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Effect of platelet-rich plasma on the healing of intrabony defects treated with an enamel matrix protein derivative and a natural bone mineral

Ferenc Döri[1], Dimitris Nikolidakis[2], Tamás Húszár[3], Nicole B. Arweiler[4], István Gera[1] and Anton Sculean[2]

[1]Department of Periodontology, Semmelweis University, Budapest, Hungary; [2]Department of Periodontology Radboud University Medical Center, Nijmegen, The Netherlands; [3]Department of Oral and Maxillofacial Surgery, Semmelweis University, Budapest, Hungary; [4]Department of Operative Dentistry and Periodontology, Albert-Ludwigs University, Freiburg, Germany

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Abstract

Background: Regenerative periodontal surgery utilizing a combination of an enamel matrix protein derivative (EMD) and a natural bone mineral (NBM) and platelet-rich plasma (PRP) has been shown to enhance the outcomes of regenerative surgery significantly. At present, it is unknown whether root conditioning with EMD, followed by defect fill with a combination of NBM+PRP may additionally enhance the clinical results obtained with EMD+NBM.

Aim: To compare clinically the treatment of deep intrabony defects with either EMD+NBM+PRP or EMD+NBM.

Material and Methods: Twenty-six patients suffering from advanced chronic periodontitis, and each of whom displayed one advanced intrabony defect were randomly treated with either EMD+NBM+PRP (test) or EMD+NBM (control). The following clinical parameters were evaluated at baseline and at 1 year after treatment: plaque index (PI), gingival index (GI), bleeding on probing (BOP), probing depth (PD), gingival recession (GR) and clinical attachment level (CAL). The primary outcome variable was CAL.

Results: Healing was uneventful in all patients. At 1 year after therapy, the test sites showed a reduction in mean PD from 8.8 ± 1.9 mm to 3.1 ± 0.9 mm ($p<0.001$) and a change in mean CAL from 10.8 ± 2.0 mm to 6.0 ± 1.5 mm ($p<0.001$). In the control group the mean PD was reduced from 8.8 ± 2.0 mm to 2.8 ± 1.6 mm ($p<0.001$) and the mean CAL changed from 10.5 ± 1.6 mm to 5.5 ± 1.4 mm ($p<0.001$). CAL gains of ⩾4 mm were measured in 77% (i.e. in 10 out of 13 defects) of the cases treated with EMD+NBM+PRP and in 100% (i.e. in all 13 defects) treated with EMD+NBM. No statistically significant differences in any of the investigated parameters were observed between the two groups.

Conclusions: Within its limits, the present study has shown that (i) 1 year after regenerative surgery, both treatments resulted in statistically significant PD reductions and CAL gains and (ii) the use of PRP failed to enhance the results obtained with EMD+NBM.

Key words: controlled clinical trial; enamel matrix protein derivative; intrabony defects; natural bone mineral; platelet-rich plasma; regenerative periodontal therapy

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Regenerative periodontal therapy aims at the restitution of the tooth's supporting periodontal tissues [i.e. new periodontal ligament (PDL), new cementum with inserting connective tissue fibres and new bone] that have been lost due to periodontal disease (Karring et al. 2003). In human intrabony defects, periodontal regeneration has been demonstrated following treatment with an enamel matrix protein derivative (EMD), guided tissue regeneration

Conflict of interest and source of funding statement

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(GTR) or certain combination modalities such as EMD+ a natural bone mineral (NBM) or NBM+GTR (Nyman et al. 1982, Gottlow et al. 1986, Heijl 1997, Camelo et al. 1998, Mellonig 1999, 2000, Sculean et al. 1999, 2000, 2003, 2004, Yukna & Mellonig 2000, Majzoub et al. 2005). Recent clinical research has attempted to develop new techniques consisting of minimally invasive surgery or the use of various combinations of biologically active factors, bone substitutes/bone grafts with or without barrier membranes to improve the outcome of regenerative therapy additionally (Lekovic et al. 2000, 2002, Camargo et al. 2002, 2005, Velasquez-Plata et al. 2002, Zucchelli et al. 2003, Bokan et al. 2006, Christgau et al. 2006b, Cortellini & Tonetti 2007, Döri et al. 2007, Sculean et al. 2007).

Results from controlled clinical studies have indicated that treatment of deep intrabony defects with a combination of EMD+NBM may lead to higher CAL gains and osseous fill than treatment with EMD alone (Lekovic et al. 2000, Velasquez-Plata et al. 2002, Zucchelli et al. 2003).

Polypeptide growth factors (PGFs) have been shown to play an important role in the growth and differentiation of cells involved in periodontal wound healing (Lynch et al. 1989, 1991, Rutherford et al. 1992, 1993, Caffesse & Quinones 1993, Giannobile et al. 1994, Wang et al. 1994). Platelet-rich plasma (PRP) is an autologous volume of plasma with a four- to five-fold increased platelet concentration above baseline, and it is a proven source of growth factors (Marx et al. 1998). The positive impact of PRP on bone healing could be attributed to the angiogenetic, proliferative and differentiating effects on osteoblasts of transforming growth factor-β (TGF)-β and platelet-derived growth factor (PDGF) that are present in PRP in high concentrations (Marx 2004). In the last few years, PRP, combined with different types of grafting materials and barrier membranes, has also been used in regenerative periodontal therapy (De Obarrio et al. 2000, Camargo et al. 2002, 2005, Lekovic et al. 2002, Hanna et al. 2004, Okuda et al. 2005, Christgau et al. 2006a, b, Döri et al. 2007). It was also suggested that the use of PRP in combination with a bone graft/bone substitute may enhance the clinical management of the graft material and may also serve as a membrane barrier (Camargo et al. 2002, 2005, Lekovic et al. 2002).

Although the EMD+NBM combination has been shown to result in higher clinical improvements compared with treatment with EMD alone (Lekovic et al. 2000, Velasquez-Plata et al. 2002, Zucchelli et al. 2003), the question arises as to whether the results may be further improved with the use of PRP. It may be speculated whether EMD application onto the root surface can stimulate the migration of PDL fibroblasts and promote the formation of cementum with inserting collagen fibres. A subsequent defect fill with a combination of NBM+PRP might lead to an increase of growth factors in the wound area while, at the same time, PRP might act as a barrier membrane inhibiting epithelial cell proliferation and improving wound stability.

However, at present, no data from controlled clinical studies are available evaluating the healing of deep intrabony defects following treatment with a combination of EMD+NBM+PRP.

Therefore, the aim of the present prospective, randomized, controlled clinical study was to compare the treatment of deep intrabony defects with EMD+NBM+PRP with EMD+NBM.

Material and Methods

Patient population

Twenty-six patients (14 females and 12 males) (aged from 32–56 years) suffering from advanced chronic periodontal disease were included in this parallel-design study (i.e. 13 patients in each group) after having signed an informed consent. The study was performed in accordance with the Helsinki Declaration of 1975, as revised in 2000. The study protocol has been reviewed and approved by the university ethical board (Semmelweis University Budapest, Hungary). All patients were treated at the Department of Periodontology, Semmelweis University Budapest. The patients initially received cause-related periodontal therapy, consisting of oral hygiene instruction, motivation and subgingival scaling/root planing under local anaesthesia performed by the same experienced periodontist (F. D.). The patients were consecutively enrolled in the study when the following inclusion criteria were met: (1) no systemic diseases that could influence the outcome of the therapy; (2) a good level of oral hygiene [plaque index (PI) <1] (Löe 1967); (3) compliance with the maintenance programme, and (4) the presence of one intrabony defect with a probing depth of at least 6 mm and an intrabony component of $\geqslant 4$ mm as detected on the radiographs. None of the patients was a smoker (Tonetti et al. 1995). All regenerative surgical procedures were performed by the same experienced periodontist (F. D.) between September 2004 and September 2005. The following clinical parameters were assessed 1 week before and 1 year after the surgical procedure using the same type of periodontal probe (UNC 15, Hu-Friedy, Chicago, IL, USA): PI (Löe 1967), gingival index (GI) (Löe 1967), bleeding on probing (BOP), probing depth (PD), gingival recession (GR) and clinical attachment level (CAL). The measurements were made at six sites per tooth: mesiovestibular (mv), midvestibular (v), distovestibular (dv), mesiolingual (ml), midlingual (l) and distolingual (dl) by the same calibrated investigator (I. G.). The examiner was not aware, in any of the cases, of the type of treatment administered. The cemento-enamel junction (CEJ) was used as the reference point. In cases where the CEJ was not visible, a restoration margin was used for these measurements. In the calculations, only measurements at the same (at baseline the deepest, in terms of PD) site of the included defect were included. If two sites within a defect exhibited the same PD and CAL, it was decided by a toss of coin as to which site should be included in the analysis.

Intra-examiner reproducibility

Five patients, each showing 10 teeth (single and multi rooted) with probing depths >6 mm on at least one aspect of each tooth, were used to calibrate the examiner. The examiner evaluated the patients on two separate occasions, 48 h apart. Calibration was accepted if measurements at baseline and at 48 h were similar to the millimetre at the $>90\%$ level. The examiner was not aware of the surgical procedure to be performed.

Randomization

The defects were randomly assigned before surgery to the two treatment groups with the randomized block approach. Blocking to control for the effects of the prognostic variables INTRA and CAL was used to decrease outcome variability (Fleiss 1986). To allow randomization, INTRA (defined as the distance from the alveolar bone crest to the bottom of the defect) was estimated before surgery on pre-operative radiographs and by performing transgingival bone sounding. In each case, the surgeon was informed of the assigned treatment option after completion of flap elevation and defect debridement.

PRP preparation

In this study, the PRP preparation was performed using the Curasan PRP kit (Curasan AG, Kleinostheim, Germany) immediately before the operation. The Curasan system consists of a standard laboratory centrifuge with eight monovettes, a vortex mixer and a kit with disposable material. One monovette was filled with an 8.5 ml solution [7 ml blood and 1 ml citrate–phosphate–dextrose–adenine (CPDA) solution for anticoagulation]. The first spin was performed at $547 \times$ g. for 10 min. This procedure divided the blood into three basic components: red blood cells, PRP and platelet-poor plasma (PPP). The red blood cell layer formed at the lowest level, the PRP layer in the middle and the PPP layer at the top. PRP and PPP were collected in a second monovette. Then, a second spin was performed at $1231 \times$ g for 15 min. The platelet pellet concentrated at the bottom of the monovette, whereas the PPP concentrated on top. The PPP was removed so that the PRP remained in the monovette. After re-suspending the platelet pellet within the remaining volume of plasma with the vortex mixer, a 0.4 ml volume of PRP was ready for use. Appel et al. (2002) have shown that PRP volumes prepared with this technique contain a mean platelet count value of $2520 \times 10^3/\mu$l and high mean concentration values of growth factors (i.e. 295 ng/μl PDGF-AB and 500 ng/μl TGF-β1).

Surgical procedure

Following local anaesthesia, intracrevicular incisions were performed extending to the neighbouring teeth. Then, full-thickness mucoperiosteal flaps were raised vestibularly and orally. Vertical-releasing incisions were performed if deemed necessary for better access to the surgical site or to achieve a better closure. All granulation tissue was removed from the defects and the roots were thoroughly scaled and planed by means of manual and ultrasonic instruments. During surgery, the following measurements were made: distance from the CEJ to the bottom of the defect (CEJ–BD) and distance from the CEJ to the most coronal extension of the alveolar bone crest (CEJ–BC). The intrabony component (INTRA) of the defects was defined as (CEJ–BD) − (CEJ–BC).

After defect debridement, in both groups the root surfaces adjacent to the defects were conditioned for 2 min. with ethylenediaminetetraacetic acid (EDTA) gel (pH 6.7) (PrefGel®, previously BIORA, Sweden now Straumann, Basel, Switzerland) in order to remove the smear layer (Blomlöf et al. 1996). The defects and the adjacent mucoperiosteal flaps were then thoroughly rinsed with sterile saline in order to remove all EDTA residues.

In the EMD+NBM+PRP group, EMD (Emdogain®, Straumann, Basel, Switzerland) was first applied on the root surfaces, immediately followed by defect fill with NBM+PRP. At the time of application, coagulation of PRP was achieved by combining it with an equal volume of a sterile saline solution containing 10% calcium chloride and 100 U/ml of sterile bovine thrombin. The PRP displayed a sticky consistency within a few seconds. Afterwards, bovine porous bone mineral (NBM) granules (particle size 0.25 to 1.0 mm, BioOss®, Geistlich, Wolhusen, Switzerland) were mixed with the coagulated PRP. In the defects treated with EMD+NBM, EMD was first applied onto the root surfaces and then the defects were filled with NBM. In both groups, care was taken not to overfill the defects. Finally, the flaps were repositioned coronally and closed with vertical or horizontal mattress sutures.

Postoperative care

All patients received antibiotics for 1 week (3×500 mg amoxicillin/day). The postoperative care consisted of 0.2% chlorhexidine rinses twice a day for 4 weeks. Sutures were removed 14 days after the surgery. Recall appointments were scheduled weekly during the first 6 weeks after surgery and 1 per month following the rest of the observation period of 1 year. The recall appointments consisted of reinforcement in oral hygiene measures and professional supragingival tooth cleaning.

Statistical analysis

The statistical analysis was performed using a commercially available software program (SPSS® for Windows version 12.0, SPSS Inc., Chicago, IL, USA). The primary outcome variable was the CAL gain, whereas PD and GR changes were the secondary outcome variables. For the statistical evaluation of the changes from baseline to one year in each treatment group, the paired *t*-test was used. For comparisons between the groups, the unpaired *t*-test was used. The α error was set at 0.05. The power of the study, considering 1 mm as a significant difference between the groups, was calculated to be 0.80.

Results

All patients completed the study. The postoperative healing was uneventful in all cases. No complications such as allergic reactions, abscesses or infections were observed throughout the entire study period. A slight wound dehiscence, however, without exposure of the graft particles, occurred in the third week at two sites in the EMD+NBM+PRP and at three sites in the EMD+NBM group. All dehiscences epithelialized within a few days without any side effects.

There were no differences in the gender distribution between the groups (i.e. seven females and six males) in each of the two groups.

Table 1 illustrates for both groups the mean PI, GI and BOP. GI and BOP improved statistically significantly compared with baseline, but no statistically significant differences were found between the two groups.

The defects displayed a comparable distribution and configuration in the two groups (Table 2). The depth of the intrabony component as measured during surgery is presented in Table 3. There were no differences in the depth of the intrabony component between the two groups.

At baseline, the mean PD was similar in the two groups and no statistically

Table 1. Mean (± SD) plaque, gingival and bleeding scores at the treated sites at baseline and the 1-year examination

	EMD+NBM+PRP ($N = 13$)	EMD+NBM ($N = 13$)
Plaque index scores		
Baseline	0.7 ± 0.3	0.9 ± 0.1
12 months	0.7 ± 0.2	0.6 ± 0.3
Gingival index scores		
Baseline	1.3 ± 0.2	1.4 ± 0.3
12 months	0.7 ± 0.3	0.8 ± 0.2
Bleeding scores		
Baseline (%)	58	60
12 months (%)	19	18

EMD, enamel matrix protein derivative; NBM, natural bone mineral; PRP, platelet-rich plasma.

Table 2. Distribution and configuration of treated defects

	EMD+NBM+PRP ($N = 13$)	EMD+NBM ($N = 13$)
Maxilla	9	8
Mandible	4	5
Anterior teeth	6	5
Premolars	4	4
Molars	3	4
1–2 wall	7	8
2 wall	6	5

EMD, enamel matrix protein derivative; NBM, natural bone mineral; PRP, platelet-rich plasma.

Table 3. Baseline defect characteristics expressed in mm (mean ± SD)

Treatment	CEJ–BD (mm)	CEJ–BC (mm)	INTRA (mm)
EMD+NBM+PRP ($N = 13$)	12.2 ± 1.2	7.1 ± 1.1	5.1 ± 1.1
EMD+NBM ($N = 13$)	12.1 ± 1.3	6.9 ± 1.2	5.2 ± 1.2

EMD, enamel matrix protein derivative; NBM, natural bone mineral; PRP, platelet-rich plasma; CEJ–BD, distance from the cemento-enamel junction (CEJ) to the bottom of the defect; CEJ–BC, distance from CEJ to the most coronal extension of the alveolar bone crest; INTRA, intrabony component.

Table 4. Clinical parameters at baseline and 1 year expressed in mm ($n = 13$ for each group)

	Baseline	1 Year	Difference	Significance
Probing depth				
EMD+NBM+PRP	8.8 ± 1.9	3.1 ± 0.9	5.8 ± 1.8	$p < 0.001$
EMD+NBM	8.8 ± 2.0	2.8 ± 1.6	5.9 ± 1.3	$p < 0.001$
			NS	
Gingival recession				
EMD+NBM+PRP	1.9 ± 1.4	2.9 ± 1.5	1.0 ± 1.0	< 0.01
EMD+NBM	1.8 ± 1.4	2.7 ± 1.5	0.9 ± 1.3	< 0.01
			NS	
Clinical attachment level				
EMD+NBM+PRP	10.8 ± 2.0	6.0 ± 1.5	4.8 ± 1.3	$p < 0.001$
EMD+NBM	10.5 ± 1.6	5.5 ± 1.4	5.0 ± 0.9	$p < 0.001$
			NS	

EMD, enamel matrix protein derivative; NBM, natural bone mineral; PRP, platelet-rich plasma.

significant difference was found. At 1 year, the mean PD was decreased significantly in both groups compared with the baseline data ($p < 0.001$). The mean PD reduction was 5.8 ± 1.8 mm in the EMD+NBM+PRP group and 5.9 ± 1.3 mm in the EMD+NBM group. No statistically significant difference between the groups was found (Table 4).

At baseline, the mean GR was similar between the two groups, with no statistically significant difference (Table 4). At 1 year, the mean GR increase was 1.0 ± 1.0 mm in the EMD+NBM+PRP group and 0.9 ± 1.3 mm in the EMD+NBM group. The increase in GR was statistically significant for both groups ($p < 0.01$), but no difference between the groups was observed.

No statistically significant difference was also found between the groups regarding the baseline mean value of CAL (Table 4). The mean CAL gain was 4.8 ± 1.3 mm in the EMD+NBM+PRP group and 5.0 ± 0.9 mm in the EMD+NBM group ($p < 0.001$). In both groups, the CAL improved significantly compared with baseline but no statistically significant difference was observed between the two groups.

The frequency distribution of CAL gain for both treatment groups is shown in Table 5. In both groups, all sites gained at least 3 mm of CAL. CAL gains of ⩾4 mm were measured in 77% (i.e. in 10 out of 13 defects) of the cases treated with EMD+NBM+PRP and in 100% (i.e. in 13 out of 13 defects) treated with EMD+NBM.

Discussion

The results of the present study have shown that regenerative periodontal surgery in deep intrabony defects with both combination approaches may lead to significant PD reduction and CAL gain compared with baseline values. No adverse reactions such as allergies, abscesses or rejection of the implanted materials were observed throughout the entire study period of 1 year, which in turn indicates that the materials used and their combinations were well tolerated. No statistically significant differences in any of the investigated parameters were found between the treatments. CAL gains of ⩾4 mm were measured in 77% (i.e. in 10 out of 13 defects) of the cases treated with EMD+NBM+PRP and in 100% (i.e. in all 13 defects) treated with EMD+NBM. These results indicate that, at least from a clinical point of view, treatment with EMD+NBM+PRP does not confer a significant benefit to treatment with EMD+NBM. However, when interpreting these findings, it has to be kept in mind that at present being no other data evaluating the treatment of intrabony defects with EMD+NBM+PRP are available, and therefore, direct comparisons with other studies are not possible.

Table 5. Frequency distribution of CAL gain expressed in mm ($n = 13$ for each group)

CAL gain (mm)	EMD+NBM+PRP		EMD+NBM	
	N°	%	N°	%
0	0	0	0	0
1	0	0	0	0
2	0	0	0	0
3	3	23	0	0
4	2	15	4	31
5	5	39	6	46
6	1	8	2	15
7	2	15	1	8

EMD, enamel matrix protein derivative; NBM, natural bone mineral; PRP, platelet-rich plasma; CAL, clinical attachment level.

The results from controlled clinical studies evaluating the effect of a combination of PRP with different types of bone substitutes and GTR in regenerative periodontal therapy are somewhat controversial. While some reports have shown significantly higher CAL gains and defect fill following the combination of bone substitutes PRP and GTR (Camargo et al. 2002, 2005), others have failed to show a significant benefit of PRP (Christgau et al. 2006b, Döri et al. 2007). Furthermore, the study design of the papers by Camargo et al. (2002, 2005) includes too many different variables between the groups, which makes it impossible to draw any definitive conclusions regarding the sole effect of PRP. In a controlled split-mouth study, comparing treatment with NBM+PRP with NBM alone, statistically significantly higher CAL gain was obtained in the combination group (i.e. 3.15 mm in the NBM+PRP group *versus* 2.31 mm in the NBM group) (Hanna et al. 2004). On the other hand, the results of a recent controlled clinical study comparing treatment of intrabony defects with NBM+PRP+GTR with NBM+GTR have demonstrated excellent clinical outcomes after both combination approaches, but no statstically significant differences in any of the investigated parameters were found between the groups (Döri et al. 2007). At 1 year after therapy, the mean CAL gains were 4.5 ± 1.1 mm in the NBM+PRP+GTR group and 4.6 ± 1.1 mm in the NBM+GTR, respectively. CAL gains of $\geqslant 4$ mm were found in 80% (i.e. in 12 out of 15 defects) of the cases treated with NBM+PRP+GTR and in 87% (i.e. in 13 out of 15 defects) treated with NBM+GTR.

The present results obtained in the EMD+NBM group are in line with those obtained in other controlled clinical studies, which have shown that treatment of intrabony defects with EMD+NBM may result in significantly higher CAL gains compared with treatment with EMD alone (Lekovic et al. 2000, Velasquez-Plata et al. 2002, Zucchelli et al. 2003). Results from a split-mouth study have indicated a higher increase in gingival recession following treatment with EMD (i.e. 0.8 ± 0.8 mm) than following treatment with EMD+NBM (i.e. 0.3 ± 0.6 mm) while the re-entry demonstrated significantly higher bone fill in the EMD+NBM group (i.e. 4.0 ± 0.8 mm) compared with the EMD one (i.e. 3.1 ± 1.0 mm) (Velasquez-Plata et al. 2002). In a further controlled clinical trial, 60 deep intrabony defects in 60 patients with chronic periodontitis were treated with the simplified papilla preservation flap and defect fill with either EMD+NBM or EMD alone (Zucchelli et al. 2003). Both treatments resulted in clinically and statistically significant improvements in terms of CAL gain, PD reduction and radiographic bone fill when compared with baseline. However, treatment with EMD+NBM resulted in a significantly higher CAL gain (5.3 ± 1.1 mm *versus* 4.3 ± 1.0 mm) and less increase in gingival recession (0.4 ± 0.6 mm *versus* 0.9 ± 0.5 mm) than treatment with EMD.

Slight differences between the present results obtained with EMD+NBM and those referred to might be related to differences in the initial depth of the defects. It is well documented that in deeper defects, a greater CAL gain may be achieved (Tonetti et al. 1996). Furthermore, in the present study, the application of EMD+NBM was slightly different compared with those referred to (Lekovic et al. 2000, Velasquez-Plata et al. 2002, Zucchelli et al. 2003). While in this study, EMD was first applied on the root surfaces and subsequently followed by defect fill with NBM, in the referred studies, the defects were filled by a the mixture of EMD+NBM.

When interpreting the healing result obtained with EMD+NBM, the results of a human histologic study that has demonstrated formation of cementum, PDL and bone formation following this regenerative treatment modality need to be mentioned (Sculean et al. 2003). Thus, the clinical results obtained following this treatment approach may not only represent a clinical improvement but also, at least to a certain extent, a regenerative type of healing.

There might be several explanations for the lack of difference between the two treatment groups. One aspect may be related to the incomplete understanding of the precise mechanism of PRP upon periodontal regeneration. It was suggested that PRP contains high concentrations of several growth factors such as PDGF and TGF-β, which may strongly modulate the regeneration process (De Obarrio et al. 2000, Camargo et al. 2002, 2005, Lekovic et al. 2002, Okuda et al. 2003, Kawase et al. 2005, Christgau et al. 2006a). Data from in vitro studies have shown that PRP stimulates the proliferation of PDL and osteoblastic cells while, at the same time, epithelial cell proliferation is inhibited (Okuda et al. 2003, Kawase et al. 2005). It was also speculated that due to its fibrinogen content, PRP reacts with thrombin and induces fibrin clot formation, which in turn is capable of upregulating collagen synthesis in the extracellular matrix and provides a favourable scaffold for cellular migration and adhesion (Camargo et al. 2005). In the present study, no blood parameters or growth factor concentrations were evaluated. This in turn may comport the risk of a production of PRP volumes with low platelet/growth factor concentrations. On the other hand, it has been shown previously that PRP volumes prepared with this technique contain an optimal platelet count (Appel et al. 2002). When addressing this issue, it should also be kept in mind that in a clinical practice setting it is difficult to evaluate the blood of every patient and subsequently decide as to whether an application of PRP is preferable or not. Thus, from a clinician's point of view, the practical aspects related to PRP preparation, which involves an additional step to the surgical procedure, should also be pointed out.

Furthermore, data from in vitro studies indicate that EMD may also influence periodontal wound healing by an indirect stimulatory effect on the release of growth factors during periodontal wound healing and by inhibiting or at least retarding epithelial downgrowth (Kawase et al. 2000, Schwartz et al. 2000, Van der Pauw et al. 2000, Lyngstadaas et al. 2001, Okubo et al. 2003). Because both PRP and EMD seem to have a stimulatory effect on wound healing, it may be speculated that the stimulatory effect provided by only one of these two materials might be sufficient to create an optimal healing environment and thus, it questions the additional benefit of this combination.

On the other hand, it should be kept in mind that the lack of a difference between the two groups may also be related to the rather limited number of treated defects (e.g. 13 defects in each group) and therefore, the study may not have the statistical power to rule out the possibility of a difference between the 2 groups (Gunsolley et al. 1998). For superiority trials in the treatment of periodontal intrabony defects using regenerative materials, a sample size of approximately 30 persons per group has been estimated to be needed, considering a desirable difference between groups of 1.0 ($\pm$ 1.3) mm CAL gain (Gunsolley et al. 1998). However, it needs to be pointed out that from a practical point of view, it is very difficult to recruit such a large number of patients for a mono-centre randomized-controlled clinical trial.

In conclusion, within its limits, the present study has shown that (i) at one year after regenerative surgery, both treatments resulted in statistically significant PD reductions and CAL gains and (ii) the use of PRP failed to enhance the results obtained with EMD+NBM.

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Address:
Anton Sculean
Department of Periodontology
Radboud University Medical Center
P.O. Box 9101, Internal Postal Code 117
6500 Nijmegen, Philips van Leydenlaan 25
The Netherlands
E-mail: a.sculean@dent.umcn.nl

Clinical Relevance

Scientific rationale for the study: EMD and NBM have been shown to enhance periodontal regeneration. PRP, combined with different types of grafting materials and barrier membranes, has also been successfully used in regenerative periodontal therapy. At present, there are no data regarding to what extent root conditioning with EMD, followed by defect fill with a combination of NBM+PRP may additionally enhance the clinical results obtained with EMD+NBM.

Principal findings: At 1 year after regenerative surgery, both treatments resulted in significant clinical improvements compared with baseline. No statistically significant differences in any of the investigated parameters were found between the two groups.

Practical implications: The use of PRP has failed to enhance the results obtained with EMD and NBM.