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Regenerative treatment with platelet-rich plasma combined with a bovine-derived xenograft in smokers and non-smokers: 12-month clinical and radiographic results

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Abstract

Aim: The purpose of this study was to assess the healing response of intrabony defects following regenerative treatment with platelet-rich plasma (PRP) combined with a bovine-derived xenograft (BDX) in smokers and non-smokers.

Materials and Methods: A total of 24 advanced chronic periodontitis patients, 12 smokers and 12 non-smokers, with 113 intrabony defects with an intrabony component of $\geqslant 3$ mm were included in this study. Defects were surgically treated with PRP/BDX. At baseline and 12 months after surgery, the following parameters were recorded: plaque and sulcus bleeding indices, probing depth (PD), relative attachment level, marginal recession, probing and radiographic bone levels.

Results: Considering the soft tissue measurements, smokers and non-smokers presented a mean PD reduction of 3.97 ± 0.76 and 4.63 ± 0.52 mm, recession of 0.76 ± 0.44 and 0.50 ± 0.12 mm and attachment gain of 3.26 ± 0.42 and 4.06 ± 0.40 mm, respectively. Evaluation of the hard tissue findings revealed that the mean clinical and radiographic bone gains in smokers and non-smokers were 2.83 ± 0.47 and 3.63 ± 0.38 mm, 2.98 ± 0.38 and 3.67 ± 0.48 mm, respectively. Inter-group differences for PD reduction ($p < 0.05$), attachment ($p < 0.001$), clinical ($p < 0.001$) and radiographic bone gains ($p < 0.001$) were found to be significant between smokers and non-smokers.

Conclusions: Within the limits of this study, the results indicate that treatment outcome following PRP/BDX application in intrabony defects is impaired with smoking.

Key words: bovine-derived xenograft; intrabonydefects; platelet-rich plasma; smoking

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Conflict of interest and source of funding statement

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Smoking is one of the factors that has a detrimental effect on periodontal treatment outcomes (Bergstrom 1989, Bergstrom et al. 2000). Smokers showed a significantly less favourable response compared with non-smokers after scaling/root planing (Grossi et al. 1996), adjunctive anti-microbial therapy (Kinane & Radvar 1997), modified Widman flap surgery (Preber & Bergstrom 1990), root coverage (Erley et al. 2006) and regenerative procedures (Tonetti et al. 1995, Trombelli & Scabbia 1997, Trombelli et al. 1997, Zucchelli et al. 2002, Stavropoulos et al. 2004). The precise mechanisms by which smoking interferes with

periodontal regenerative healing are not completely understood. It can be hypothesized that any substance that might jeopardize the function of cells capable of periodontal regeneration could also impair tissue repair and regeneration (Balaji 2008). Smoking byproducts such as nicotine and cotinine may inhibit the attachment, proliferation and chemotaxis of human periodontal ligament fibroblasts (Giannopoulou et al. 1999, James et al. 1999). In the literature, there is a body of clinical evidence supporting the negative influence of smoking on the outcome of regenerative procedures, mostly guided tissue regeneration (GTR) (Tonetti et al. 1995, Trombelli & Scabbia 1997, Trombelli et al. 1997, Zucchelli et al. 2002, Stavropoulos et al. 2004). However, there are no data on the effect of smoking status on the clinical and radiographic outcomes of a procedure based on the usage of platelet-rich plasma (PRP) in intrabony defects.

PRP is an established treatment methodology that has been used in periodontal regeneration. The regeneration requires a sequence of biological events including cell adhesion, migration, proliferation and differentiation (Giannobile 1996).

The use of polypeptide growth factors (PGFs) to regulate these biological events has recently attracted the attention of researchers (Giannobile 1996, Howell et al. 1997, Giannobile & Somerman 2003). Among all PGFs, platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β) have been studied most extensively. PDGF and TGF-β have been shown to promote cell growth, differentiation and periodontal regeneration (Oates et al. 1993, Howell et al. 1997, Giannobile & Somerman 2003).

PDGF and TGF-β are abundant in the α granules of platelets (Assoian et al. 1984). Platelets are involved in the wound-healing process and represent a natural source of PGFs (Giannobile & Somerman 2003). A convenient approach to obtain autologous PDGF and TGF-β is the use of PRP that can be easily prepared from patient's blood by centrifugation (Marx et al. 1998, Okuda et al. 2003). It can be rationalized that by increasing the local concentrations of PGFs with the application of PRP, the periodontal healing outcome would be enhanced (de Obarrio et al. 2000, Camargo et al. 2002, Lekovic et al. 2002, Hanna et al. 2004, Okuda et al. 2005, Yılmaz et al. 2007).

PRP has been used successfully to enhance the clinical outcome obtained with GTR and bone grafts in the treatment of intrabony defects (de Obarrio et al. 2000, Camargo et al. 2002, Lekovic et al. 2002, Hanna et al. 2004, Okuda et al. 2005, Yılmaz et al. 2007). However, other studies suggested that the use of PRP failed to improve the results obtained with GTR and bone substitutes (Christgau et al. 2006, Döri et al. 2007a, b, 2008a, b, Yassıbağ-Berkman et al. 2007, Piemontese et al. 2008). The purpose of the present study was to evaluate the 12-month clinical and radiographic outcomes of a procedure based on the usage of PRP in combination with a bovine-derived xenograft (BDX) in smokers and non-smokers as measured by clinical and radiographic parameters.

Materials and Methods

Subjects and defects

The present clinical study was designed as a controlled clinical trial with a parallel design. Each selected patient, who applied to the clinics of Department of Periodontology at the Faculty of Dentistry, Yeditepe University, first received non-surgical periodontal therapy consisting of oral hygiene instructions, full-mouth supra-/sub-gingival scaling and root planing under local anaesthesia and occlusal adjustment by the same experienced periodontist (B. Y.). Two months following the initial periodontal therapy, patients underwent a re-evaluation examination.

Patients with systemic diseases that could interfere with periodontal healing and the number of platelets, who used antibiotics and anti-inflammatory agents at least 6 months before treatment and those who used anti-coagulants and/or anti-aggregans were excluded. Patients who met the inclusion criteria were systemically healthy, and had no contraindications for periodontal therapy. Surgical treatment was not scheduled if the patient could not demonstrate an adequate standard of supra-gingival plaque control. The patient was considered as eligible if plaque index (PI) < 1. Twenty-four advanced chronic periodontitis patients were included (12 smokers and 12 non-smokers). Patients who smoked regularly $\geqslant 10$ cigarettes on a daily basis were defined as smokers. Occasional and light smokers (< 10 cigarettes/day) were excluded. Two- and combined three-wall intrabony periodontal defects with a probing depth (PD) $\geqslant 6$ mm, radiographic defect depth $\geqslant 3$ mm and intrabony defect depth $\geqslant 3$ mm confirmed during the surgery were treated. When molar teeth were implicated, defects with furcation involvement were excluded. The selected patients underwent full-mouth periodontal surgeries due to generalized horizontal and vertical bone destruction pattern of the advanced chronic periodontitis. All surgeries were performed by the same experienced periodontist (S. Y.) between December 2006 and December 2007. After an explanation of all aspects of the study as well as alternative treatment regimens, an informed consent to participate in the study was obtained from all patients. The study design and consent were approved by the University Institutional Review Board.

Study groups

The study groups were designated as follows:

Group 1: 12 smoker patients treated with PRP (Harvest Technologies Corp., Plymouth, MA, USA)+BDX (Bio-Oss, Geistlich, Wolhusen, Switzerland).
Group 2: 12 non-smoker patients treated with PRP+BDX.

Clinical and radiographic assessments

For all patients, the following clinical parameters were recorded pre-operatively and at 12 months post-operatively by the same calibrated examiner (G. C.). A calibration exercise was carried out to obtain acceptable intra-examiner reproducibility as described previously by Sculean et al. (2005).

PI was measured according to Silness & Löe (1964), and sulcular bleeding index (SBI) according to Mühlemann & Son (1971). PD, relative attachment level (RAL), probing bone level (PBL) and marginal recession (REC) were measured to the nearest millimetre with a calibrated periodontal probe (PCP 15 UNC, Hu-Friedy, Chicago, IL, USA) using an individual occlusal stents as a reference point for probe placement. Occlusal stents for positioning measuring probes were fabricated with cold-cured acrylic resin on a cast model obtained from an alginate impression. It was made to cover the occlusal surfaces of the tooth being treated and the occlusal surfaces of at least one tooth in the mesial and distal directions. It was also extended apically on the buccal and lingual surfaces to cover the coronal third of the teeth. Six grooves were placed so that the post-

surgical measurements could be at the same position and angulation as those made before surgery. PD was the distance between the free gingival margin and the probeable bottom of the pocket, RAL was the distance between the probeable bottom of the pocket and the edge of the stent, REC was the distance between the free gingival margin and the edge of the stent and PBL was the distance between the probeable bone crest and the edge of the stent. PBL was measured under local anaesthesia by trans-gingival probing (sounding). The probe was forced through the soft tissue towards the bone until definite resistance was met (Kersten et al. 1992). PI was evaluated at four periodontal sites (mesio-buccal, mid-buccal, disto-buccal and mid-lingual), whereas other measurements were made at six points (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual). The present study reported only local measurements at the deepest inter-proximal point of the selected defects regarding PD, RAL, REC and PBL. Measurements where the edge of the stents was taken as the reference point were relative values (RAL, REC, PBL) to evaluate the attachment loss/gain, marginal soft tissue level change and clinical bone loss/gain.

Pre-operative and 12-month post-operative intra-oral radiographs (Kodak Ultra Speed, Readymatic, X-omet, Paris Cedex, France) were taken by the paralleling technique using a film holder device for the evaluation of radiographic bone level (RBL) (RWT® Standard Film Holder System; bite blocks, indicator arms, aiming rings; Kentzler-Kaschner Dental GmbH, Ellwangen/Jagst, Germany) connected to an acrylic dental splint (individual) to achieve identical film placement at each evaluation with the aim of standardization. The film holder was coupled to the X-ray tube via an adapter (RWT® Standart Film Holder System; aiming rings). Pre- and post-operative radiograph pairs were independently assessed on a light box by three experienced clinicians who were not told which radiograph was which. The mode (most frequent) count was accepted (Christgau et al. 1995). When measuring RBL, the three investigators were blinded with respect to the clinical measurements and had to reach an agreement in terms of the location of both anatomical and bone loss landmarks. Radiographic measurements were obtained as described elsewhere (Kuru et al. 2006) utilizing an adhesive millimetre grid (X-ray Grid, 3–4 cm, Meyer Haake GmbH, Oberursel, Germany). The differences between pre- and post-operative RBL measurements were considered as the radiographic bone loss/gain.

PRP preparation

PRP was prepared 1 h before surgery using the SmartPReP device (Harvest Technologies Corp., Plymouth, MA, USA). Briefly, 30 ml blood was withdrawn by puncture of an antecubital vein and processed according to the manufacturer's instruction. The procedure included an activation of platelets by autologous thrombin yielding a release of the various PGFs (Leitner et al. 2006). The clotted PRP was then mixed with cancellous deproteinized BDX granules.

Surgical procedure

Flap surgery for pocket elimination in sites different from the experimental ones was planned to be performed during the regenerative treatment. Therefore, surgical procedure was performed either in the maxilla or in the mandible, in one session. Following local anaesthesia, an intra-sulcular incision aiming to preserve the papillae was performed. Mucoperiostal buccal and lingual access flaps were then reflected. Granulation tissue adherent to the alveolar bone was removed to provide full access and visibility to the root surfaces. Any sub-gingival calculus was removed gently using hand instruments. No osseous recontouring was performed. Then, PRP and BDX combination was packed into the defects. Finally, PRP was applied to the wound area again. Care was taken not to overfill the defects. Finally, the flaps were replaced and sutured appropriately with a 3-0 silk material using the inter-dental suture technique. The sutures were free of tension, obtaining a complete coverage of the intrabony defects. A deep horizontal mattress suture was additionally used only if there was any residual tension from the flap margins. After a healing period of 2 weeks, the sutures were removed.

Post-operative care

Post-surgical care was directed at the maintenance of wound stability and infection control. All patients received systemic antibiotic therapy for a period of 7 days post-operatively (amoxicillin + potassium clavulanate, 2×1000 mg/day). In addition, all patients were advised to avoid hard chewing in the surgical areas and to rinse twice daily with a 0.2% solution of chlorhexidine digluconate for 2 weeks. Recall appointments were scheduled every second week during the first 2 months following the surgical procedure and all patients were recalled once a month for the remaining observation period.

During the 12-month follow-up period, neither sub-gingival instrumentation nor probing of the operated areas was performed.

Intra-surgical measurements

After defect debridement, distance from the edge of the occlusal stent to the bottom of the defect (A) and distance from the edge of the stent to the most coronal extension of the alveolar bone crest (B) were measured. The intrabony component of the defects was defined as A–B.

Statistical analysis

The statistical analysis was performed using a commercially available NCSS statistical and power analysis software (Kaysville, UT, USA). The average results of defects treated in each patient were taken into consideration and the findings were analysed considering the patient as the unit of evaluation. Each parameter was expressed as the mean value $\pm$ standard deviation. The primary outcome variable was the attachment gain, whereas PD reduction and clinical/radiographic bone gains were the secondary outcome variables. The baseline values to assess the homogeneity of the groups (excluding the relative values) were compared using the unpaired *t*-test. For the statistical evaluation of changes from baseline to 12 months in each treatment group, the paired *t*-test was used. For comparisons between the groups, the unpaired *t*-test was used. In the calculations, the deepest site per defect measurements were included. The α error was set at 0.05. The power of the study, with $\geqslant 1$ mm indicating a significant difference between the groups, was calculated to be 0.83.

Results

Fifty advanced chronic periodontitis patients were screened against the criteria listed for eligibility for this study.

Fifteen patients did not meet the inclusion criteria, and 11 patients refused to participate in the study. The remaining 24 patients received the intended treatment. No patients were lost to follow-up and returned for clinical and radiographic evaluation at 12 months. Clinical evaluation of post-surgical healing revealed a good soft tissue response to the combination with no adverse complications. Subject gender, defect distribution, number of osseous walls and baseline defect characteristics are presented in Table 1. Both groups presented similar baseline characteristics in terms of PD and RBL values.

The age of the patients ranged from 32 to 50 years. Table 2 illustrates the measurements of the PI and SBI parameters. All patients maintained a good level of oral hygiene and gingival status throughout the recall periods. Low PI (0.44 ± 0.13 and 0.62 ± 0.21, respectively) and SBI (0.50 ± 0.18 and 0.75 ± 0.27, respectively) values in smokers and non-smokers at the pre-operative examination further reduced at 12-months post-operatively (0.28 ± 0.08, 0.31 ± 0.09, and 0.31 ± 0.10, 0.41 ± 0.15, respectively). Inter-group comparison revealed a statistically significant difference for PI and SBI scores, revealing better oral hygiene levels in non-smokers (Table 2, $p < 0.05$).

At 12 months, smokers and non-smokers presented a mean PD reduction of 3.97 ± 0.76 mm ($p < 0.01$) and 4.63 ± 0.52 mm ($p < 0.01$), recession of 0.76 ± 0.44 mm ($p < 0.01$) and 0.50 ± 0.12 mm ($p < 0.01$), attachment gain of 3.26 ± 0.42 mm ($p < 0.01$) and 4.06 ± 0.4 mm ($p < 0.01$), respectively (Table 3). The inter-group comparison revealed a statistically significant difference for PD ($p < 0.05$) and RAL ($p < 0.001$) measurements (Table 3). Greater PD reduction and attachment gain occurred in non-smokers.

Evaluation of the hard tissue findings indicated that the treatment modality resulted in bone gain at 12 months in both groups. Radiographic assessments demonstrated defect fill when compared with the pre-operative images (Figs 1–4). The mean clinical and radiographic bone gains in smokers and non-smokers were 2.83 ± 0.47 mm ($p < 0.01$) and 3.63 ± 0.38 mm ($p < 0.01$), 2.98 ± 0.38 mm ($p < 0.01$) and 3.67 ± 0.48 mm ($p < 0.01$), respectively (Table 3). The inter-group comparison revealed a statistically significant difference for PBL ($p < 0.001$) and RBL ($p < 0.001$) measurements (Table 3). Greater clinical and radiographic bone gains occurred in non-smokers.

Table 1. Subject gender, defect distribution, number of osseous walls and baseline defect characteristics

Variable	Smokers	Non-smokers
Total number (patients/defects)	12/56	12/57
Female/defects	5/21	6/28
Male/defects	7/35	6/29
Maxilla	30	33
Mandible	26	24
Anterior	18	22
Pre-molar	20	17
Molar	18	18
Patient number with 4 defects	8	7
Patient number with 5 defects	2	3
Patient number with 7 defects	2	2
3-2 wall	18	20
3-2-1 wall	15	13
2 wall	23	24
Initial PD (mm)	6.72 ± 1.03	6.89 ± 0.95^{NS}
Initial RBL (mm)	5.80 ± 1.88	4.54 ± 0.53^{NS}
Intrabony component (mm)	3.72 ± 0.86	3.94 ± 0.64^{NS}

NS, $p > 0.05$, not significant.

Table 2. Full-mouth PI and SBI values at baseline and 12 months

	Smokers	Non-smokers	*p* value
PI			
Baseline	0.44 ± 0.13	0.62 ± 0.21	<0.05
12 months	0.28 ± 0.08	0.31 ± 0.09	NS
p value	<0.01	<0.01	
SBI			
Baseline	0.50 ± 0.18	0.75 ± 0.27	<0.05
12 months	0.31 ± 0.10	0.41 ± 0.15	NS
p value	<0.01	<0.01	

PI, plaque index; SBI, sulcular bleeding index; NS, not significant.

Table 3. Clinical and radiographic outcomes at 12 months

	Smokers	Non-smokers	*p* value
Increase in REC	$-0.76 \pm 0.44^{\#\#}$	$-0.50 \pm 0.12^{\$\$}$	0.082
Decrease in PD	$3.97 \pm 0.76^{\#\#}$	$4.63 \pm 0.52^{\$\$}$	0.019^{*}
Gain in RAL	$3.26 \pm 0.42^{\#\#}$	$4.06 \pm 0.40^{\$\$}$	0.0001^{***}
Gain in PBL	$2.83 \pm 0.47^{\#\#}$	$3.63 \pm 0.38^{\$\$}$	0.001^{***}
Gain in RBL	$2.98 \pm 0.38^{\#\#}$	$3.67 \pm 0.48^{\$\$}$	0.001^{***}

Intra-group difference (smokers):
$^{\#\#}p < 0.01$
Intra-group difference (non-smokers):
$^{\$\$}p < 0.01$.
Inter-group difference (*p*) (smokers versus non-smokers):
$^{*}p < 0.05$,
$^{***}p < 0.001$.
REC, marginal recession; PD, probing depth; RAL, relative attachment level; PBL, probing bone level; RBL, radiographic bone level.

Discussion

The 12-month results of the present clinical study demonstrated that a procedure based on the usage of PRP in combination with BDX provides benefits for both smokers and non-smokers, in terms of PD reduction, attachment and bone gains compared with baseline. However, as reported previously with respect to other periodontal therapy modalities (Preber & Bergstrom 1990, Tonetti et al. 1995, Grossi et al. 1996,

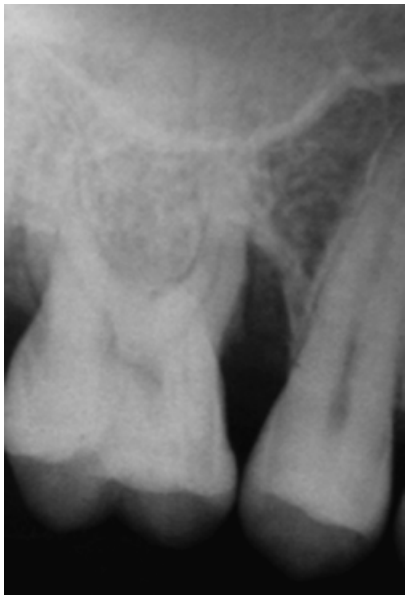

Fig. 1. Baseline radiographic view of a defect from the smokers group.

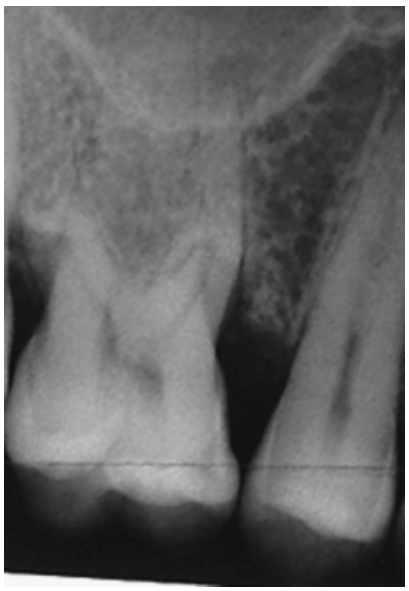

Fig. 2. 12-month radiographic view of a defect from the smokers group.

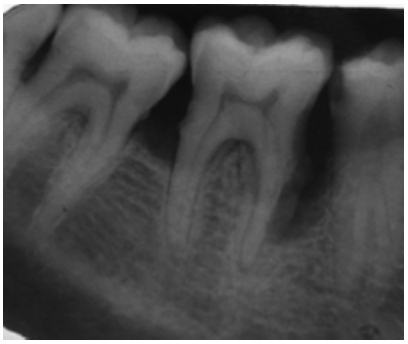

Fig. 3. Baseline radiographic view of a defect from the non-smokers group.

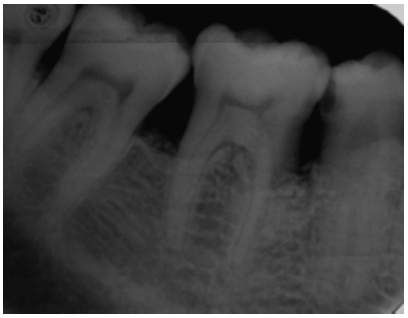

Fig. 4. 12-month radiographic view of a defect from the non-smokers group.

Kinane & Radvar 1997, Trombelli & Scabbia 1997, Trombelli et al. 1997, Zucchelli et al. 2002, Stavropoulos et al. 2004, Erley et al. 2006), cigarette smoking significantly affected therapy outcome in the present investigation. Smokers presented less PD reduction, less attachment and bone gains than non-smokers.

All patients participating in this study tolerated the surgical procedures well. No complications were observed at any treated site. This observation is in accordance with the results of other studies that have shown that PRP and BDX do not elicit any allergic or foreign body reactions (Lekovic et al. 2002, Hanna et al. 2004). It has been reported that the biological, physical and chemical properties of PRP may affect wound healing (Oates et al. 1993, Marx et al. 1998, Giannobile & Somerman 2003, Okuda et al. 2003). The effects of PGFs on cells and the high content of fibrinogen (fibrin glue), which promotes a favourable scaffold for cellular migration, are essential steps in the regeneration of periodontal defects. The PRP preparation presents a sticky characteristic, which works as a haemostatic and stabilizing agent, and may aid immobilization of the blood clot and bone graft in the defect area (Kawase et al. 2003).

In the last few years, PRP combined with different types of grafting materials, barrier membranes and enamel matrix derivative has been used in 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. 2006, Döri et al. 2007a, b, 2008a, b, Yassıbağ-Berkman et al. 2007, Yılmaz et al. 2007, Piemontese et al. 2008). In this study, PRP and BDX were chosen as the combination materials to enhance certain properties of each material, with the overall goal of enhancing the desired outcome of therapy. Bone substitutes of bovine origin are widely used for the treatment of bone defects in dental and orthopaedic surgery. Because of the occurrence of bovine spongiform encephalopathy (BSE) and the new variant of Creutzfeldt–Jakob Disease, the risks of transmitting diseases through the use of such materials need to be carefully evaluated. BDX is a deproteinized grafting material with proven safety and is prepared by the protein extraction of bovine bone, but maintains the natural structure of the bone. The material undergoes a low heat (300°C) chemical extraction process that extracts the organic components, leaving the architecture of bone intact (Gross 1997). Theoretical and experimental data indicate that the use of this material does not carry a risk of transmitting BSE to patients (Wenz et al. 2001).

The mechanism of action of PRP is attributed to the biologic effects of its PGF content (Marx et al. 1998, Giannobile & Somerman 2003, Okuda et al. 2003). On the other hand, BDX, with its bone morphogenetic protein content, which is a member of TGF superfamily, will add to the effects of the PGFs within the platelets, exerting a synergistic impact on the cell population of the wound (Schwartz et al. 2000).

The present results compared well with those obtained in previous studies with a similar combination, which have shown that treatment of intrabony defects with PRP+BDX improves PD reduction and attachment gain (Lekovic et al. 2002, Hanna et al. 2004). In these studies, the authors demonstrated a PD reduction in the range of 3.5–3.9 mm and an attachment gain in the range of 3.1–3.7 mm for PRP+BDX application. The results of the current study demonstrated a mean PD reduction of 3.97 ± 0.76 mm and a mean attachment gain of 3.26 ± 0.42 mm in smokers, whereas in non-smokers, these values were 4.63 ± 0.52 and 4.06 ± 0.40 mm, respectively. Our results obtained in the non-smokers group appear to be superior to the results obtained by other investigators (Lekovic et al. 2002, Hanna et al. 2004). This difference may be explained by the initial PDs, defect configurations and surgical technique. On the other hand, the results obtained in the smokers group for the same soft tissue parameters are significantly less

favourable than the results presented in the non-smokers group. This result may be attributed to the hypothesis that there may be a differential susceptibility of PRP to the negative effects of smoking (due to nicotine and its cytotoxic and vasoactive effects). In the literature, PRP was accepted as a practical source for PGFs required in regenerative procedures (Marx et al. 1998). Therefore, any factor that might jeopardize the regenerative potential of PRP by adversely affecting PGF production could also influence the regenerative outcomes (Balaji 2008). It has been reported that smoking down-regulates hydroxyproline and collagen production (Jorgensen et al. 1998). Hydroxyproline and collagen are essential for the production and maintenance of connective tissue. The presence of nicotine on root surfaces in smokers has also been documented (Cuff et al. 1989). This nicotine can be stored in fibroblasts, which alters fibroblast function and proliferation (Peacock et al. 1993, Tipton & Dabbous 1995). When fibroblasts are exposed to nicotine, cellular changes can occur (Hanes et al. 1991). This altered function of fibroblasts due to nicotine exposure could also be the cause of the poor periodontal wound healing. These combined effects of smoking may lead to less favourable outcomes following regenerative periodontal procedures.

Evaluation of the hard tissue findings revealed that the mean clinical and radiographic bone gains in smokers and non-smokers were 2.83 ± 0.47 and 3.63 ± 0.38 mm, 2.98 ± 0.38 and 3.67 ± 0.48 mm, respectively. A limited number of literatures presented data on hard tissue regeneration in humans (Camargo et al. 2005, Yassıbağ-Berkman et al. 2007). Only one study using BDX demonstrated a 3.4–3.5 mm defect fill with BDX+PRP+GTR (Camargo et al. 2005). This result corraborates the values reported in our study. It is difficult to compare our hard tissue results with the other study because β-tricalcium phosphate was used as a combination material (Yassıbağ-Berkman et al. 2007). The hard tissue results obtained from our study were found to be in favour of the non-smokers group. Although the precise mechanism by which smoking interferes with bone healing is not completely well understood, Hollinger et al. (1999), based on an experimental study, hypothesized that nicotine has a negative effect on bone healing by reducing osteoblast function. This hypothesis may also be valid to support our hard tissue results. For the radiographic evaluation of the hard tissue changes, a film holder device (RWT® Standard Film Holder System) was used in the present study. This system was modified by connecting the bite block to an individual acrylic dental splint aiming to achieve identical film placement at each evaluation and to obtain reproducible radiographs with regard to optimal standardization (Yılmaz et al. 2003, Kuru et al. 2006).

In the present study, no blood parameters or PGF concentrations were evaluated. This in turn may comport the risk of a production of PRP volumes with low platelet/PGF concentrations in smokers. When addressing this issue, in clinical practice, although it is difficult to evaluate the blood of every patient, it has utmost importance to draw definitive conclusions on the impaired effects of smoking.

Because PRP preparation utilizes the patient's own blood, the risk of human-to-human disease transmission is virtually eliminated, making it a safe treatment modality. When PRP is considered for use in the clinical practice of periodontics, several factors have to be taken into account, including the fact the clinician has to be proficient in drawing the necessary blood, the cost of the centrifugation machine and disposable materials and extra time and steps to prepare the coagulated PRP for its actual use. To avoid technical and financial restrictions, some clinicians choose to have blood laboratories perform the centrifugation of platelets before the surgical procedure. However, this decision should be carefully evaluated because some studies have shown that regular centrifugation of blood to separate the platelets may yield concentrations different from those obtained by a specialized machine (Weibrich et al. 2002, 2003). The centrifugation machine used in the present study has been tested to yield high percentages of platelet concentration with less blood drawn from the patient and with less complex procedures.

Conclusions

Within the limits of this study, the combination of PRP and BDX seems to be promising for periodontal regenerative therapy in advanced chronic periodontitis patients with intrabony defects. The results support the view that smoking impairs the healing outcome of the PRP treatment of intrabony defects significantly.

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Clinical Relevance

Scientific rationale for the study: PRP combined with different types of bone graft materials has been shown to enhance periodontal regeneration and has been successfully used in periodontal regenerative procedures. At present, there are no data on the effects of smoking on the clinical and radiographic outcomes of a procedure based on the usage of PRP and BDX combination in intrabony periodontal defects.

Principal findings: At 12 months after regenerative surgery, PRP+ BDX treatment resulted in significant soft and hard tissue healing when compared with baseline. Statistically significant differences were found in terms of PD reduction, attachment and bone gains between smokers and non-smokers in favour of non-smokers.

Practical implications: Cigarette consumption should be carefully considered while planning periodontal surgical procedures comprising PRP+BDX application.