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Effect of platelet-rich plasma on the healing of intra-bony defects treated with a natural bone mineral and a collagen membrane

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Abstract

Background: Regenerative periodontal therapy with a combination of platelet-rich plasma (PRP)+a natural bone mineral (NBM)+guided tissue regeneration (GTR) has been shown to result in significantly higher probing depth reductions and clinical attachment-level gains compared with treatment with open flap debridement alone. However, at present, it is unknown to what extent the use of PRP may additionally enhance the clinical outcome of the therapy compared with treatment with NBM+GTR.

Aim: To clinically compare treatment of deep intra-bony defects with NBM+PRP+GTR with NBM+GTR.

Material and Methods: Thirty patients suffering from advanced periodontal disease, and each of whom displayed one advanced intra-bony defect were randomly treated with a combination of either NBM+PRP+collagen membrane (GTR) or NBM+GTR. The following clinical parameters were evaluated at baseline and at 1 year after treatment: plaque index, gingival index, bleeding on probing, probing depth (PD), gingival recession and clinical attachment level (CAL). CAL changes were used as the primary outcome variable.

Results: No differences in any of the investigated parameters were observed at baseline between the two groups. Healing was uneventful in all patients. At 1 year after therapy, the sites treated with NBM+PRP+GTR showed a reduction in mean PD from 8.9 ± 2.3 mm to 3.4 ± 2.0 mm ($p < 0.001$) and a change in mean CAL from 10.9 ± 2.2 mm to 6.4 ± 1.8 mm ($p < 0.001$). In the group treated with NBM+GTR, the mean PD was reduced from 8.9 ± 2.5 mm to 3.4 ± 2.3 mm ($p < 0.001$), and the mean CAL changed from 11.1 ± 2.5 mm to 6.5 ± 2.3 mm ($p < 0.001$). In both groups, all sites gained at least 3 mm of CAL. CAL gains of $\geqslant 4$ mm were measured 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. 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) at 1 year after regenerative surgery with both NBM+PRP+GTR and NBM+GTR, significant PD reductions and CAL gains were found, and (ii) the use of PRP has failed to improve the results obtained with NBM+GTR.

Key words: collagen membrane; intra-bony defects; natural bone mineral; platelet-rich plasma; randomized controlled clinical trial

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Conflict of interest and source of funding statement

The authors declare that they have no conflict of interest.

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Regenerative periodontal therapy aims to reform tooth's supporting tissues (i.e. root cementum, periodontal ligament and alveolar bone), which have been lost following periodontitis or trauma (Caton & Greenstein 1993, Karring et al. 2003). Human histologic studies have shown that regenerative surgery involving defect fill with either a natural bone mineral (NBM) in combination with an enamel matrix protein derivative or a collagen membrane may promote a regenerative type of healing characterized by formation of cementum, periodontal ligament and bone (Camelo et al. 1998, Mellonig 2000, Sculean et al. 2003b, 2004). Results from controlled clinical studies have demonstrated that treatment of intra-bony defects with NBM combined with a collagen membrane or other regenerative materials (i.e. enamel matrix derivative) may lead to significantly higher clinical attachment level (CAL) gains and osseous fill than access flap surgery (Camargo et al. 2000, Paolantonio 2002, Sculean et al. 2003a, Tonetti et al. 2004, Bokan et al. 2006).

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). Findings from animal studies have demonstrated periodontal regeneration following short-term application of a combination of platelet-derived growth factors (PDGF) and insulin-like growth factors (IGF; Lynch et al. 1989, 1991, Rutherford et al. 1992, 1993, Giannobile et al. 1994). Data from controlled clinical studies have indicated that regenerative periodontal surgery and application of a combination of recombinant human (rh) platelet-derived growth factor-BB (PDGF-BB) and rh insulin-like growth factor-I (IGF-I) or rhPDGF-BB on a β-tricalciumphosphate (TCP) vehicle resulted in significantly higher improvements in terms of defect fill and gain of clinical attachment (CAL) compared with controls (i.e. application of the vehicle alone; Howell et al. 1997, Nevins et al. 2005). On the other hand, platelet-rich plasma (PRP), which is an autologous volume of plasma with a four to fivefold-increased platelet concentration above baseline, is a proven source of growth factors (Marx et al. 1998). PRP has been suggested for use to increase the rate of bone deposition and bone volume in combination with bone grafts during bone augmentation procedures (Sanchez et al. 2003). It has also been suggested that the application of the PRP in dentistry may have successful results and beneficial outcomes in the tissue regeneration, bone repair and general wound healing (Tozum & Demiralp 2003). 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 PDGF that are present in PRP in high concentrations (Marx 2004). Some human clinical trials have already reported a beneficial effect of PRP on bone formation regarding bone augmentation procedures (Marx et al. 1998, Anitua 1999, Wiltfang et al. 2003, Kassolis & Reynolds 2005). 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). Observations from a case report series have suggested that treatment of deep intra-bony periodontal defects with PRP combined with a bone allograft and guided tissue regeneration (GTR) may result in significant clinical improvements compared with baseline values (De Obarrio et al. 2000), although this study could not clarify the potential effect of PRP. Findings from controlled clinical studies comparing treatment of deep intra-bony defects with a combination of a NBM, PRP and GTR with either open-flap debridement (OFD), GTR or NBM have shown significantly higher CAL gains and defect fill following the combination approach (Camargo et al. 2002, 2005, Hanna et al. 2004). However, the CAL gain after GTR in these studies was far less compared with other studies (Cortellini & Tonetti 2005).

At present, it is still unknown to what extent a combination of NBM+PRP +GTR may additionally enhance the clinical outcomes compared with treatment with NBM+GTR. Thus, the aim of the present controlled clinical study was to evaluate the additional effect of PRP on the treatment of deep intra-bony defects comparing the use of NBM+PRP+GTR with NBM+GTR.

Material and Methods

Patient population

Thirty patients (16 females and 14 males; aged from 28 to 56 years), suffering from advanced periodontal disease, were included in this parallel-design study (i.e. 15 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, in Semmelweis University by the same experienced surgeon (D. F.). 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 program and (4) presence of one intra-bony defect with a probing depth (PD) of at least 6 mm and an intra-bony component of at least 3 mm as detected on the radiographs. None of the patients was a smoker (Tonetti et al. 1996). 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 CAL. The measurements were made at six sites per tooth: mesiovestibular, midvestibular, distovestibular, mesiolingual, midlingual and distolingual by a calibrated investigator (I. G.). The examiner was not aware, in any of the cases, of the type of treatment rendered. 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. Only one measurement per tooth, the deepest site of the selected defect at baseline, was included in the result calculations.

Intra-examiner reproducibility

Five patients, each showing 10 teeth (single and multirooted) with PDs >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 $>90\%$ 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 was performed to control for the effects of the prognostic variables INTRA and CAL to decrease outcome variability (Fleiss 1986). For allowing 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. Also, blood samples were collected from all patients regardless of the subsequent PRP application.

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 8.5 ml solution [7 ml blood and 1 ml citrate–phosphate–dextrose–adenine (CPDA) solution for anticoagulation]1. The first spin was performed at 2400 r.p.m. 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 3600 r.p.m. 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

After administering local anaesthesia, intra-crevicular 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 a 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 hand and ultrasonic instruments. No conditioning of the root surfaces was performed. 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 intra-bony component (INTRA) of the defects was defined as (CEJ–BD)–(CEJ–BC).

In the NBM+PRP+GTR group, at the time of the application, coagulation of PRP was achieved by its combination 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 0.25–1.0 mm, BioOss®, Geistlich, Wolhusen, Switzerland) were mixed with the coagulated PRP. Care was taken not to overfill the defects. Following grafting, a bioresorbable collagen membrane of porcine origin (BioGide Perio®, Geistlich, Wolhusen, Switzerland) was trimmed and adapted over the entire defect so as to cover 2–3 mm of the surrounding alveolar bone and to ensure stability of the wound and of the graft material. No sutures or pins were used for membrane fixation or stabilization. The same surgical protocol was also used for NBM+GTR sites, without using PRP.

Finally, the flaps were re-positioned coronally and closed with vertical or horizontal mattress sutures.

Post-operative care

All patients received antibiotics for 1 week (3×500 mg amoxicillin/day). The post-operative 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 months after surgery and once per month following the rest of the observation period of 1 year.

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 secondary outcome variables. For the statistical evaluation of the changes from baseline to 1 year in each treatment group, the paired *t*-test was used. For the comparisons between the groups, the unpaired *t*-test was used. The α error was set at 0.05. The power of the study, given 1 mm as a significant difference between the groups, was calculated to be 0.80.

Results

The post-operative healing was uneventful in all cases. No complications such as allergic reactions, abscesses or infections were observed throughout the study period. Membrane exposure occurred at three sites in the NBM+PRP+GTR group and at four sites in the NBM+GTR group. The exposed parts of the membranes disintegrated without any side effects.

There were no differences in the gender distribution between the groups (i.e. nine females and six males in the NBM+PRP+GTR and 10 females and five males in the NBM+GTR group).

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 intra-

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

	NBM+PRP+GTR ($N = 15$)	NBM+GTR ($N = 15$)
Plaque index scores		
Baseline	0.8 ± 0.3	0.7 ± 0.6
12 months	0.7 ± 0.5	0.8 ± 0.5
Gingival index scores		
Baseline	1.1 ± 0.5	1.2 ± 0.5
12 months	0.5 ± 0.4	0.6 ± 0.4
Bleeding scores		
Baseline	52%	54%
12 months	18%	19%

GTR, guided tissue regeneration; NBM, natural bone mineral; PRP, platelet-rich plasma.

Table 2. Distribution and configuration of treated defects

	NBM+PRP+GTR ($N = 15$)	NBM+GTR ($N = 15$)
Maxilla	8	7
Mandible	7	8
Anterior teeth	6	7
Premolars	3	3
Molars	6	5
1–2 wall	9	10
2 wall	4	3
3 wall	2	2

GTR, guided tissue regeneration; 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)
NBM+PRP+GTR ($N = 15$)	12.2 ± 2.2	7.0 ± 2.0	5.2 ± 2.1
NBM+GTR ($N = 15$)	12.5 ± 2.4	7.2 ± 2.2	5.3 ± 2.2

CEJ–BD, cemento-enamel junction to the bottom of the defect; CEJ–BC, distance from the CEJ to the most coronal extension of the alveolar bone crest; GTR, guided tissue regeneration; INTRA, distance from the alveolar bone crest to the bottom of the defect, NBM, natural bone mineral; PRP, platelet-rich plasma.

Table 4. Clinical parameters at baseline and 1 year expressed in mm ($n = 15$ for each group)

	Baseline	1 Year	Difference	Significance
Probing depth				
NBM+PRP+GTR	8.9 ± 2.3	3.4 ± 2.0	5.5 ± 1.3	$p < 0.001$
NBM+GTR	8.9 ± 2.5	3.4 ± 2.3	5.5 ± 1.7	$p < 0.001$
			n.s.	
Gingival recession				
NBM+PRP+GTR	1.9 ± 0.7	3.0 ± 1.1	1.1 ± 0.7	$p < 0.01$
NBM+GTR	1.9 ± 1.2	3.2 ± 1.5	1.3 ± 0.8	$p < 0.01$
			n.s.	
Clinical attachment level				
NBM+PRP+GTR	10.9 ± 2.2	6.4 ± 1.8	4.5 ± 1.1	$p < 0.001$
NBM+GTR	11.1 ± 2.5	6.5 ± 2.3	4.6 ± 1.1	$p < 0.001$
			n.s.	

GTR, guided tissue regeneration; NBM, natural bone mineral; PRP, platelet-rich plasma.

bony component as measured during surgery is presented in Table 3. There were no differences in the depth of the intra-bony component between the two groups.

At baseline, the mean PD was similar in the two groups and no statistically 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 5.5 ± 1.3 mm in the NBM+PRP+GTR group and 5.5 ± 1.7 mm in the NBM+GTR group. No 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 1.1 ± 0.7 mm in the NBM+PRP+GTR group and 1.3 ± 0.8 mm in the NBM+GTR 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 4.5 ± 1.1 mm in the NBM+PRP+GTR group and 4.6 ± 1.1 mm in the NBM+GTR 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 $\geqslant 4$ mm were measured in 80% (i.e. in 12 out of 15 defects) of the cases treated with PRP+NBM+GTR and in 87% (i.e. in 13 out of 15 defects) treated with NBM+GTR.

Discussion

The results of this study have shown that treatment of deep intra-bony defects with both the combination of NBM+PRP+GTR and NBM+GTR may lead to significant PD reduction and CAL gain compared with baseline values. However, no statistically significant differences in any of the investigated parameters were found between the two treatment modalities. The lack of adverse reactions such as allergies, abscesses or rejection of the implanted materials indicates that both combination techniques were well tolerated. These findings are in agreement with findings from previous studies, which have failed to show that any of the used materials may elicit any allergic or foreign body reactions. The present results obtained in the NBM+PRP+GTR group compare well with those reported by others (Camargo et al. 2002, 2005; Lekovic et al. 2002). Results from a recent controlled clinical

Table 5. Frequency distribution of CAL gain expressed in mm ($n = 15$ for each group)

CAL gain (mm)	NBM+PRP+GTR		NBM+GTR	
	no.	%	no.	%
0	0	0	0	0
1	0	0	0	0
2	0	0	0	0
3	3	20	2	13
4	4	27	6	40
5	6	40	3	20
6	1	6	4	27
7	1	6	0	0

CAL, clinical attachment level; GTR, guided tissue regeneration; NBM, natural bone mineral; PRP, platelet rich plasma.

study have demonstrated that treatment of deep intra-bony defects with NBM+PRP+GTR resulted in a significantly higher PD reduction, CAL gain and defect fill than treatment with OFD alone (Camargo et al. 2005). In another controlled clinical study, treatment with NBM+PRP+GTR yielded significantly higher clinical improvements compared with treatment with GTR alone (Camargo et al. 2002). Moreover, in a comparative split-mouth study comparing treatment with NBM+PRP to 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 data available on a comparison between treatment with NBM+PRP+GTR and NBM+PRP have failed to show significant differences between the two groups (Lekovic et al. 2002). In that split-mouth study, 21 patients, each with one pair of intra-bony defects, were randomly treated with either NBM+PRP+GTR or NBM+PRP. The clinical measurements revealed, at 6 months following therapy, a mean CAL gain of 4.12 ± 0.78 mm on buccal and 4.16 ± 0.83 mm on lingual sites in the NBM+PRP+GTR group. In the NBM+PRP group, the mean CAL gain was 3.78 ± 0.72 mm on buccal and 3.84 ± 0.76 mm on lingual sites. No statistically significant differences were detected between the two groups (Lekovic et al. 2002).

The present results obtained in the NBM+GTR group compare well with those obtained in other controlled clinical studies, which have shown that treatment of intra-bony defects with NBM+GTR may result in significantly higher PD reductions and CAL gains compared with OFD (Camargo et al. 2000, Sculean et al. 2003a, Tonetti et al. 2004). These studies have reported a mean CAL gain of 2.1, 4.0 and 3.3 mm, respectively, following treatment with NBM+GTR (Camargo et al. 2000, Sculean et al. 2003a, Tonetti et al. 2004).

Slight differences between the present results and those reported by others following treatment of intra-bony defects with NBM+PRP+GTR or NBM+GTR may be explained by differences in the initial depth of the defects. Based on the literature, in deeper defects, a greater CAL gain is achieved (Cortellini et al. 1998, Cortellini & Tonetti 2005). In this study, bony defects with a moderate depth (i.e. approximately 5 mm) were included. Also, data from previous clinical studies suggest that the surgical technique used, including careful preservation of supracrestal soft tissues, may have a significant influence upon post-operative soft tissue recession and subsequent CAL gain (Trombelli et al. 2002, Cortellini & Tonetti 2005).

Furthermore, in the present study, in both groups, all sites gained at least 3 mm of CAL. CAL gains of $\geqslant 4$ mm were measured in 80% (i.e. in 12 out of 15 defects) of the cases treated with PRP+NBM+GTR and in 87% (i.e. in 13 out of 15 defects) treated with NBM+GTR. These results seem to indicate that, at least from a clinical point of view, treatment with NBM+PRP+GTR does not seem to confer a significant benefit to treatment with NBM+GTR. When interpreting the healing type obtained with NBM+GTR, it needs to be mentioned that human histologic studies have provided evidence of cementum, periodontal ligament and bone formation following this regenerative treatment modality (Camelo et al. 1998, Mellonig 2000, Sculean et al. 2004). 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. Moreover, when interpreting the results obtained in the NBM+PRP+GTR group, it should be kept in mind that the precise mechanism of PRP on periodontal regeneration is still not well understood. 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). Data from in vitro studies have shown that PRP stimulated the proliferation of PDL and osteoblastic cells while, at the same time, epithelial cell proliferation was 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 up-regulating collagen synthesis in the extra-cellular matrix and promotes a favourable scaffold for cellular migration and adhesion (Camargo et al. 2005). In this study, no blood parameters or growth factors' concentration were evaluated. Of course, this entails the risk of a production of PRP volumes with low platelet/growth factors concentrations' that can result in disappointing results. It has previously been shown that PRP volumes prepared with this technique contain an optimal platelet count (Appel et al. 2002). Moreover, in a clinical practice setting, it appears difficult to evaluate the blood of every patient and subsequently decide whether an application of PRP is preferable or not. The rather limited results obtained in the PRP group may be explained by the use of the anorganic bone mineral, which does not contain any vital bone cells and thus, may be either not or only to a minor extent be influenced by PRP. On the other hand, it should be kept in mind that this assumption was recently questioned as it was shown that the addition of PRP to a bovine-derived xenograft may significantly improve the clinical outcomes in intra-bony defects (Hanna et al. 2004).

A lack of beneficial effects of PRP on periodontal and bone regeneration has also been reported by others (Pryor et al. 2005, Christgau et al. 2006a, b, Klongnoi et al. 2006). Very recent findings of a randomized prospective clinical split-mouth study have failed to show a

positive influence of autologous platelet concentrate (APC) on clinical and radiographic outcomes at 12 months following GTR therapy (Christgau et al. 2006a, b). In the study referred to, the application of APC was evaluated in the treatment of deep infra-bony defects additional to a bioresorbable membrane combined with β-TCP (Christgau et al. 2006a, b). Furthermore, the study also included testing of the growth factor levels in the APCs used in their study and confirmed a high concentration of PDGF-AB, PDGF-BB, TGF-β1 and IGF-I (Christgau et al. 2006a).

Other authors have suggested that following coagulation, the PRP preparation exhibits a ''sticky consistency'' that may improve the clinical handling properties of the combination of PRP and the grafting material (Anitua 1999, De Obarrio et al. 2000, Camargo et al. 2002, 2005, Lekovic et al. 2002, Okuda et al. 2005). Another property that was attributed to PRP is its possible haemostatic activity, which in turn may enhance blood-clot stabililty (Lekovic et al. 2002, Camargo et al. 2005). As PRP preparation uses the patient's own blood, the risk of disease transmission is practically eliminated and until now, neither systemic nor any other adverse reactions related to PRP treatment have been reported. However, the PRP application involved the use of bovine thrombin, which is not an autologous material. Regarding this material, until now no disease transmission or immunogenic reactions have been reported. Also, the risk for coagulopathies is minimal due to low dose, high product purity and topical application of PRP (Marx 2004). Some authors have questioned the necessity of using thrombin (Dugrillon et al. 2002), whereas others insist on using it (Marx 2004). According to the previous literature, PRP is expected to have an influence especially on the bone tissue (Marx et al. 1998, Sanchez et al. 2003, Marx 2004). On the other hand, it is well known that a real regeneration process can only be demonstrated histologically (Caton & Greenstein 1993) and thus a surgical re-entry or bone sounding are of limited value when evaluating this issue. This may be especially true when a non-resorbable bone substitute such as NBM has been used to fill the defects (Camelo et al. 1998, Camargo et al. 2002, 2005, Lekovic et al. 2002, Sculean et al. 2003b, 2004). Furthermore, it has been demonstrated previously that periodontal regeneration and defect fill will, in most cases, lead to CAL gain (Karring et al. 2003, Sculean et al. 2004).

Additionally, from a clinician's point of view, it is important to point to the practical aspects related to PRP preparation, which involves an additional step to the surgical procedure. Furthermore, at present, no data from human histologic material are available evaluating healing following treatment with NBM+PRP+GTR and thus, it is still unclear to what extent the use of PRP may enhance or impair the regeneration process compared with other, less complicated, treatment modalities such as NBM+GTR.

At present, according to our knowledge, no other data from controlled clinical studies are available comparing treatment with NBM+PRP+GTR with treatment with NBM+GTR, which makes direct comparisons difficult. Additionally, the post-healing evaluation period was longer in this study (i.e. 1 year) compared with the other studies mentioned above (i.e. 6 months). Long-term evaluation is preferable as it may indicate the clinical stability of the results obtained.

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

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Clinical Relevance

Scientific rationale for the study: Regenerative periodontal surgery using a combination of PRP, a NBM and GTR has been proposed as a modality to enhance treatment outcome in intra-bony defects. At present, it is unknown to what extent the use of PRP may additionally enhance the clinical results obtained with NBM+GTR. This study has compared clinically the healing of deep intra-bony defects treated with either NBM+PRP+GTR or NBM+GTR.

Principal findings: The results have shown that although both treatment modalities resulted in significant clinical improvements, the use of PRP has failed to additionally improve the clinical outcome.

Practical implications: The use of PRP does not seem to enhance additionally the outcome of regenerative periodontal surgery obtained with NBM+GTR.