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Enamel matrix derivative and low-level laser therapy in the treatment of intra-bony defects: a randomized placebo-controlled clinical trial

Onur Ozcelik[1], Mehmet Cenk Haytac[1] and Gulsah Seydaoglu[2]

[1]Department of Periodontology, Faculty of Dentistry, Cukurova University, Adana, Turkey; [2]Department of Biostatistics, Faculty of Medicine, Cukurova University, Adana, Turkey

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Abstract

Aim: The aim of this study was to evaluate the immediate post-operative pain, wound healing and clinical results after the application of an enamel matrix protein derivative (EMD) alone or combined with a low-level laser therapy (LLLT) for the treatment of deep intra-bony defects.

Material and Methods: This study was an intra-individual longitudinal test of 12 months' duration conducted using a blinded, split-mouth, placebo-controlled and randomized design. In 22 periodontitis patients, one intra-bony defect was randomly treated with EMD+LLLT, while EMD alone was applied to the contra-lateral defect site. LLLT was used both intra- and post-operatively. Clinical measurements were performed by a blinded periodontist at the time of surgery, in the first week and in the first, second, sixth and 12th month. Visual analogue scale (VAS) scores were recorded for pain assesment.

Results: The results have shown that the treatment of intra-bony defects with EMD alone or EMD+LLLT leads to probing depth reduction and attachment-level gain. In addition, EMD+LLLT had resulted in less gingival recession ($p<0.05$), less swelling ($p<0.001$) and less VAS scores ($p<0.02$) compared with EMD alone.

Conclusion: This study shows that EMD is an effective, safe and predictable biomaterial for periodontal regeneration and LLLT may improve the effects of EMD by reducing post-operative complications.

Key words: enamel matrix derivative; low-level laser/therapy; periodontal regeneration; surgical periodontal therapy; wound healing

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Various treatment techniques have been used to improve the regeneration of periodontal tissues and intra-bony defects in particular (Nyman et al. 1982, Cortellini et al. 1993, 1995, 1999, 2001, Tonetti et al. 1993, 1998, 2004a, Kilic et al. 1997, Cortellini & Tonetti 2000, 2007, Harrel et al. 2005, Laurell et al. 2006, Slavkin & Bartold 2006, Zeichner-David 2006, Sculean et al. 2007b). The use of an Emdogain® enamel matrix protein derivative (EMD) has been introduced recently as a new treatment alternative for periodontal regeneration, where it stimulates attachment and proliferation of periodontal ligament (PDL) fibroblasts (Brett et al. 2002, Cattaneo et al. 2003, Chano et al. 2003, Rincon et al. 2003), accelerates early wound healing (Hoang et al. 2000, Tonetti et al. 2004b) and promotes gingival healing (Hammarström 1997, Keila et al. 2004, Sanz et al. 2004, Sculean et al. 2004, Bosshardt et al. 2005) and vascularization (Yuan et al. 2003). Clinically, the application of EMD has shown that EMD facilitates regeneration and is superior to access flap alone in intra-bony defects. It was established by some human studies that application of EMD onto a previously

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debrided and conditioned root surface enhances wound healing (Wennström & Lindhe 2002, Hagenaars et al. 2004, Tonetti et al. 2004b, Cortellini & Tonetti 2007, Ozcelik et al. 2007) and the formation of a new connective tissue attachment (i.e. new cementum with inserting collagen fibres) (Sculean et al. 1999, Yukna & Mellonig 2000, Windisch et al. 2002) and of new alveolar bone (Heijl et al. 1997, Tonetti et al. 2002, Sculean et al. 2003, 2005, 2006, 2007a, Francetti et al. 2004, Tsitoura et al. 2004, Cortellini & Tonetti 2007). The broad spectrum of activities of EMPs can explain both the often-observed enhanced wound healing and the regeneration of periodontal tissues following therapeutic application of EMD.

Low-level lasers (LLLs) work in the milliwatt range with wavelengths usually in the red or near-infrared spectrum (500–900 nm) without inducing thermal effects. LLLs do not cut or ablate the tissues (Walsh 1997, Reddy 2004). The therapy performed with such lasers is often called ''LLL therapy'' (LLLT) or ''therapeutic lasers therapy'' whereas the therapy has been referred to as ''biostimulation'' and ''biomodulation'' (Abergel et al. 1984, Walsh 1997, Dortbudak et al. 2000, Yilmaz et al. 2002, Rubinov 2003, Khadra et al. 2004, Sun & Tuner 2004, Posten et al. 2005). Laser-enhanced biostimulation has been reported to induce intracellular metabolic changes, resulting in faster cell division, proliferation rate, migration of fibroblasts and rapid matrix production (Basford 1989, Loevschall & Arenholt-Bindslev 1994, Kreisler et al. 2002, Khadra 2005, Khadra et al. 2005a, Pourzarandian et al. 2005). In addition, the ability of LLLT to promote a favourable soft tissue-healing response has been described in several experimental (Balboni et al. 1986, Loevschall & Arenholt-Bindslev 1994, Conlan et al. 1996, Webb et al. 1998, Almeida-Lopes et al. 2001, Nascimento et al. 2004, Posten et al. 2005) and a few clinical studies (Neiburger 1999, Amorim et al. 2006). Some recent meta-analyses of the literature concerning the potential of low-energy laser treatment revealed a highly significant positive effect on wound healing in general and a significant shortening of healing time (Bouneko et al. 2000, Woodruff et al. 2004).

Although the data on the low-power laser irradiation effect on the proliferation rate of PDL cell cultures are not clear, the responsiveness of PDL fibroblasts to LLL energy has been demonstrated by some in vitro studies (Kreisler et al. 2001, 2003, Van der Pauw et al. 2002). In particular, the results obtained in Kreisler's study (2003) revealed that an 809-nm LLL light had a stimulatory effect on the proliferation of PDL fibroblasts. In addition, numerous authors have reported in clinical and experimental studies about the potential enhancement of bone regeneration by laser irradiation in general (Silva et al. 2002, Stein et al. 2005) and in dental medicine (Saito & Shimizu 1997, Dortbudak et al. 2000, Kawasaki & Shimizu 2000, Khadra et al. 2004, Jakse et al. 2007). These findings might be of clinical relevance in periodontal therapy in terms of regeneration.

Thus, both EMD and LLLT demonstrate potential attributes that may contribute to the regeneration of periodontal tissues and periodontal wound healing. Treating intra-bony defects with a combination of EMD+LLLT may further enhance the healing process. The benefits of EMD on periodontal regeneration have been thoroughly investigated in both in vivo and in vitro studies; however, the effects of LLLT on periodontal wound healing and regeneration are unknown. The aim of this study was to evaluate the periodontal regeneration of deep intra-bony defects, wound healing and immediate post-operative pain after application of EMD alone or a combination of EMD+LLLT.

Material and Methods

Study population and experimental design

The participants of this study were chosen from chronic periodontitis patients who referred to the Periodontology Department of Cukurova University between January 2006 and June 2006. The exclusion criteria of this study were identified as the presence of uncontrolled or poorly controlled diabetes, pregnancy or any other systemic diseases known to affect periodontal tissues. Patients who had received periodontal treatment in the last 6 months and smokers were also excluded. The approval of the Local Ethics Committee of Cukurova University Faculty of Dentistry approval was obtained. All patients were briefly informed about the biologic effects of LLLT and EMD before their use and they gave an informed consent to cooperate on this project. This study was planned as an intra-individual longitudinal test of 12 months' duration conducted using a blinded, split-mouth, placebo-controlled and randomized design. The flow chart of the study design is shown in Fig. 1. Patients who had two contra-lateral intra-bony defects (same type of tooth on the sides of the same jaw) with a probing depth of at least 6 mm and an intra-bony component of at least 3 mm evident on intra-oral radiographs (Linares et al. 2006) and that were located in the interproximal area of mandibular pre-molar/molar region were included in the study (Table 1 and Fig. 2a). A total of 22 chronic periodontitis patients (12 males, 10 females and age range between 31 and 49 years) were treated in the present investigation. Twenty-two intra-bony periodontal defects were treated with EMD+LLLT (test) and the results were compared with 22 contra-lateral defects treated with EMD alone (control).

Patients were included after completion of cause-related therapy consisting of scaling and root planing, motivation and oral hygiene instructions. The criterion for surgery was optimal plaque control with a full-mouth plaque score (FMPS) (O'Leary et al. 1972) of 10% or less.

Clinical measurements

The clinical measurements used in this study and their recording time periods are shown in Table 2. Swelling of the soft tissues, colour of the gingiva, gingival recession (REC), bleeding index (BI), probing pocket depth (PPD) and clinical attachment level (CAL) were recorded. Swelling of the gingiva was scored as follows: 0 = no swelling, 1 = moderate swelling, and 2 = pronounced swelling. The colour of the gingiva was recorded as follows: 0 = no redness, 1 = moderate redness, and 2 = pronounced redness (Hagenaars et al. 2004). The probing variables were measured by a manual probe (CP-15UNC Probe, Hu-Friedy, Chicago, IL, USA). The contra-lateral gingiva was used as a reference to assess changes in tissue colour. Bleeding was assessed for the area that had been operated and scored at six sites per tooth (or at the appropriate number of sites that are only partially involved in the flap procedure). The presence or absence of bleeding was recorded on

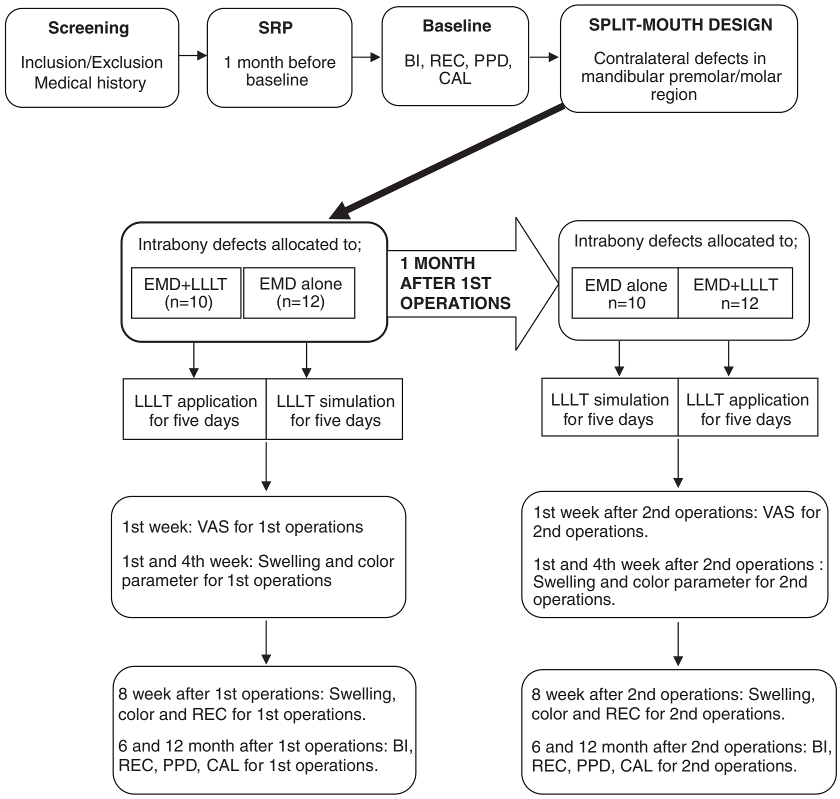

Fig. 1. Flow chart of the study.

Table 1. Distribution of defect and tooth categories in test and control sites

	Test sites ($n = 22$)	Control sites ($n = 22$)
Defect categories		
1-wall	0	0
2-wall	4	5
3-wall	12	12
Combined 2-/3-wall	3	3
Combined 1-/2-/3-wall	3	2
Tooth categories		
Pre-molars	10	11
Molars	12	11

a two-point scale (no, or within 30 s after probing) (Hagenaars et al. 2004) REC was recorded to the nearest millimetre at the deepest location of the selected inter-proximal site at six points around each tooth (buccal: mesial, mid-point and distal; palatal/lingual: mesial, mid-point and distal). CAL was calculated as the sum of PPD and REC. All measurements at all time points were performed by the same periodontist (MCH) who was blinded to the treatment techniques and to the test and control sites in order to assure an unbiased determination. The calibration of the examiner was performed by the evaluation of all study parameters including PPD, CAL and REC on two separate occasions on 10 patients who were not enrolled in the study. Calibration was accepted if the measurements were similar to the millimetre at 90% level on these two separate examinations. In addition, same calibration procedures were performed for the ordinal-scaled data of swelling and colour.

Randomization and timing of the surgery for test and control sites

At the baseline, the contra-lateral surgical sites were randomly assigned for receiving EMD+LLLT or EMD alone by the periodontist (O. O.) with the toss of a coin. The surgeries between sites were performed at least at 1-month intervals in order to evaluate and compare the post-operative pain between the two surgical sites.

Defect debridement, EMD and LLLT application

Following intracrevicular incisions, full mucoperiostal buccal and lingual access flaps were raised in each surgery patient. The defects were debrided with a combined use of curettes and ultra-sonic power-driven instruments (EMS Piezon Master 600, Nyon, Switzerland) and the roots were carefully planed. During the instrumentation, the flaps were slightly reflected, carefully protected with periosteal elevators and frequent saline irrigations (Fig. 2b and c). At the end of instrumentation, ethylenediamine tetra-acetic acid was applied on the instrumented root surface for 2 min. Then, the defect area was carefully rinsed with saline, air-dried and meticulously isolated from blood and saliva in order to prevent contamination. After that, EMD was applied on the dried root surface (Fig. 2d). Immediately after EMD application, the defect area was irradiated with a LLL for 10 min. on the test sites (Fig. 2e). The laser device used in this study was a diode laser (ULOCKS, Voronezh State University Laboratories, Voronezh, Russia) with a wavelength of 588 nm. The overall energy density per irradiation of 5 min. was 4 J/cm^2. During irradiation, the tip of the laser probe was placed on both the buccal and the lingual side of the periodontal defect area (5 min. for each). The flaps were then replaced and sutured with modified internal mattress sutures. LLLT was again applied for 10 min. from the outer buccal and lingual surfaces (again for 5 min. for each) of the flaps immediately after suturing for the test sites (Fig. 2f and g). To ensure uniform exposure of the whole defect region, the probe was positioned perpendicularly and in slight contact with the flaps. The control sites were subjected to the same procedure, except for irradiation with a diode laser. For these control sites, the laser application was simulated, without starting the laser machine. All surgical procedures and LLLT application were performed by the same periodontist (O. O.) in order to prevent inter-operator variations. The total time frame for the treatments including LLLT ranged between 65 and 75 min.

Post-operative laser irradiation

All test sites received post-operative LLLT daily for 5 days from the outer buccal and lingual surfaces of the flaps (5 min. for each). LLLT simulation without starting the laser machine was also performed for 5 days for the control sites in order to assure patient blindness.

Post-operative maintenance

A protocol for the control of bacterial contamination consisting of doxycycline (100 mg b.i.d. for 1 week), 0.12% chlorhexidine mouth rinsing three times per day and weekly prophylaxis was prescribed (Tonetti et al. 2002). Patients were requested to avoid brushing, flossing and chewing in the treated area for a period of 2 weeks. Then, patients resumed full-oral hygiene. At the end of the ''early healing phase'', patients were placed on a 3-month recall system for 12 months.

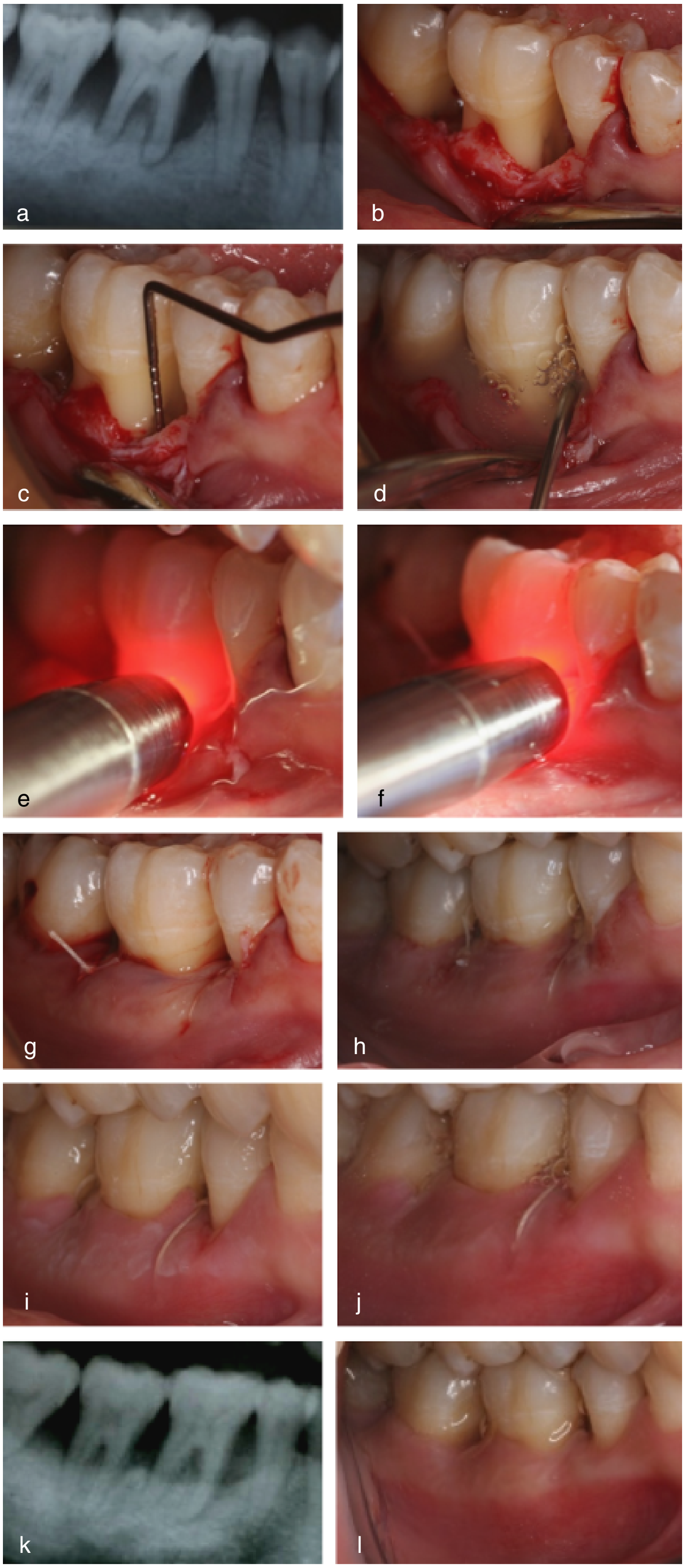

Fig. 2. Preoperative radiograph of the intrabony defects at the mandibular pre-molar molar region (a). Intra-operative view of the defect after debridment (b and c) and during EMD application (d). The defect was irradiated by a low-level laser after EMD application (e) and after suturing (f). low-level laser therapy-applied area on the immediate post-operative (g), first (h), third (i) and seventh (j) days. Radiographic (k) and clinical (l) views of the operation site at the 12th postoperative month. VAS, visual analogue scale; LLLT, low-level laser therapy; EMD, enamel matrix protein derivative.

Immediate post-operative pain assessment

Postoperatively, all patients were strictly instructed not to use any analgesics in order to evaluate immediate post-operative pain. The patients were given a diary each to record their pain perceptions in the first week following surgery. These were assessed on a 100 mm visual analogue scale (VAS). The boundaries of the VAS were anchored by the phrases ''no pain'' and ''unbearable pain''.

Statistical analysis

Statistical analysis was performed using the statistical package SPSS v 12.0. For each continuous variable, normality was checked by the Shapiro–Wilks test. Non-parametric tests were chosen for continuous variables because the data were not distributed normally. Comparisons between groups were applied using the Wilcoxon sum rank test. Time-dependent data were analysed by the Friedman test within groups. Analysis of covariance (ANCOVA) was used to compare differences in responses between treatment groups, accounting for pre-existing differences at baseline. χ^2 statistics (McNemar test) were calculated to assess the statistical significance of differences in dichotomous response variables between treatment groups. Data were expressed as median (25–75% percentiles) or mean ± (SE of mean). The percentages of the frequencies of degrees in ordinal-scaled data of

Table 2. Flow chart of the study parameters

Parameters:						
Period of time	Swelling	Colour	BI	REC	PPD	CAL
Baseline	–	–	X	X	X	X
1 week	X	X	–	–	–	–
1 month	X	X	–	–	–	–
2 months	X	X	–	X	–	–
6 months	–	–	X	X	X	X
12 months	–	–	X	X	X	X

BI, bleeding index; REC, gingival recession; PPD, probing pocket depth; CAL, clinical attachment level.

Table 3. Distribution of REC, PPD, CAL and BI of according to groups and time

	EMD Median (25–75% percentiles)	EMD+LLLT Median (25–75% percentiles)	*p* value (Wilcoxon)
REC			
Baseline	2.3 (1.8–3.0)	1.9 (1.1–2.4)	0.098
2 months	2.4 (2.1–3.3)	2.0 (0.9–2.5)	0.046**
6 months	2.6 (2.3–3.4)	2.2 (0.9–2.6)	0.016**
12 months	2.9 (2.4–3.8)	2.3 (1.1–2.8)	0.015**
p value*	0.0001	0.0001	
PPD			
Baseline	6.5 (5.9–6.7)	6.6 (5.9–6.8)	0.750
6 months	2.9 (2.3–3.7)	2.5 (2.2–3.9)	0.903
12 months	1.7 (1.3–3.3)	1.3 (1.2–2.3)	0.218
p value*	0.0001	0.0001	
CAL			
Baseline	7.2 (6.5–7.5)	7.2 (6.8–7.5)	0.727
6 months	4.4 (3.5–6.5)	4.4 (3.0–6.0)	0.478
12 months	3.2 (2.8–4.7)	3.1 (2.4–3.7)	0.046
p value*	0.0001	0.0001	
BI			
Baseline	1.0 (1.0–1.3)	1.0 (1.0–1.3)	0.627
6 months	0.0 (0.0–1.0)	0.0 (0.0–1.0)	0.564
12 months	0.0 (0.0–0.0)	0.0 (0.0–0.0)	1.000
p value*	0.0001	0.0001	

*Friedman test between baseline and 12 months in each group.
**$p<0.05$.
EMD, enamel matrix protein derivative; BI, bleeding index; REC, gingival recession; PPD, probing pocket depth; CAL, clinical attachment level; LLLT, low-level laser therapy.

swelling and colour were expressed to be *n* (%). A *p*-value of <0.05 was considered as significant.

Results

The results of the study are summarized in Tables 3 and 4. All patients complied with the requirements of the study and sutures were removed on the seventh day (Fig. 2h–j). In general, the post-operative healing was uneventful. The immediate post-operative VAS scores have shown that patients had significantly lower pain in the first and second post-operative days in EMD+LLLT-treated sites compared with EMD alone ($p<0.02$ for both days) (Fig. 3). In addition, there was no evidence of any carry-over effect, period effect or a treatment by period interaction ($p>0.05$).

Statistically significant differences in the swelling response existed between the treatment groups in the first post-operative week. Patients had significantly less swelling on the LLLT-applied sites ($p<0.01$). There were no statistically significant differences between the dichotomous response variables (swelling and colour) at months 1 and 2 (Table 4) ($p>0.05$).

The long-term results of this study (Table 3) have shown that the treatment of intra-bony defects with EMD alone or EMD+LLLT led to a statistically significant PPD reduction ($p<0.0001$) and CAL gain ($p<0.0001$) compared with the baseline and there was no significant difference between the two treatment modalities ($p>0.05$ for all time points). In addition, the EMD+LLLT-applied sites had less gingival recession at all evaluation time points compared with EMD alone, with the statistical significance increasing with time (Fig. 2k and l).

Discussion

To date, more than 100 cell biology studies about EMD and periodontal regeneration have been performed and all these studies have revealed its positive effects on the progenitor cells of osteoblasts and cementoblasts, on the growth on PDL and on wound healing (Hoang et al. 2000, Wennström & Lindhe 2002, Hagenaars et al. 2004, Mirastschijski et al. 2004, Myhre et al. 2006). In the light of these studies, the aim of the present study was to assess whether LLLT application, together with EMD, would further improve periodontal regeneration and wound healing.

Because periodontal regeneration requires the repopulation of the wound area adjacent to the root surface with cells from the PDL (Oates et al. 2001), it is important to know the effects of LLLT and EMD on PDL cells. EMD has been shown to influence a number of properties of PDL cells including proliferation, cell attachment and matrix synthesis (Van der Pauw et al. 2000, Davenport et al. 2003). An in vitro investigation of the effects of EMD on the behaviour of human PDL cells and

Table 4. Comparison of the frequencies of the degrees of swelling and colour between groups

	Swelling *n* (%)			Colour (redness) *n* (%)		
	0 (none)	1 (moderate)	2 (pronounced)	0 (none)	1 (moderate)	2 (pronounced)
Week 1						
EMD+LLLT	11 (50.0)	11 (50.0)	0 (0)*	16 (72.7)	6 (27.3)	0 (0)
EMD	0 (0)	13 (59.1)	9 (40.9)	10 (45.5)	11 (50.0)	1 (4.5)
Month 1						
EMD+LLLT	19 (86.4)	3 (13.6)	–	16 (72.7)	6 (27.3)	–
EMD	20 (90.9)	2 (9.1)	–	18 (81.8)	4 (18.2)	–
Month 2						
EMD+LLLT	20 (90.9)	2 (9.1)	–	17 (77.3)	5 (22.7)	–
EMD	21 (95.5)	1 (4.5)	–	19 (86.4)	3 (13.6)	–

*$p<0.001$ between groups.
EMD, enamel matrix protein derivative; LLLT, low-level laser therapy.

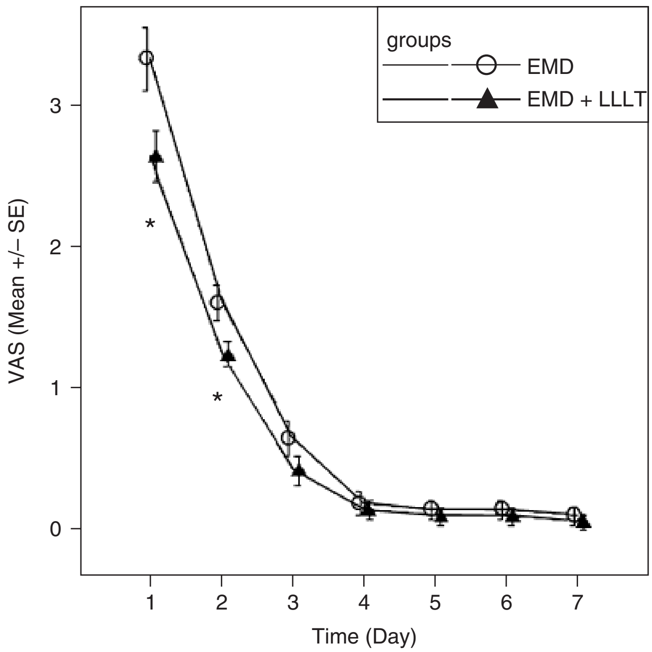

Fig. 3. Comparison of post-operative first-week visual analogue scale scores for EMD+LLLT and EMD alone. VAS, visual analogue scale; LLLT, low-level laser therapy; EMD, enamel matrix protein derivative.

gingival fibroblasts indicated that both cell types release significantly higher levels of transforming growth factor β 1 (TGF-β 1) under the influence of EMD than in control cultures (Van der Pauw et al. 2000, 2002, Lyngstadaas et al. 2001, Grayson et al. 2006). In another in vitro study, Hoang et al. (2000) demonstrated that PDL cell wound fill rates increased significantly compared with those of gingival fibroblasts when EMD is added to a medium containing both cell types. The responsiveness of PDL fibroblasts to a LLL energy in vitro has also been demonstrated in several studies (Kreisler et al. 2001, 2003). Ozawa et al. (1997) described a laser-induced inhibition of the plasminogen activator (PA) plasmin proteolytic system, concluding that laser irradiation may reduce collagen breakdown around the PDLF associated with traumatic occlusion. In addition to these findings, another recent study revealed that 809-nm LLL light had a stimulatory effect on the proliferation of PDL fibroblasts (Kreisler et al. 2003) and the production of basic fibroblast growth factor (bFGF) (Yu et al. 1993).

The results of this study have shown that the treatment of intra-bony defects with EMD alone or EMD+LLLT led to a statistically significant PD reduction and CAL gain compared with the baseline. In addition, the combination of EMD+LLLT had resulted in significantly less gingival recession in the post-operative second, sixth and 12th month compared with EMD alone. Several beneficial effects of both EMD and LLLT on early wound healing may explain the reduction of gingival recession in the EMD+LLLT-treated defects. Tonetti et al. (2004b) have reported that 2 weeks following access flap surgery alone, the tissue density (expressed as changes in CADIA units) decreased below the pre-surgical level and remained below baseline values for the first 6 weeks of healing. Following application of EMD, on the other hand, already at week 2, the tissue density appeared to be higher than the level before surgery. During the initial 6 weeks of healing, soft-tissue CADIA values of EMD-treated sites were significantly higher than those of the control treatment. In addition to the increased tissue density obtained by EMD, the major changes seen in wounds treated with LLLT include increased granulation synthesis, enhanced neovascularization of tissue, increased fibroblast proliferation, maturation, attachment and matrix synthesis (Bisht et al. 1994, Mirsky et al. 2002). In addition, both EMD (Yuan et al. 2003) and LLLT (Mirsky et al. 2002, Tuby et al. 2006) improve angiogenesis and microcirculation (Kozlov et al. 1995, Schaffer et al. 2000). All these biologic effects of EMD and LLLT may contribute to the higher tensile strengths of gingival flap margins, rapid neovascularization and protection and stability of the granulation tissue under the wound margins, which may subsequently prevent the collapse of healing wound, thus minimizing soft-tissue recession. This result may also support the hypothesis that the enhanced healing effect of LLLT is associated primarily with the early, most sensitive stages of the healing process in which the wound has started remodelling (Khadra et al. 2005b).

Although not statistically significant, EMD+LLLT-treated defects had more CAL gains in the 12th month compared with EMD alone. This finding may be related to the capability of both EMD (Narukawa et al. 2007) and LLLT (Abramovitch-Gottlib et al. 2005, Tuby et al. 2007) in promoting the proliferation of mesenchymal stem cells or progenitor cells of PDL. Furthermore, some studies proved the promotive effect of EMD on bone formation (Keila et al. 2004, Carinci et al. 2006). Similarly, experimental in vivo studies indicate accelerated bone repair in standardized bone defects and enhanced healing of artificial fractures following LLLT (Barushka et al. 1995, Saito & Shimizu 1997, Silva et al. 2002, Khadra et al. 2004, Jakse et al. 2007). In vitro LLL irradiation results in significantly stimulated bone matrix formation in osteoblast cultures (Dortbudak et al. 2000). In particular, in vitro laser irradiation of osteoblast cultures enhances both cellular proliferation, especially the proliferation of nodule-forming cells of the osteoblast lineage, and cellular differentiation, resulting in an increase in the number of more differentiated osteoblastic cells and finally an increase in bone matrix formation (Ozawa et al. 1998). A recent in vitro study of Stein et al. (2005) confirmed that low-energy laser irradiation promotes proliferation and maturation of human osteoblasts as well.

In the post-operative healing period, LLLT-applied sites produced less pain and swelling. The ability of LLLT to exert analgesic effects has historically been a major clinical application of the technique (Bouneko et al. 2000, Enwemeka et al. 2004) and there are reports that have shown pain reduction effects of LLLT for peri-apical pathology following root canal treatment and post-extraction pain (Kawakami et al. 1989). In vivo, LLLT selectively inhibits a range of nociceptive signals arising from peripheral nerves, including neuronal discharges elicited by pinch, cold, heat stimulation and chemical irritation (Sato et al. 1994, Tsuchiya et al. 1994). There have been claims that successful analgesia following oral surgery can be achieved with all major LLLT wavelengths (Armida 1989). Therefore, LLLT may contribute to the already well-known beneficial effects of EMD on the post-operative complication reduction and improvement of the patients' quality of life (Tonetti et al. 2004b, Ozcelik et al. 2007). In addition, Oliver et al. (1969) and Qadri et al. (2005) have revealed that the application of LLLT reduced periodontal gingival inflammation, which may explain the reduction of swelling found in this study.

In the present study, the periodontal defect area was irradiated immediately after EMD application and after completion of sutures. Therefore, laser light had been diffused directly into the root surface and bone surface and this may increase its effectiveness. Kreisler et al. (2003) clearly revealed that repeated laser applications are necessary to achieve a positive effect on the proliferation of fibroblasts. On this basis, in the present study periodontal defect areas were lased during surgery and for 5 consecutive days. However, extreme variations of the application parameters such as dose, wavelength, the amount of applied energy and required time periods have been used in previous studies with LLLT (Tuner & Hode 1998). Therefore, there is currently an urgent need for the standardization of the specific wavelength and dose for each procedure and for each case.

The only physical risk in LLLT is the risk of eye damage. While it has never been reported to occurr, the risk of eye damage must be considered, especially when using an invisible and collimated (parallel) beam. Suitable protective glasses should be worn by the dentist and the patient. Because the therapeutic lasers are well above the ionizing spectrum, there is no risk of cancerous changes.

Besides the LLLT application, there are several other approaches for reducing the post-operative morbidity such as minimally invasive flaps (Cortellini et al. 1999, 2001, Cortellini & Tonetti 2007). In the present study, the time needed for LLLT application (intrasurgically and once a day for 5 days after surgery) added up to 70 min. per defect. This is a considerable amount of time that is required in addition to the time of the surgery and complicates the justification of the use of LLLT with the small additional benefits of this technique. Therefore, further studies, which analyse the optimal dose and time for maximum benefit from LLLT, are required.

The reason for choosing a split-mouth design was to facilitate the comparison of both EMD and LLLT under similar healing conditions by eliminating patient-specific conditions (Hujoel & Moulton 1988). The relatively small study population constitutes a major limitation to the generalizability of the present results, and does not allow for definitive conclusions to be drawn. It should also be mentioned that no analgesic drugs were used in this study in order to evaluate pain perceptions of the patients exclusively. With the use of these drugs, as it would be in the clinical setting, pain scores for both treatments would be less. Another limitation of the study is the lack of bone-fill measurements as no re-entry procedures were performed.

In summary, within the limits of this study, the results have shown that (i) the use of EMD results in favuorable clinical outcomes in intra-bony defects and (ii) LLLT may improve the effects of EMD by reducing gingival recession and post-operative pain and swelling. In order to better understand the utility of LLLT for wound healing and regeneration, clinical studies that correlate cellular effects and biologic processes are needed. Future studies should be well-controlled investigations with a rational selection of laser type and treatment parameters.

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Address:
Onur Ozcelik
Department of Periodontology
Faculty of Dentistry
Cukurova University
Balcali, 01330 Adana
Turkey
E-mail: oozcelik@cu.edu.tr

Clinical Relevance

Scientific rationale for the study: Because both EMD and LLLT demonstrate potential clinical benefits, it is possible that combined use of EMD+LLLT for the treatment of deep intra-bony defects may improve wound healing and clinical results and may reduce immediate post-operative pain.

Principal findings: Despite a small sample population, this study has demonstrated that the combination of EMD+LLLT may result in greater improvements in wound healing and pain perceptions than those obtained following treatment with EMD alone.

Practical implications: Based on these preliminary data, application of EMD in combination with LLLT in the treatment of deep intrabony defects could help improve the rate of wound healing and clinical success and reduce the immediate post-operative pain. However, further studies of larger populations are needed in order to make ultimate conclusions and clinical recommendations.