol 2003;30:197-206.
39. Heard RH, Mellonig JT, Brunsvold MA, Lasho DJ, Meffert RM, Cochran DL. Clinical evaluation of wound healing following multiple exposures to enamel matrix protein derivative in the treatment of intrabony periodontal defects. J Periodontol 2000;71:1715-21.
40. Arweiler NB, Auschill TM, Donos N, Sculean A. Antibacterial effect of an enamel matrix protein derivative on *in vivo* dental biofilm vitality. Clin Oral Investig 2002;6:205-9.
41. Sculean A, Auschill TM, Donos N, Brecx M, Arweiler NB. Effect of an enamel matrix protein derivative (Emdogain) on *ex vivo* dental plaque vitality. J Clin Periodontol 2001;28:1074-8.
42. Myhre AE, Lyngstadaas SP, Dahle MK, Stuestøl JF, Foster SJ, Thiemermann C, *et al.* Anti-inflammatory properties of enamel matrix derivative in human blood. J Periodontal Res 2006;41:208-13.
43. Kuru B, Yilmaz S, Argin K, Noyan U. Enamel matrix derivative alone or in combination with a bioactive glass in wide intrabony defects. Clin Oral Investig 2006;10:227-34.
44. Lekovic V, Camargo PM, Weinlaender M, Nedic M, Aleksic Z, Kenney EB. 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 2000;71:1110-6.
45. Tonetti MS, Lang NP, Cortellini P, Suvan JE, Adriaens P, Dubravec D, *et al.* Enamel

matrix proteins in the regenerative therapy of deep intrabony defects. J Clin Periodontol 2002;29:317-25.
46. Windisch P, Sculean A, Klein F, Tóth V, Gera I, Reich E, *et al.* Comparison of clinical, radiographic, and histometric measurements following treatment with guided tissue regeneration or enamel matrix proteins in human periodontal defects. J Periodontol 2002;73:409-17.
47. Zucchelli G, Bernardi F, Montebugnoli L, De SM. Enamel matrix proteins and guided tissue regeneration with titanium-reinforced expanded polytetrafluoroethylene membranes in the treatment of infrabony defects: A comparative controlled clinical trial. J Periodontol 2002;73:3-12.
48. Camargo PM, Lekovic V, Weinlaender M, Vasilic N, Kenney EB, Madzarevic M. The effectiveness of enamel matrix proteins used in combination with bovine porous bone mineral in the treatment of intrabony defects in humans. J Clin Periodontol 2001;28:1016-22.
49. Isidor F, Karring T, Attström R. The effect of root planing as compared to that of surgical treatment. J Clin Periodontol 1984;11:669-81.
50. Pihlstrom BL, Oliphant TH, McHugh RB. Molar and nonmolar teeth compared over 6 1/2 years following two methods of periodontal therapy. J Periodontol 1984;55:499-504.
51. Sculean A, Windisch P, Chiantella GC, Donos N, Brecx M, Reich E. Treatment of intrabony defects with enamel matrix proteins and guided tissue regeneration. A prospective controlled clinical study. J Clin Periodontol 2001;28:397-403.
52. Silvestri M, Ricci G, Rasperini G, Sartori S, Cattaneo V. Comparison of treatments of infrabony defects with enamel matrix derivative, guided tissue regeneration with a nonresorbable membrane and Widman modified flap. A pilot study. J Clin Periodontol 2000;27:603-10.
53. Reddy MS, Jeffcoat MK. Methods of assessing periodontal regeneration. Periodontol 2000 1999;19:87-103.
54. Froum SJ, Weinberg MA, Rosenberg E, Tarnow D. A comparative study utilizing open flap debridement with and without enamel matrix derivative in the treatment of periodontal intrabony defects: A 12-month re-entry study. J Periodontol 2001;72:25-34.
55. Lekovic V, Camargo PM, Weinlaender M, Kenney EB, Vasilic N. Combination use of bovine porous bone mineral, enamel matrix proteins, and a bioabsorbable membrane in intrabony periodontal defects in humans. J Periodontol 2001;72:583-9.
56. Lindhe J, Westfelt E, Nyman S, Socransky SS, Heijl L, Bratthall G. Healing following surgical/non-surgical treatment of periodontal disease. A clinical study. J Clin Periodontol 1982;9:115-28.
57. Esposito M, Grusovin MG, Papanikolaou N, Coulthard P, Worthington HV. Enamel matrix derivative (Emdogain(R)) for periodontal tissue regeneration in intrabony defects. Cochrane Database Syst Rev 2009;4:CD003875.
58. De Leonardis D, Paolantonio M. Enamel matrix derivative, alone or associated with a synthetic bone substitute, in the treatment of 1- to 2-wall periodontal defects. J Periodontol 2013;84:444-55.
59. Miron RJ, Guillemette V, Zhang Y, Chandad F, Sculean A. Enamel matrix derivative in combination with bone grafts: A review of the literature. Quintessence Int 2014;45:475-87.
60. Zucchelli G, Amore C, Montebugnoli L, De Sanctis M. Enamel matrix proteins and bovine porous bone mineral in the treatment of intrabony defects: A comparative controlled clinical trial. J Periodontol 2003;74:1725-35.
61. Gurinsky BS, Mills MP, Mellonig JT. 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 2004;75:1309-18.
62. Hoidal MJ, Grimard BA, Mills MP, Schoolfield JD, Mellonig JT, Mealey BL. Clinical evaluation of demineralized freeze-dried bone allograft with and without enamel matrix derivative for the treatment of periodontal osseous defects in humans. J Periodontol 2008;79:2273-80.
63. Velasquez-Plata D, Scheyer ET, Mellonig JT. 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 2002;73:433-40.
64. Sculean A, Chiantella GC, Windisch P, Gera I, Reich E. Clinical evaluation of an enamel matrix protein derivative (Emdogain) combined with a bovine-derived xenograft (Bio-Oss) for the treatment of intrabony periodontal defects in humans. Int J Periodontics Restorative Dent 2002;22:259-67.
65. Scheyer ET, Velasquez-Plata D, Brunsvold MA, Lasho DJ, Mellonig JT. A clinical comparison of a bovine-derived xenograft used alone and in combination with enamel matrix derivative for the treatment of periodontal osseous defects in humans. J Periodontol 2002;73:423-32.
66. Jepsen S, Topoll H, Rengers H, Heinz B, Teich M, Hoffmann T, *et al.* Clinical outcomes after treatment of intra-bony defects with an EMD/synthetic bone graft or EMD alone: A multicentre randomized-controlled clinical trial. J Clin Periodontol 2008;35:420-8.
67. Bokan I, Bill JS, Schlagenhauf U. Primary flap closure combined with Emdogain alone or Emdogain and Cerasorb in the treatment of intra-bony defects. J Clin Periodontol 2006;33:885-93.
68. Peres MF, Ribeiro ED, Casarin RC, Ruiz KG, Junior FH, Sallum EA, *et al.* Hydroxyapatite/ß-tricalcium phosphate and enamel matrix derivative for treatment of proximal class II furcation defects: A randomized clinical trial. J Clin Periodontol 2013;40:252-9.
69. Crea A, Dassatti L, Hoffmann O, Zafiropoulos GG, Deli G. Treatment of intrabony defects using guided tissue regeneration or enamel matrix derivative: A 3-year prospective randomized clinical study. J Periodontol 2008;79:2281-9.
70. Heijl L, Heden G, Svärdström G, Ostgren A. Enamel matrix derivative (EMDOGAIN) in the treatment of intrabony periodontal defects. J Clin Periodontol 1997;24(9 Pt 2):705-14.
71. Heden G, Wennström J, Lindhe J. Periodontal tissue alterations following Emdogain treatment of periodontal sites with angular bone defects. A series of case reports. J Clin Periodontol 1999;26:855-60.
72. Minabe M, Kodama T, Kogou T, Takeuchi K, Fushimi H, Sugiyama T, *et al.* A comparative study of combined treatment with a collagen membrane and enamel matrix proteins for the regeneration of intraosseous defects. Int J Periodontics Restorative Dent 2002;22:595-605.
73. Gupta G. Gingival crevicular fluid as a periodontal diagnostic indicator – I: Host derived enzymes and tissue breakdown products. J Med Life 2012;5:390-7.
74. Javed F, Ahmed HB, Mikami T, Almas K, Romanos GE, Al-Hezaimi K. Cytokine profile in the gingival crevicular fluid of rheumatoid arthritis patients with chronic periodontitis. J Investig Clin Dent 2014;5:1-8.
75. Javed F, Al-Askar M, Al-Hezaimi K. Cytokine profile in the gingival crevicular fluid of periodontitis patients with and without type 2 diabetes: A literature review. J Periodontol 2012;83:156-61.
76. Javed F, Al-Hezaimi K, Salameh Z, Almas K, Romanos GE. Proinflammatory cytokines in the crevicular fluid of patients with peri-implantitis. Cytokine 2011;53:8-12.
77. Iwata T, Morotome Y, Tanabe T, Fukae M, Ishikawa I, Oida S. Noggin blocks osteoinductive activity of porcine enamel extracts. J Dent Res 2002;81:387-91.
78. Kawase T, Okuda K, Momose M, Kato Y, Yoshie H, Burns DM. Enamel matrix derivative (EMDOGAIN) rapidly stimulates phosphorylation of the MAP kinase family and nuclear accumulation of smad2 in both oral epithelial and fibroblastic human cells. J Periodontal Res 2001;36:367-76.
79. Kawase T, Okuda K, Yoshie H, Burns DM. Anti-TGF-beta antibody blocks enamel matrix derivative-induced upregulation of p21WAF1/cip1 and prevents its inhibition of human oral epithelial cell proliferation. J Periodontal Res 2002;37:255-62.
80. Suzuki S, Nagano T, Yamakoshi Y, Gomi K, Arai T, Fukae M, *et al.* Enamel matrix derivative gel stimulates signal transduction of BMP and TGF-{beta}. J Dent Res 2005;84:510-4.
81. Wada Y, Yamamoto H, Nanbu S, Mizuno M, Tamura M. The suppressive effect of enamel matrix derivative on osteocalcin gene expression of osteoblasts is neutralized by an antibody against TGF-beta. J Periodontol 2008;79:341-7.