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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

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Abstract

Aim: The purpose of the present study was to investigate the effectiveness of a regenerative procedure based on supra-crestal soft tissue preservation in association with combined autogenous bone (AB) graft/enamel matrix derivative (EMD) application in the treatment of deep periodontal intra-osseous defects.

Methods: Thirteen consecutively treated patients, seven females and six males, aged 30–65 years, three smokers, were included. A total of 15 deep, one- to two-wall intra-osseous defects were selected. Immediately before surgery and 6 months after surgery, pocket probing depth (PPD), clinical attachment level (CAL), and gingival recession (REC) were recorded.

Results: PPD amounted to 9.4 ± 1.8 mm before surgery, and decreased to 4.7 ± 1.2 mm post-surgery ($p < 0.0000$). CAL varied from 10.5 ± 2.0 mm pre-surgery to 6.2 ± 1.7 mm post-surgery ($p < 0.0000$), with CAL gain averaging 4.3 ± 1.4 mm. Fourteen (93.3%) defects presented CAL gain $\geqslant 3$ mm. REC change was 0.4 ± 0.7 mm.

Conclusions: Results from the present study indicated that a regenerative procedure based on supra-crestal soft tissue preservation and combined AB/EMD treatment leads to a clinically and statistically significant improvement of soft tissue conditions of deep periodontal intra-osseous defects.

Key words: autogenous bone graft; enamel matrix derivative; intra-osseous defects; periodontal regeneration; periodontal treatment/surgery

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Emdogain® (EMD; Biora AB, Malmö, Sweden) constitutes a commercially available compound that mainly consists of amelogenin and related proteins that are derived from porcine tooth buds. During fetal life, these enamel matrix proteins are secreted and temporarily deposited on the root surface by the cells of Hertwig's epithelial root sheath, being essential for the formation of acellular cementum and the development of associated periodontal ligament and alveolar bone (Hoffman 1960, Ten Cate et al. 1971, Ten Cate & Mills 1972, Hammarström 1997a,b). EMD has been shown to be effective in regenerating the periodontal attachment apparatus both in animals (Hammarström et al. 1997b, Sculean et al. 2000) and humans (Heijl 1997, Mellonig 1999, Sculean et al. 1999, Yukna & Mellonig 2000). The efficacy of EMD in the treatment of periodontal intra-osseous defects with respect to open flap debridement (OFD) has been well documented by three systematic reviews (Trombelli et al. 2002a, Esposito et al. 2003, Giannobile & Somerman 2003). Overall, the surgical application of EMD resulted in statistically significant improvements in attachment levels and probing depth reduction when compared with access flap procedure (Trombelli 2005).

Because of its gel-like consistency, EMD possesses limited space-making potential which, in turn, may potentially affect its regenerative potential (Mellonig 1999). Two methods have been proposed to maximize the clinical effect of EMD. Firstly, adequate preservation of supra-crestal inter-dental soft tissues has been shown to limit the collapse of the flap into the bone defect, thus optimising available space for regeneration. Previous data have suggested that the provision of a secluded space by means of proper inter-dental tissue management and primary closure may potentially allow for an EMD-induced healing process to occur undisturbed, thus enhancing clinical outcome (Trombelli et al. 2002b). Recently, a regenerative strategy based on (i) the soft and hard tissue characteristics of the defect, and (ii) four different regenerative methods, including EMD, to optimise the clinical outcome has been elegantly validated in a cohort study (Cortellini & Tonetti 2005).

Secondly, a combined approach based on EMD plus a graft biomaterial has been suggested, particularly when the regenerative treatment is addressed in deep, non-contained intra-osseous defects (Froum et al. 2001). Consistently, recent studies indicated that the combination of EMD with bone substitutes, such as bovine porous bone mineral and demineralized freeze-dried bone allograft, has the potential to enhance the reconstructive outcome compared with EMD alone in terms of clinical attachment level (CAL) gain (Lekovic et al. 2000, Zucchelli et al. 2003) or bone fill (Velasquez-Plata et al. 2002, Gurinsky et al. 2004).

In view of these considerations, we decided to study a consecutive series of cases to explore the effectiveness of a regenerative procedure based on the preservation of supra-crestal soft tissue in association with an autogenous cortical bone (AB) graft/EMD combination for the treatment of deep intra-osseous defects. The additional use of an AB graft with EMD was supported by data from a randomized-controlled trial (RCT) showing a greater CAL gain after an access flap procedure with an AB graft compared with an access flap procedure alone for the reconstructive treatment of deep intra-osseous defect (Movin & Borring-Møller 1982). Evidence stemming from human biopsies of intra-osseous defects revealed the regenerative potential for bone autograft in terms of new bone and cementum formation (Nabers et al. 1972, Hiatt et al. 1978, Froum et al. 1983, Stahl et al. 1983). Furthermore, an anecdotal report showed that the adjunctive application of an AB graft with EMD resulted in a clinically significant gain of attachment on diseased root surfaces and bone fill on radiographs in intra-osseous defects (Leung & Jin 2003).

Material and Methods

Patient population

Patients were enrolled from among those seeking care for moderate to advanced periodontal disease at the Research Centre for the Study of Periodontal Diseases, University of Ferrara, and at the Department of Odontostomatological, Orthodontic and Surgical Disciplines, Second University of Naples.

The criteria for inclusion in the study were as follows: (1) no systemic diseases that contraindicated periodontal surgery; (2) no medications affecting periodontal status; (3) no pregnancy or lactation; (4) presence of at least one intra-bony defect with a PPD $\geqslant 6$ mm, a radiographic depth of the defect $\geqslant 4$ mm and a predominantly one- and two-wall intra-osseous component (as assessed through bone sounding); (5) full-mouth plaque score (O'Leary et al. 1972) and full-mouth bleeding score (calculated as the percentage of sites that bled after probing on the total number of probed sites) $<20\%$ at the time of the surgical procedure. Third molars, teeth with degree III mobility, furcation involvement or inadequate endodontic treatment and/or restoration were excluded.

Clinical recordings

Immediately before surgery and at 6 months after surgery, the following clinical parameters were assessed:

(i) local plaque score (LPS) and local bleeding score (LBS): recorded as positive when detectable plaque or bleeding on probing was present at the surgically treated site;
(ii) pocket probing depth (PPD), CAL and REC: recorded at six aspects per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual).

Probing measurements were performed by two calibrated examiners (S. B. and A. S.) using a manual pressure-sensitive probe (at approximately 0.3 N force) with 1 mm increments (UNC 15, Hu-Friedy Mfg. Inc, Chicago, IL,USA). Measurements were approximated to the nearest millimetre. An investigator meeting was performed as described previously (Tonetti et al. 1998). In brief, a calibration exercise was conducted to obtain acceptable intra- and inter-examiner reproducibility for pocket depth, recession of the gingival margin and evaluation of defect anatomy. However, the intra- and inter-examiner reproducibility was not assessed. Examiners were not blinded to the surgical procedure.

Intra-surgical measurements

Intra-surgical mesurements, recorded following debridement of the defect, included:

(i) probing bone level (PBL) – as the distance from the cemento–enamel junction to the bottom of the defect;
(ii) intra-bony component of the defect (IBD) – as the distance from the most coronal extension of the inter-proximal bone crest to the bottom of the defect (Fig. 1).

Pre-surgical phase

Prior to surgery, patients received a complete periodontal examination**,** oral hygiene instructions and multiple scaling and root planing sessions. At least 4 weeks elapsed from the completion of the non-surgical therapy until re-examination (pre-surgery recordings) and surgery.

Surgical procedure

Surgical sessions were performed by two operators (L.T. and L.G.). The areas selected for surgery were anesthetized. Sulcular incisions were made on both buccal lingual surfaces in an attempt to preserve the marginal and inter-dental tissues to the maximum possible extent. Flap design in the inter-dental area consisted of one of the following alternatives: (1) sulcular incisions with the split of buccal and lingual papilla; (2) inci-

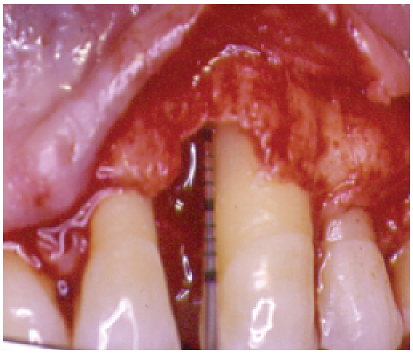

Fig. 1. The intra-surgical view of the defect revealed a pre-dominant one- to two-wall intra-osseous component, 9 mm deep.

sion with the preservation of the buccal papilla, according to the simplified papilla preservation technique (Cortellini et al. 1999); (3) incision with the preservation of the buccal papilla, according to the modified papilla preservation technique (Cortellini et al. 1995); and (4) incision with the preservation of the lingual/palatal papilla, according to the inter-proximal tissue maintenance procedure (Murphy 1996).

Selection of the flap design was based on the following: (1) width of the inter-dental space, evaluated as the distance from the cemento–enamel junction of the tooth presenting the bone defect to the cemento–enamel junction of the adjacent tooth; (2) distance from the contact point/area to the bone crest, as assessed radiographically; (3) apico–coronal width of the inter-dental keratinized tissue in the area of the intra-bony defect; and (4) location and morphology of the bone defect, as determined by bone sounding. The gingival tissues were incised at least one tooth mesial and distal to the defect site to provide access for visualization and instrumentation of the bone defect as well as for bone graft harvesting. Vertical releasing incisions were placed mesial or distal to the treated defects, if they were considered necessary for better access and/or primary closure of the surgical wound.

Full-thickness mucoperiosteal flaps were raised buccally and lingually up to 3–4 mm apical to the bone crest, and then a partial-thickness flap was performed on the buccal aspect to allow the coronal displacement of the buccal flap. Maximum care was taken to preserve inter-dental soft tissues. After flap reflection, all granulation tissue was removed from the defect, and the root surface was scaled and planed with hand and using ultrasonic instruments. An adequate amount of the AB graft was harvested from the buccal cortical plate by means of a bone scraper (Micross®, Meta C.G.M., Reggio Emilia, Italy). In all cases, the bone graft was collected from the surgical site adjacent to the intra-osseous defect. The exposed root surfaces were conditioned with 24% EDTA gel for 2 min. The defects and the adjacent mucoperiosteal flaps were then thoroughly rinsed with saline to remove the gel remnants. A first layer of EMD was then injected to condition the bone defect and the more apical portion of the root surface (Fig. 2). Then, the AB graft was positioned to fill only the IBD (Fig. 3). Finally, a second layer of EMD was injected to cover the grafted AB particles and to condition the portion of the root surface coronal to the bone crest (Fig. 4). Therefore, a ''sandwich'' technique was adopted to treat the defect, i.e. apical layer of EMD, AB graft and coronal layer of EMD. Therefore, a ''sandwich'' technique was adopted to treat the defect, i.e. apical layer of EMD, AB graft and coronal layer of EMD.

In relation to flap design, flaps were positioned at the pre-surgery level or slightly coronal in order to achieve primary closure of the inter-dental area without any tension. Monofilament non-resorbable # 5-0 or 6-0 (Gore-Tex Suture CV 5 or CV 6; W.L. Gore & Associates Inc., Flagstaff, AZ, USA) or polypropylene (Perma Sharp Suture 6–0, Hu-Friedy Mfg. Inc.) suturing material was used. Selection of the suturing technique was based on flap design (Cortellini et al. 1995, 1999 Murphy 1996).

The patients received systemic antibiotic therapy: 2 g/day for 6 days (Augmentin, SmithKline Beecham, Milan, Italy), and 0.12% chlorhexidine mouth rinses, twice a day for 6 weeks. Mechanical tooth cleaning was not allowed in the surgical area for the first six post-operative weeks. Sutures were removed at least 14 days following surgery. Patients were then scheduled on monthly recall visits, including supra-gingival tooth cleaning, until the 6-month re-evaluation.

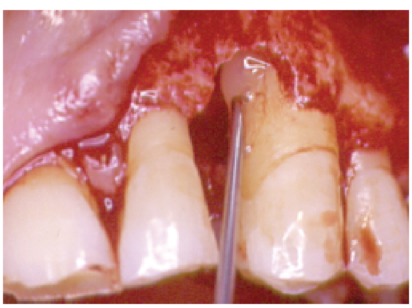

Fig. 2. A first layer of enamel matrix derivative (EMD) was injected to condition the bone defect and the more apical portion of the root surface.

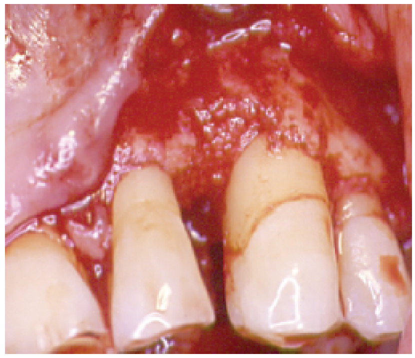

Fig. 3. An autogenous bone graft was positioned to fill only the intra-bony component of the defect.

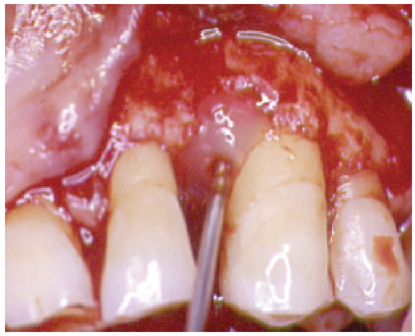

Fig. 4. A second layer of enamel matrix derivative (EMD) was then injected to condition the bone graft and the portion of the root surface coronal to the bone crest.

Statistical analysis

The defect was regarded as the statistical unit. The aspect of the tooth (topographically related to the intra-osseous defect) presenting the largest CAL value at the time of the pre-surgery recordings was used for further comparison and statistical analysis of clinical variables. Data were expressed as mean $\pm$ standard deviation.

Pre- and post-surgery recordings were compared by means of χ^2-test or student's t-test for paired observations. The level of significance was set at 5%.

Results

The patient and defect characteristics of the test and control groups are summarized in Table 1.

The study population comprised of 13 consecutively treated subjects, seven females and six males, recruited in the period between June 2003 and June 2004. The mean age of the patients was 50.7 years (range: 30–65 years). Three patients were smokers (daily cigarette consumption $\leqslant 5$).

Eleven patients had one defect, and two patients had two defects. A total of 15 defects were therefore analysed. Three defects were located in lateral incisors, two in canines, and 10 in premolars or molars. Neither infective complications nor adverse reactions related to AB/EMD treatment were recorded during the healing phase. All patients complied with the recall programme until re-evaluation. The PBL ranged from 8 to 15 mm (mean: 11.1 ± 2.3 mm), and the IBD varied from 5 to 9 mm (mean: 6.9 ± 1.1 mm).

Twelve (80%) defects were LPS negative both pre- and post-surgery. The incidence of LBS-positive defects shifted from 53.3% pre-surgery to 26.7% post-surgery, the difference not being statistically significant ($p = 0.269$).

The pre- and post-surgery clinical recordings are reported in Table 2. CAL varied from 10.5 ± 2.0 mm pre-surgery to 6.2 ± 1.7 mm post-surgery ($p < 0.0000$), with the CAL gain averaging 4.3 ± 1.4 mm. Eight defects presented a CAL gain of at least 5 mm, six defects of 3–4 mm, and one defect of 2 mm.

PPD amounted to 9.4 ± 1.8 mm before surgery, and decreased to 4.7 ± 1.2 mm post-surgery ($p < 0.0000$).

The REC change was 0.4 ± 0.7 mm. Five (33.3%) defects showed an REC increase of 1 mm, eight (53.3%) defects showed no change and one defect showed an REC decrease of 1 mm. Only one defect showed an REC increase of 2 mm.

Representative pre-surgery and 6-month-post-surgery periapical X-rays of the intra-osseous defect are presented in Figs 5a and b.

Table 1. Baseline characteristics of patients and intraosseous defects treated with a combined autogenous bone graft/enamel matrix derivative

Variable	Value
Subject number	13
Age (years)	50.7 ± 10.0
Males/females	6/7
Smokers	3 (23.1%)
Defect number	15
Upper/lower teeth	8/7
Anterior/posterior teeth	5/10
Probing bone level (mm)	11.1 ± 2.3
Intrabony component of defect (mm)	6.9 ± 1.1

Discussion

The purpose of the present study was to explore the effectiveness of a regenerative procedure based on supra-crestal soft tissue preservation in association with AB/EMD in the treatment of deep intra-bony defects. Fifteen consecutively treated osseous defects were analysed 6 months following surgery. A statistically significant and clinically relevant CAL gain was observed with respect to baseline condition, with more than 50% of the defects presenting a CAL gain of at least 5 mm. Interestingly, a limited apical shift of the gingival margin was consistently observed in all cases.

In our material, only deep non-containing intra-osseous defects, with a predominant one- to two-wall intra-osseous component, were included. A reconstructive approach based on the combined use of EMD and a graft was previously suggested in the treatment of defects with such morhological characteristics (Froum et al. 2001). The rationale for this approach was that, while EMD would exert a biological effect on the cascade of events leading to periodontal regeneration, the use of the graft may, to a certain extent, hinder the collapse of the flap into the bone defect during the early healing phase. It has been repeatedly demonstrated that the space available between the root surface and the mucoperiosteal flap strongly affects the outcome of reconstructive procedures, in general, and the extent of newly formed bone, in particular (Sigurdsson et al. 1994, 1995). In this respect, the use of either a bovine porous bone mineral or a demineralized freeze-dried bone allograft in combination with EMD produced a significantly enhanced outcome compared with EMD alone in terms of CAL gain (Lekovic et al. 2000, Zucchelli et al. 2003) or bone fill (Velasquez-Plata et al. 2002, Gurinsky et al. 2004).

A synergistic biological effect between the graft and EMD can also be hypothesized following the combined approach. It should be, however, stressed that while the use of EMD has been shown to be effective in improving the hard and soft tissue condition of an intra-osseous defects at clinical and histologic assessment, for some of the graft biomaterials the regenerative potential is still a matter of debate (Trombelli 2005). Findings from RCTs have shown that the hard and/or soft tissue measurements following the application of EMD in the treatment of deep intra-osseous defects are improved by the additional use of some graft biomaterials (Lekovic et al. 2000, Velasquez-Plata et al. 2002, Zucchelli et al. 2003, Gurinsky et al. 2004), but not others (Sculean et al. 2005). Moreover, the magnitude of the treatment outcome (CAL gain, REC change), as observed in our case series, differs from other previous reports where intra-osseous defects of similar severity had been treated by different graft biomaterials combined with EMD application (Scheyer et al. 2002, Sculean et al. 2002, Velasquez-Plata et al. 2002, Gurinsky et al. 2004). The differences in treatment outcome between studies using different combined EMD +graft approaches may be partly

Table 2. Pre- and post-surgery pocket probing depth (PPD), clinical attachment level (CAL) and gingival recession (REC) in intraosseous defects treated with a combined autogenous bone graft/enamel matrix derivative as measured (in mm) immediately pre-surgery and 6 months post-surgery (mean ± standard deviation)

PPD				CAL				REC			
Pre	Post	Difference	p^*	Pre	Post	Difference	p^*	Pre	Post	Difference	p^*
9.4 ± 1.8	4.7 ± 1.2	4.7 ± 1.5	0.0000	10.5 ± 2.0	6.2 ± 1.7	4.3 ± 1.4	0.0000	1.1 ± 0.8	1.5 ± 1.3	0.4 ± 0.7	0.0541

*Significance of differences from baseline recordings (the Student *t*-test was used for paired observations)

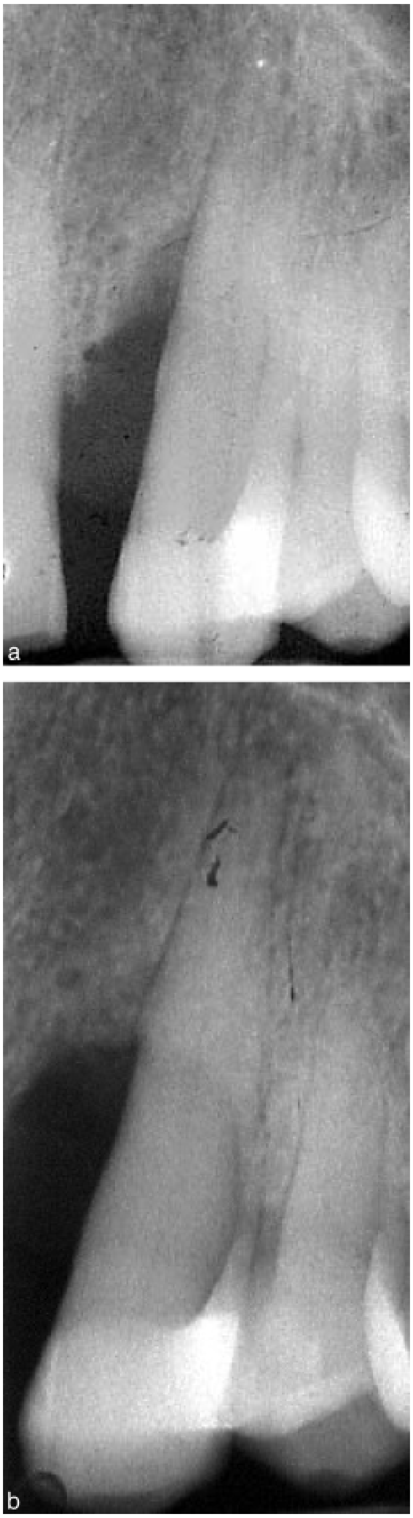

Fig. 5. Pre-surgical (a) and 6 months –post-surgery (b) radiographic aspect of the intra-osseous defect.

because of differences in the study populations, surgical procedures and maintenance strategies adopted. However, an enhancement in the treatment outcome dependent on the nature of the graft with respect to only EMD application may also be hypothesized.

A recent metanalysis demonstrated that the additional use of an AB graft with OFD results in a greater CAL gain when compared with OFD alone (Trombelli 2005). Previous histologic studies based on human biopsies indicated a true and complete periodontal regeneration, including bone, cementum and periodontal ligament, following AB graft in different types of periodontal defects (Froum et al. 1975, Hiatt et al 1978, Stahl et al. 1983). Experimental studies in non-human primates have shown that the combined application of EMD plus cortical AB graft results in a significantly greater bone and cementum formation compared with access flap surgery alone in small periodontal defects (Cochran et al. 2003). Therefore, in the present study the selection of AB as an adjunct to an EMD-supported reconstructive procedure accounted for the physico-mechanical properties of the cortical bone chips used, as well as the potential biological effect of AB to implement the regenerative outcome. Further investigations are needed to determine whether and to what extent this specific type of AB, mainly of cortical nature, may promote periodontal regeneration.

In our material, a limited amount of post-operative recession was observed in all cases despite the morphological characteristics of the selected defect population (mainly one- to two-wall, non-self-supporting defects). We have previously demonstrated that the selection of a specific flap design, in relation to the anatomical characteristics of inter-dental space and the location/morphology of intra-osseous defect, as well as a proper suturing technique, may effectively contribute in limiting the apical shift of the gingival margin following an EMD-only treatment (Trombelli et al. 2002b). Interestingly, the magnitude of REC increase was smaller (0.4 ± 0.7 mm) in the present study compared with the previous EMD report (0.7 ± 0.8 mm) where a similar defect population had been treated with a similar surgical procedure (i.e. preservation of the inter-dental soft tissues) (Trombelli et al. 2002b). These data agrees with recent studies where a combined approach (EMD+graft) resulted in less REC compared with EMD treatment only (Velasquez-Plata et al. 2002, Zucchelli et al. 2003). Overall, these observations seem to emphasize the clinical relevance of the selection of the surgical approach to the anatomy of the treated area, as well as the physical and biological characteristics of the regenerative materials adopted (Froum et al. 2001, Cortellini & Tonetti 2005).

In conclusion, the results of the present case series seem to support the use of a combined AB graft/EMD application in the treatment of deep, non-containing intra-osseous defects. However, because of the high resorption rate of the AB graft, a longer observation interval may be needed to confirm the stability of the clinical outcome, as observed at 6 months. Further, randomised-controlled studies are needed to evaluate the clinical benefit, if any, of the additional use of an AB graft to EMD application when compared with EMD alone.

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Clinical Relevance

Scientific rationale for the study: A combined approach based on an EMD plus a graft biomaterial has been recently suggested. Therefore, we explored the effectiveness of a regenerative procedure based on the use of an AB graft/EMD combination for the treatment of deep intra-osseous defects.

Principal findings: Treatment resulted in significant and consistent attachment level gain and probing depth reduction at 6-month follow-up, while post-operative gingival recession was minimized.

Practical implications: Preliminary results from the present case series seem to support the use of a combined AB graft/EMD application in the treatment of deep, non-containing intra-osseous defects.