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Five years clinical results following treatment of human intra-bony defects with an enamel matrix derivative: A randomized controlled trial

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Abstract

Introduction. Emdogain® represents an extracellular matrix derivative that controls and promotes periodontal regeneration. Several studies have demonstrated that the treatment of periodontal defects with Emdogain® leads to improvements in clinical parameters. However, long-time clinical trials establishing clinical usefulness of Emdogain® are scarce. Therefore, the present randomized split mouth, controlled study was undertaken to evaluate the effectiveness of Emdogain® as an adjunct to open flap debridement for the treatment of intra-bony defects over a period of 5 years. ***Materials and methods.*** The study population consisted of 15 chronic periodontitis patients with bilateral interproximal osseous defects. The test group was treated by open flap debridement with EDTA 24% (PrefGel®) followed by enamel matrix derivative (Emdogain®). The control group was treated by open flap debridement with EDTA 24% (PrefGel®). ***Results.*** After 1 and 5 years, both the test and control groups showed significant mean PPD reduction. A greater reduction in mean Probing Pocket Depth (PPD) was observed in the test group (3.84 ± 1.05) as compared to the control group (1.92 ± 0.35). The mean Clinical Attachment Level (CAL) gain of 3.18 ± 0.87 mm was observed in the test group, while the control group displayed mean CAL gain of 1.60 ± 0.54 mm. The observed differences were found to be statistically significant in both the groups ($p < 0.05$). Percentage bone fill was significantly increased at 12 months post-surgery in test group (66.66 ± 7.8%) as compared to control group (31.71 ± 4.1%). ***Conclusion.*** The treatment with Emdogain resulted in a significantly higher CAL gain and PPD reduction in comparison with OFD and PrefGel®.

Key Words: *enamel matrix derivative/protein, osseous defects, randomized controlled trial, regenerative therapy*

Introduction

A primary goal of periodontal therapy consists not only of arresting the tissue destruction caused by periodontal disease but also to regenerate the tooth supporting tissues including alveolar bone, periodontal ligament and cementum [1]. The first revolutionary stage of periodontal regeneration was focused on the regeneration of osseous lesions utilizing a variety of bone replacement grafts [2]. Although bone grafts play a positive role in bone regeneration, the ability for regeneration of cementum and periodontal ligament was very limited with bone grafts [3].

Guided tissue regeneration (GTR) was a pioneering technique that set the second stage for the evolution of periodontal regeneration [4]. However, the GTR technique presents certain unresolved problems. These primarily include; limited amount of regeneration achievable and membrane contamination in cases of exposure of non-absorbable membranes [5].

To overcome the problems encountered with currently available regenerative techniques, an enamel matrix proteins derivative (Emdogain ®) was introduced as an alternative for obtaining periodontal regeneration. Emdogain® represents an extracellular matrix derivative that seems to control and promote periodontal regeneration [6,7]. Emdogain® is derived from porcine tooth buds and is composed principally of amelogenin and related proteins [6,7].

Human histological data from a few studies have shown that the treatment with enamel matrix derivative (Emdogain®) results in the formation of a new layer of acellular cementum with inserting collagen fibers and the formation of new alveolar bone [8,9]. However, few other human histologic studies have questioned both the consistency of the histologic

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outcomes and the ability of EMD to predictably stimulate formation of acellular cementum [2]. Several studies have demonstrated that the treatment of intra-bony periodontal defects with enamel matrix derivative (EMD) leads to significant gain of clinical attachment level (CAL), reduction in probing depth (PD) and bone fill as observed on the radiographs [10–13]. However, long-time prospective controlled clinical trials establishing scientific evidence for the clinical usefulness of Emdogain® are scarce.

Therefore, the present randomized split mouth, controlled clinical and radiographic study was undertaken to comparatively evaluate the effectiveness of enamel matrix proteins (Emdogain®) as an adjunct to open flap debridement (OFD) in the treatment of intra-bony osseous defects.

Materials and methods

The study population consisted of 15 systemically healthy chronic periodontitis patients (aged 37–45 years, mean age 40.66 ± 2.96 years), with bilateral interproximal osseous defects in the same arch and exhibiting clinical attachment loss ≥6 mm from the outpatient department of Periodontics, S.P. Dental College, Wardha, India.

The patients excluded were (1) smokers, (2) pregnant females and lactating mothers, (3) patients non-compliant to phase 1 therapy and (4) history of periodontal surgical treatment of the selected quadrant.

Prior to initiation of the study, the purpose and design of this clinical trial were explained to the patients and informed consent was obtained from every patient. The study protocol was approved by the ethical committee of J.N. Medical College, Wardha, India.

The study was a randomized, controlled, split mouth study performed over 5 years. Prior to surgery, two bilateral defects of each patient were randomly assigned by a coin flip to the test group and control group. The test sites were treated by open flap debridement with EDTA 24% (PrefGel®) followed by enamel matrix derivative (Emdogain®).The control sites were treated by open flap debridement with EDTA 24% (PrefGel®).

Initial therapy

After proper examination and diagnosis, initial therapy consisting of oral hygiene instructions, supragingival and subgingival scaling and root planing under local anesthesia and, occlusal adjustment, if necessary, was performed. A re-evaluation examination was performed 6 weeks following completion of initial therapy to determine patient's response to the therapy and to confirm the need for periodontal surgery.

The clinical parameters recorded were plaque index [14], papillary bleeding index [15] and the probing measurements. The probing measurements recorded were probing pocket depth (PPD), clinical attachment level (CAL) and the gingival recession (GR). The probing measurements were recorded using a computerized constant pressure probe (Florida probe, Florida probe corporation, Gainesville, FL). All the probing measurements were recorded at six sites per tooth; for later calculations the mean of the six sites was used. Custom-made occlusal acrylic stents were used to standardize the probe angulation and position. The probing measurements were recorded at Baseline, 1 year and 5 years follow-up. The PI and PBI were recorded at 3 month, 6 month, 1 year and 5 years follow-ups (Figs. 1,2).

Intra-oral periapical radiographs (IOPA) were obtained using a long cone (XCP Rinn, Dentsply) paralleling technique at baseline, 1 year and 5 years after surgery to measure the defect depth and bone fill. Radiographic measurements were obtained utilizing a millimeter grid mount (Nix Company Ltd, Japan) (Fig. 3).

The means and standard deviations (mean ± SD) values were calculated for all clinical parameters. Student's paired *t*-test was used to compare data from baseline with those at follow-up for each treatment group. Comparisons between treatment groups were carried out using student's unpaired *t*-test.

Surgical procedure

Prior to surgery, the patients were asked to rinse with a 0.12% chlorhexidine gluconate solution for 1 min. After adequate local anesthesia was achieved, sulcular incisions were made on the facial and lingual/palatal surfaces using a B.P knife with blades no. 12 or 15. The incisions were carried as far interproximally as possible to preserve the entire papilla and to facilitate complete wound closure. Wherever required, vertical incisions were made.

In the test group, full thickness flap was elevated and granulation tissues and adherent connective tissue tags were removed from the defect. The root surfaces were scaled and planed with manual and ultrasonic instruments until a smooth and hard consistency was obtained.

Following debridement, the defects were measured with a William's graduated periodontal probe to the nearest millimeter (mm) from the crest of the bone to the deepest level of the defect (Fig. 4). The bony defects were classified as 2-wall, 3-wall or combined defect, depending on their topography in the vertical direction.

The root surfaces adjacent to defect were conditioned with 24% EDTA gel (PrefGel®, Straumann USA LLC, Andover, MA) for 2 min to remove the

smear layer and then thoroughly rinsed with normal saline to remove all EDTA residues. Emdogain® gel (Straumann USA LLC, Andover, MA) was applied to the test site according to manufacturer's directions, covering the entire root surface, and the defect was completely filled (Fig. 5). The flaps were repositioned and sutured with interrupted sutures (4-0 Mersilk, Ethicon). The control group (OFD sites) was treated with an identical surgical procedure, except the Emdogain® application.

Post-surgical instructions

After surgery, patients were prescribed amoxicillin 500 mg t.d.s for 5 days and Ibuprofen 400 mg t.d.s for 5 days. Patients were instructed to rinse twice daily with 0.12% chlorhexidine digluconate (Periogard®, Colgate Palmolive, India) for 2 weeks. Sutures were removed 8 days post-surgery. Recall appointments were scheduled every second week during the first 2 months following surgery and then once per month for the first year post-operatively. After the first year, and during the rest of the study period of 5 years, patients were recalled every 6 months. The recall appointments consisted mainly of reinforcement of oral hygiene measures and professional supragingival scaling. All the patients received oral hygiene instruction and full mouth professional prophylaxis in the form of supragingival scaling by ultrasonic scaler at each recall visit. No adverse event or complication was recorded.

Statistical analysis

Mean and standard deviation (mean ± SD) values were calculated for all the parameters. The mean data was analyzed for statistical significance by standard statistical methods. The Student Paired *t*-test was used to assess homogeneity between test and control groups at baseline for all the parameters. To evaluate difference between baseline and 5 years (within group), paired *t*-test was used. The paired *t*-test was also used to compare clinical and radiographic outcomes of test and control groups at 5 years.

Results

A total of 15 patients with 30 infra-bony defects received periodontal surgical treatment. Fifteen infra-bony defects were treated with OFD +Emdogain® and 15 with OFD alone. The post-operative healing was uneventful in all treated cases. No clinically detectable or subjectively reported side-effects were noted in any treated patient. The distribution of treated defects with respect to number of osseous walls and tooth location is shown in Table I.

Table I. Distribution and configuration of treated infra-bony defects and tooth location.

	Test group (n = 15)	Control group (n = 15)
Infra-bony defects		
3 wall	8	8
2 wall	5	4
Combined defects	2	3
Tooth location		
Mandibular premolars	3	3
Mandibular molars	12	12

The mean PI and PBI scores of the total study population remained low throughout the study. The mean PI at baseline was 0.90 ± 0.49 and at 5 years follow-up period it was found to be 0.70 ± 0.06. At baseline the mean PBI was 1.08 ± 0.08 and at 5 years follow-up it was found to be 0.73 ± 0.10. Various studies [16,17] have suggested that good oral hygiene, as reflected by low plaque score, is associated with stability of the clinical attachment gain. In the present study, a regular supportive periodontal care programme was established to maintain minimum PI and PBI.

At the baseline, no statistically significant differences in any of the investigated parameters were observed between the test and control sites, indicating that the randomization process was effective (Table II).

In the test group, the mean probing pocket depth (PPD) reduced from 7.24 ± 1.11 mm at baseline to 3.12 ± 0.87 mm at 1 year. At 5 years it was found to be 3.40 ± 0.57 mm. In the control group, PPD reduced from 6.82 ± 0.48 mm at baseline to 4.60 ± 0.93 mm at 1 year and to 4.90 ± 0.53 mm after 5 years. The mean PPD reduction at 1 year follow-up was 4.12 ± 1.11 mm in the test group and 2.22 ± 0.15 mm in the control group (Table II). The mean PPD reduction at 5 years follow-up was 3.84 ± 1.05 mm in the test group and 1.92 ± 0.35 mm in the control group. In both the groups, the mean PPD reductions after 1 and 5 years were statistically significant ($p < 0.05$) compared to the baseline data. There was statistically significant difference in mean PPD reduction between the test and control groups ($p < 0.05$). At 5 years, the test group showed significantly greater reduction of mean PPD compared to the control group, with the mean difference of 1.92 ± 0.80 mm ($p < 0.05$) (Table II).

In the test group, 1 year post-surgery, the mean clinical attachment level (CAL) was 4.12 ± 0.81 mm as compared to 8.08 ± 1.34 at baseline. Five years after surgery, mean CAL was found to be 4.90 ± 1.21 mm. In the control group, 1 year post-surgery, the mean clinical attachment level (CAL) was 5.27 ± 1.33 mm as compared to 7.32 ± 1.12 at baseline.

Table II. Comparison of periodontal probing depth (PPD), clinical attachment level (CAL) and gingival recession (GR) mean values of test and control group patients at different examination intervals.

	PPD (Mean ± SD [mm])			CAL (Mean ± SD [mm])			GR (Mean ± SD [mm])		
Examination Interval	Test	Control		Test	Control		Test	Control	
Baseline	7.24 ± 1.11	6.82 ± 0.48	$p > 0.05$, NS	8.08 ± 1.34	7.32 ± 1.12	0.292, NS	0.84 ± 0.17	0.50 ± 0.85	0.18 ± 0.09, NS
At 1 year	3.12 ± 0.87	4.60 ± 0.93		4.12 ± 0.81	5.27 ± 1.33		1.00 ± 0.13	0.67 ± 0.23	
Difference	4.12 ± 1.11	2.22 ± 0.15	1.90 ± 0.6, S	3.96 ± 0.44	2.05 ± 0.78	1.91 ± 0.65, S	0.16 ± 0.09	0.17 ± 0.11	0.1 ± 0.04, NS
At 5 years	3.40 ± 0.57	4.90 ± 0.53		4.90 ± 1.21	5.72 ± 1.09		1.50 ± 1.35	0.82 ± 1.04	
Difference	3.84 ± 1.05	1.92 ± 0.35	1.92 ± 0.8, S	3.18 ± 0.87, S	1.60 ± 0.54, S	1.58 ± 0.99, S	0.66 ± 0.01, S	0.32 ± 0.52, S	0.31 ± 0.17, NS
p-value	< 0.001	< 0.001		< 0.001	< 0.001		< 0.001	< 0.001	

Five years after surgery mean CAL was found to be 5.72 ± 1.09 mm (Table II). The mean clinical attachment level (CAL) gain at 1 and 5 years follow-up was statistically significant in both the test and control group compared to the baseline data ($p < 0.05$). There was statistically significant difference in CAL gain both at 1 and 5 years between the test and control group ($p < 0.05$). The test group showed significantly greater mean CAL gain compared to the control group, with the mean difference of 1.91 ± 0.6 at 1 year follow-up and 1.58 ± 0.99 mm ($p < 0.05$) at 5 years (Table II).

In the test group, the mean gingival recession (GR) at 1 year was 1.00 ± 0.13 mm which at 5 years was 1.50 ± 1.35 mm as compared to 0.84 ± 0.17 mm at baseline. In the control group, the mean GR increased from 0.50 ± 0.85 to 0.67 ± 0.23 at 1 year recall visit and to 0.82 ± 1.04 mm at 5 years recall visit. Thus, the mean gingival recession was increased to 0.66 ± 0.01 mm in the test group and 0.32 ± 0.52 mm in the control group at the end of 5 years recall visit. This increase in gingival recession was significantly higher in the test group but insignificant in control group compared to baseline data ($p > 0.05$). There was also no statistically significant difference between the test and control group for increase in gingival recession at any recall visit ($p > 0.05$) (Table II).

At 5 years, the mean radiographic defect depth (DD) was 1.6 ± 0.70 mm in the test group and 2.8 ± 0.79 mm in the control group (Table III). Thus, the mean DD reduction was 3.2 ± 0.63 mm (with the bone fill of 66.66 ± 7.8%) in the test group and 1.30 ± 0.68 mm (with the bone fill of 31.71 ± 4.1%) in the control group. In both the groups, DD reduction was statistically significant as compared to the baseline data ($p < 0.05$). There was statistically significant difference in DD reduction between the test and control group ($p < 0.05$). The test group showed significantly greater reduction of the mean DD with the mean difference of 1.90 ± 0.56 mm and showing 34.96% more bone fill compared to control group (Table III).

Discussion

A retrospective power analysis based on the number of the included patients was performed using the STATA software (version 10.1). The power for PPD, CAL, and bone fill was found to be 100%. However, for recession, power was found to be 71.63%. The value at or above 80% is considered significant. This shows that the sample size was adequate.

Pocket depth resolution is a key outcome of periodontal regenerative procedure. The post-operative pocket depth will have a direct impact on maintenance of treated area. Shallow pockets have a strong, negative predictive value for future disease

Table III. Comparison of mean radiographic percentage bone fill and defect depth (DD) (Mean ± SD).

Parameters	Test group	Control group	Difference
Radiographic defect depth at baseline	4.8 ± 0.54	4.1 ± 0.87	0.40 ± 0.22
Radiographic defect depth after 5 years	1.60 ± 0.70	2.80 ± 0.79	1.20 ± 1.42
Percentage bone fill	66.66 ± 7.8% (3.20 ± 0.63)	31.71 ± 4.1% (1.30 ± 0.68)	34.96 ± 3.2%, S (1.90 ± 0.56)

progression, whereas deep pockets in treated areas are a risk indicator for periodontal disease progression [18]. In the present study, pocket depth reduction was significant in both test and control groups both at 1 year and 5 years recall visit. The mean PPD reduction at both 1 year and 5 years was significantly greater ($p < 0.05$) in test group as compared to controls (4.12 ± 1.11 mm vs 2.22 ± 0.15 mm at 1 year recall and 3.84 ± 1.05 vs 1.92 ± 0.35 mm at 5 years). These findings are comparable to the previous studies. Sculean et al. [11] compared Emdogain® with GTR in intra-bony defects and reported 3.8 mm PPD reduction after 8 months in Emdogain® group. This difference in PPD reduction could be because of deeper initial PPD in their studies compared to the present study. In a similar study, Sculean et al. [19] presented the 5-year results following treatment of intra-bony defects with EMD. They reported that the sites treated with EMD demonstrated a reduction in mean PPD of 4.6 ±1.2 mm ($p < 0.001$) and of 4.3 ± 1.7 mm ($p < 0.001$) at 1 and 5 years, respectively. Heden and Wennström [20] also evaluated 5 years results following regenerative therapy with the use of enamel matrix proteins in intra-bony defects and demonstrated a reduction in mean PPD of 4.9 mm and of 5.2 mm at 1 and 5 years, respectively. Again, the greater reductions observed in these long-term studies were probably a result of greater baseline PPD.

In the present study, gain in the mean clinical attachment level was statistically significant in both test and control group at 5 years. On comparison of the mean CAL gain between the two groups, the CAL gain was significantly higher in the test group ($p < 0.05$). At 1 year recall visit, the mean CAL gain obtained was 3.96 ± 0.41 mm in the test group and 2.05 ± 0.78 mm in the control group. The mean CAL gain obtained was 3.18 ± 0.87 mm in the test group and 1.6 ± 0.54 mm in the control group at 5 years. The gain in CAL observed in the Emdogain® group compares well with those reported in other clinical trials. Sculean et al. [11] compared the results obtained with EMD or GTR in 16 patients with two symmetric intra-bony defects. They found CAL gain of 3.1 ± 0.06 mm in defects treated with Emdogain at 8 months follow-up. In a long-term study, Sculean et al. [19] presented the 5-year results following treatment of intra-bony defects with EMD and reported a mean CAL gain of 3.4 ± 1.1 mm ($p < 0.001$) and of 2.9 ± 1.6 mm ($p < 0.001$) at 1 and 5 years, respectively. These results are again comparable to the ones observed in the present study. Heden and Wennström [20] demonstrated a mean CAL gain of 4.3 mm and CAL gain of 5.3 mm at the 1-year and 5-year follow-up, respectively. The greater improvements observed might again be a result of greater baseline defect characteristics.

In a split mouth study, Velasquez-Plata et al. [21] obtained CAL gain of 2.9 ± 0.9 mm for EMD treated intra-bony defects at 6–8 months. In the present study, the mean CAL gain was higher than reported by Heijl et al. [10], who reported CAL gain of 2.1 mm in EMD treated sites and 1.5 mm in OFD alone sites at 8 months follow-up. A factor contributing to this difference could be related with the defect type; the majority of the defects in the present as well as the studies reported by Sculean et al. [11] and Velasquez-Plata et al. [21] were 2- and 3-walled, whereas the defects studied by Heijl et al. [10] were predominantly one wall configuration. Predominantly 3-wall defects have been associated with greater regenerative potential in conventional surgical procedures, as supported by various studies [22,23], although there are studies which have disputed the concept [24–26].

In the present study, increase in gingival recession was significant in test sites at 5 years as compared to baseline. The mean increase in GR was 0.66 ± 0.01 mm in the test group and 0.32 ± 0.52 mm in the control group, but the difference in two groups was not significant. This finding is in accordance with the study by Froum et al. [13], who observed 0.61 mm of increase in gingival recession at Emdogain® treated sites over the 12 months period.

Radiographic bone measurement following regenerative procedure is a non-invasive, painless, alternative to direct bone measurements. However, even with the standardized radiographic techniques, the radiograph does not show the entire topography of the area before or after treatment. Several studies have demonstrated that radiographs are less reliable than clinical techniques [27]. Direct bone measurement or surgical re-entry can provide a good view of the state of the bone crest that can be compared with the view taken during the initial surgical intervention. However, this method requires a second surgical intervention, limiting its usefulness in clinical practice.

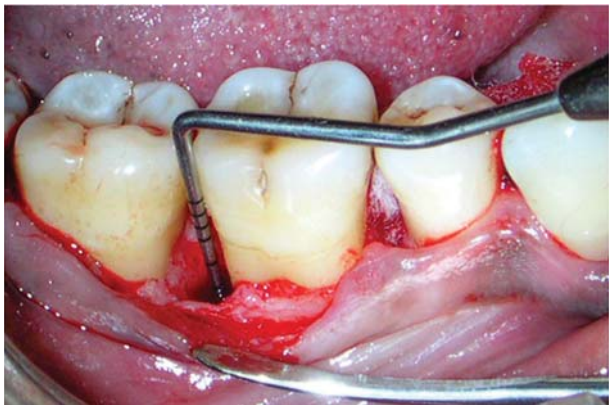

Figure 1. Pre-operative clinical view.

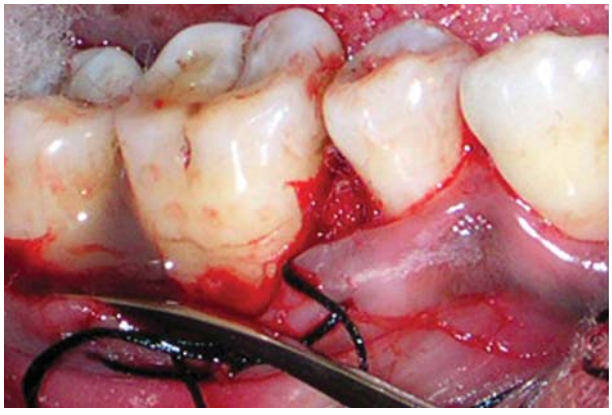

Figure 2. Post-operative clinical view.

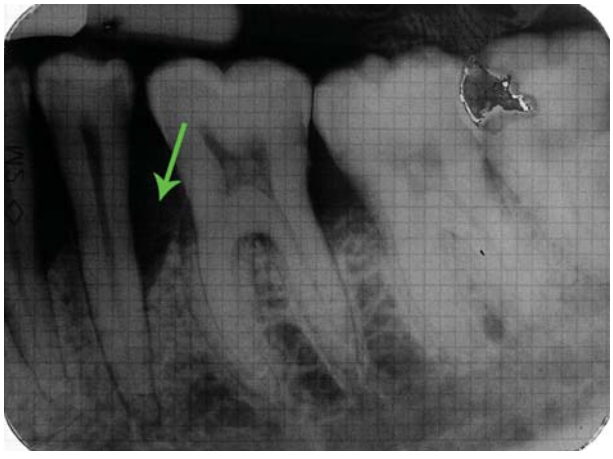

Figure 3. Baseline radiograph depicting defect.

The defect depth reduction was significant in both test and control group at 5 years. The mean defect depth reduction at 5 years was 3.20 ± 0.63 mm in the test group (with 66.6% defect fill) and 1.3 ± 0.67 mm in the control group (with 31.7% defect fill). The mean defect depth reduction was significantly greater in the test group compared to the control group ($p < 0.05$). These findings are in accordance with

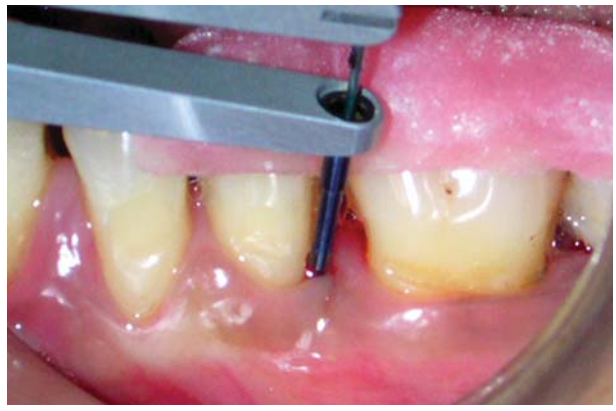

Figure 4. Intra-operative defect measurement.

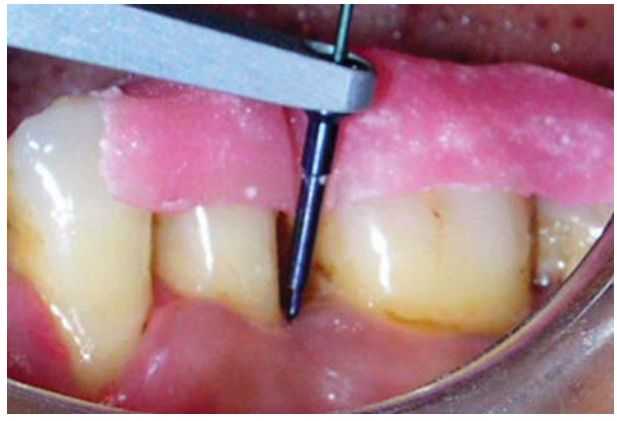

Figure 5.

previous studies. Velasquez-Plata et al. [21] obtained defect depth reduction of 3.1 ± 1.00 mm at EMD treated sites, after 6–8 months of re-entry.

Conclusion

From the analysis of the results it can be concluded that the application of Emdogain®, and PrefGel® with OFD with results in significant reduction in probing pocket depth (PPD), and gains in clinical attachment level (CAL) over a long-term follow-up as compared to OFD with PrefGel®.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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