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Platelet-rich fibrin and bovine porous bone mineral vs. platelet-rich fibrin in the treatment of intrabony periodontal defects

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Background and Objective: Bovine porous bone mineral (BPBM) is a xenograft that has been successfully utilized in periodontal regeneration. Platelet-rich fibrin (PRF) is a leukocyte and platelet preparation that concentrates various polypeptide growth factors and therefore has the potential to be used as regenerative treatment for periodontal defects. The purpose of this study was to examine the suitability of autologous PRF as regenerative treatment for periodontal intrabony defects in humans and to examine the ability of BPBM to augment the regenerative effects exerted by PRF.

Material and Methods: Using a split-mouth design, 17 paired intrabony defects were randomly treated either with PRF or with PRF–BPBM combination. Re-entry surgeries were performed at 6 mo. Primary study outcomes were changes in pocket depth, attachment level and defect fill.

Results: Preoperative pocket depths, attachment levels and transoperative bone measurements were similar for the PRF and PRF–BPBM groups. Postsurgical measurements revealed a significantly greater reduction in pocket depth in the PRF–BPBM group (4.47 ± 0.78 mm on buccal and 4.29 ± 0.82 mm on lingual sites) when compared with the PRF group (3.35 ± 0.68 mm on buccal and 3.24 ± 0.73 mm on lingual sites). The PRF–BPBM group presented with significantly greater attachment gain (3.82 ± 0.78 mm on buccal and 3.71 ± 0.75 mm on lingual sites) than the PRF group (2.24 ± 0.73 mm on buccal and 2.12 ± 0.68 mm on lingual sites). Defect fill was also greater in the PRF–BPBM group (4.06 ± 0.87 mm on buccal and 3.94 ± 0.73 mm on lingual sites) than in the PRF group (2.21 ± 0.68 mm on buccal and 2.06 ± 0.64 mm on lingual sites).

Conclusion: The results of this study indicate that PRF can improve clinical parameters associated with human intrabony periodontal defects, and BPBM has the ability to augment the effects of PRF in reducing pocket depth, improving clinical attachment levels and promoting defect fill.

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The objective of periodontal reconstructive therapy is to regenerate all tissues of the periodontium, including a functional periodontal ligament, alveolar bone and cementum (1). Currently, there is a variety of treatment modalities available for periodontal regenerative therapy, which includes bone grafts and substitutes, guided tissue regeneration, the use of growth factors, applications of tissue engineering, or combinations of two or more of the above-listed approaches (2).

Among bone grafts available for use in periodontal regenerative therapy, bovine porous bone mineral (BPBM) is a material that has been extensively researched. Bovine porous bone mineral is prepared by protein extraction from bovine bone, which results in a trabecular hydroxyapatite structure similar to human cancellous bone (3). As such, BPBM has the ability to enhance bone formation due to its osteoconductivity and has been used alone or in combination with other agents in the regenerative treatment of intrabony and furcation defects (4–10). These human clinical trials have shown that BPBM plays a positive role in reducing pocket depth, promoting gain in clinical attachment and enhancing defect fill in periodontal defects. Periodontal regeneration at the histological level has been demonstrated to be possible in defects treated with BPBM alone or when used in combination with a membrane for guided tissue regeneration (11).

Polypeptide growth factors are biological mediators that have the ability to regulate cell proliferation, chemotaxis and differentiation. Several polypeptide growth factors have been identified in human periodontal tissues by immunohistochemistry and *in situ* hybridization (12); therefore, polypeptide growth factors have a potential application in periodontal wound healing by promoting regeneration of periodontal tissues. Polypeptide growth factors have been shown to promote cell growth and differentiation *in vitro* and to induce periodontal regeneration in animals (13–22). Human periodontal regeneration studies examining the effectiveness of polypeptide growth factors, used alone or in combination with other materials and techniques, have been conducted with autologous platelet-rich plasma (5–7,10) and recombinant platelet-derived growth factor (23,24).

Autologous blood concentrates constitute a safe and convenient approach to deliver high concentrations of polypeptide growth factors to periodontal surgical wounds. Among platelet concentrates, platelet-rich fibrin (PRF) belongs to a group of second-generation blood autologous preparations that was originally described by Choukroun *et al.* (25). Platelet-rich fibrin is obtained by gentle centrifugation of peripheral blood and is characterized as being leukocyte and platelet rich and fibrin dense (26–28), besides not requiring the addition of any anticlotting agent. Dohan Ehrenfest *et al.* (29) showed that approximately 97% of platelets and 50% of leukocytes of the original blood volume were concentrated and three-dimensionally distributed in the PRF clot, which is one of the three layers resulting from the centrifugation process. After its preparation and collection, PRF can be used directly as a filler agent or compressed into a membrane. In either of those applications, PRF is believed to release polypeptide growth factors, such as transforming growth factor-β1, platelet-derived growth factor, vascular endothelial growth factor and matrix glycoproteins (such as thrombospondin-1), into the surgical wound in a sustained fashion for at least 7 d, as shown *in vitro* (30).

There are a few reports on the use of PRF in the regenerative treatment of periodontal defects in humans, but none of those investigated its effectiveness in treating interproximal intrabony defects (31–33). The purpose of this study was to evaluate the effectiveness of PRF in promoting clinical signs of periodontal regeneration in human intrabony defects and further assess the ability of BPBM to augment the regenerative effects of PRF in similar defects. The hypothesis being tested in the study was that BPBM would augment the regenerative effects of PRF in human intrabony defects.

Material and methods

Study subjects and design

The study was conducted in the Department of Periodontics, School of Dentistry at the University of Belgrade, from 10 October 2008 to 25 December 2009. The study design was approved by the Institutional Review Board at the University of Belgrade and conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2000. It was a double-blinded, controlled clinical trial that employed a split-mouth design. Patient enrollment in the study was conducted by the two therapists who performed the treatment steps described below.

Seventeen systemically healthy patients (11 women and six men, mean age 44 ± 9 years, 12 nonsmokers and five smokers) with matched pairs of interproximal, intrabony defects were enrolled in this study. The inclusion criteria were the presence of two similar interproximal, intrabony defects with probing depths ≥ 6 mm when evaluated 6 wk after the initial therapy. Osseous defects needed to have two or three walls. One-wall defects and interdental craters were excluded from the study. Only vital teeth, as revealed by a positive cold test, were included in the study. The plaque and gingival indices achieved following initial therapy had to be ≤ 1. Radiographic evidence of intrabony defects had to exist as revealed by peri-apical films taken with the long-cone parallel technique (Figs 1 and 2). The exclusion criteria were the presence of underlying systemic illnesses judged to impair normal healing, immune-compromised individuals, patients who were pregnant or lactating and patients taking any drug known to cause gingival enlargement.

Initial therapy

Initial therapy consisted of detailed oral hygiene instructions. Scaling and

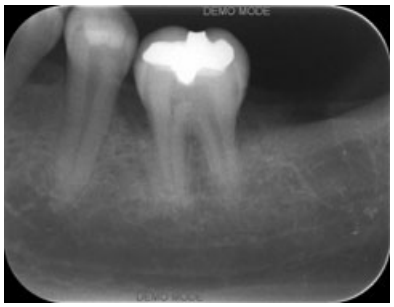

Fig. 1. Baseline radiograph of a case treated with PRF (mesial of tooth no. 19).

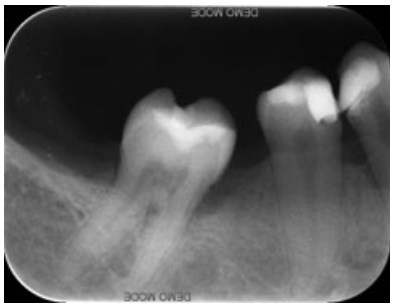

Fig. 2. Baseline radiograph of a case treated with PRF–BPBM (mesial of tooth no. 31).

root planing of the quadrants involving teeth to be treated were performed using hand curettes and an ultrasonic device under local anesthesia. Occlusal adjustment was performed if trauma from occlusion was diagnosed. Trauma from occlusion was evaluated by examining the obvious presence of fremitus in centric occlusion or in working or balancing excursions.

Six to 8 wk following phase I therapy, a periodontal re-evaluation was performed to confirm the suitability of the sites for this periodontal surgical study. The study used a split-mouth design, in which two interproximal sites were randomly (toss of a coin, performed by the study therapists) assigned to the PRF or PRF–BPBM group.

Calibration of the study examiner

All surgeries were performed by two periodontists. An examiner other than the surgeons performed all clinical measurements without knowledge of the treatment groups. Intra-examiner calibration was achieved by taking presurgical measurements on three patients undergoing periodontal therapy twice, 48 h apart, prior to beginning the study. Calibration was accepted when measurements at baseline and at 48 h were similar to the nearest whole millimeter at ≥ 90% level.

Presurgical clinical measurements

Occlusal stents to standardize the position of the periodontal probe were fabricated with cold-cured acrylic resin on a cast model obtained from an alginate impression. The occlusal stent was made to cover the occlusal surface of the tooth being treated, as well as the occlusal surfaces of at least one adjacent tooth in the mesial and distal directions. Stents were also extended apically on the buccal and lingual surfaces in order to cover the coronal third of the clinical crowns of the teeth involved. Grooves were placed so that measurements made postsurgery could be done using the same probe position and angulation as those made prior to surgery. All steps described above were developed in order to obtain an occlusal stent that was both stable when in position and easy to be removed by the therapist and the examiner.

Prior to surgery, the plaque index (34) and gingival sulcus beeding index (35) were measured. With the acrylic stent in position, the periodontal probe was inserted into the pocket at the angle necessary to reach the deepest portion of the interproximal pocket. Angulation varied between 10 and 20 degrees. A pencil mark was made where the probe made contact with the acrylic stent, and a groove was made on the pencil-marked area with a cylindrical low-speed burr. Using the groove as guide, the periodontal probe was reinserted into the pocket, and pocket depth (using the gingival margin as reference), gingival recession (using the most apical end of the stent as reference) and relative attachment level (using the most apical end of the stent as reference) were recorded. Measurements were performed with a Marquis periodontal probe and recorded to the nearest millimeter. The same measurements were repeated on buccal and lingual surfaces of each interproximal defect.

Platelet-rich fibrin preparation

Immediately before the surgical procedure, 10 mL of blood was drawn from the subject's antecubital vein. The blood sample was collected in glass-coated plastic tubes not containing any anticlotting agent. The blood-containing tubes (Z Serum Clot Activator: Vacuette, Kremsmunster, Austria) were immediately centrifuged (Labofuge 300; Heraus GmbH, Hanau, Germany) at 1000 *g* for 10 min. The centrifuged blood mass presented with a structured fibrin clot in the middle of the tube, between the red corpuscle layer on the bottom and the acellular plasma on top. The fibrin clot could easily be removed from the tube and shaped freely, and was used immediately after its collection. In the present study, PRF was compressed between two tongue blades in order to take the form of a consistent membrane, which was applied over the treatment defects whole or minced, as described in the text below.

Surgical procedures and intrasurgical measurements

The surgical procedure was performed by local infiltration of 2% lidocaine containing epinephrine at a concentration of 1:100,000. Buccal and lingual sulcular incisions were made and mucoperiosteal flaps elevated. Care was exercised to preserve as much interproximal soft tissue as possible. Complete debridement of the defects, as well as scaling and root planing to ensure root smoothness, were achieved with the use of an ultrasonic device and hand curettes (Figs 3 and 4). Measurements of the osseous defects were made utilizing the same grooves previously employed to record pocket depth and attachment levels. The distance between the most apical end of the stent and the point at which the groove-adapted probe made contact with the bottom of the defect was recorded at buccal and lingual sites.

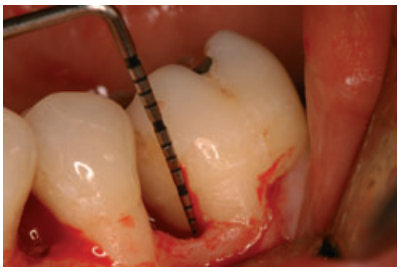

Fig. 3. Intra-operative defect of the case shown in Fig. 1 (PRF group).

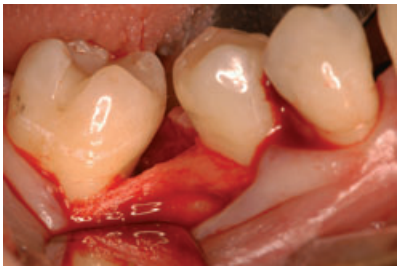

Fig. 4. Intra-operative defect of the case shown in Fig. 2 (PRF–BPBM group).

Other grooves, one buccal and one lingual, were also fabricated in order to measure the distance between the most apical end of the stent and the interproximal alveolar crest that formed the superficial border of the defect.

In the PRF–BPBM group, cancellous BPBM granules (Bio-Oss; Geistlich AG, Wolhusen, Switzerland) with particle sizes of 0.25–1.0 mm were mixed with PRF that had been minced into pieces about 0.5 mm × 0.5 mm at a proportion of 1:1 (v/v). The PRF–BPBM mixture was delivered to the defect and packed with amalgam condensers to the level of the surrounding bony walls. Care was taken not to overfill defects. A membrane of compressed PRF was trimmed and adapted over the grafted defect. Membranes were extended over the periphery of the defect in the buccal and lingual directions and secured in place using 5–0 gut sutures anchored to the adjacent teeth. Defects in the PRF group were filled with minced PRF only. A membrane of compressed PRF was adapted over the minced PRF-filled defect in the same manner as described for the PRF–BPBM group.

Flaps in both groups were repositioned and sutured with 4–0 silk sutures using an interrupted technique. Periodontal dressing was placed over the surgical area, and antibiotics (amoxicillin 500 mg every 8 h for 7 d) and 0.12% chlorhexidine gluconate rinses (every 12 h for 14 d) were prescribed. Patients were also prescribed oral analgesics (ibuprofen 400 mg, every 4 h as needed for pain).

In both groups, all defects amenable to periodontal regenerative treatment that were present in the same quadrant as the study defect were treated with the same therapeutic modality as the study defect. All sites in the quadrant that required periodontal surgical treatment but were not amenable to periodontal regenerative therapy (i.e. defects that were candidates for resective osseous treatment) would be treated during the re-entry surgery.

Postoperative follow-up care

The dressing and silk sutures were removed 1 wk postoperatively. Patients were instructed to initiate mechanical oral hygiene, consisting of brushing and flossing or interproximal brushing, at the end of the second postoperative week. Patients were examined weekly up to 1 mo after the surgeries, and then at 2, 3 and 6 mo. Postoperative care included reinforcement of oral hygiene and professional plaque removal whenever necessary.

During the first four postoperative visits, the surgically treated areas were evaluated using the healing index (HI; 36). Healing index scores healing on the basis of redness, presence of granulation tissue, bleeding, suppuration and epithelialization. A score of 1–5 is given, where 1 is associated with very poor healing, while 5 is considered excellent healing.

Re-entry surgeries

Six months (±7 d) following the initial surgery, all presurgical clinical measurements were repeated as described above and surgical re-entries were performed. Peri-apical films were taken with the long-cone parallel technique immediately before re-entry surgeries (Figs 5 and 6). Surgical re-entries consisted of buccal and lingual full-thickness flaps to access the interproximal bone (Figs 7 and 8). The criteria for hard-tissue measurements

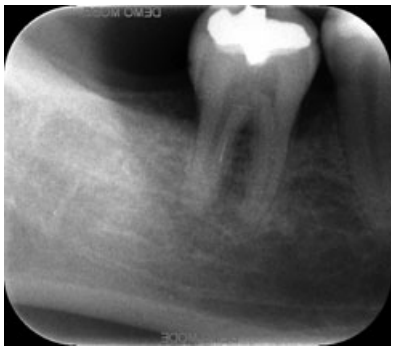

Fig. 5. Postoperative radiograph of the case treated with PRF shown in Figs 1 and 3.

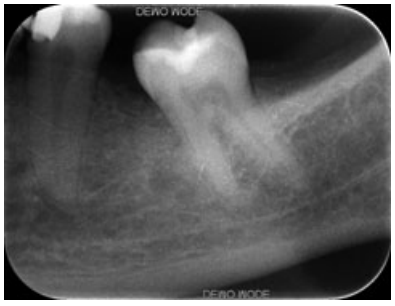

Fig. 6. Postoperative radiograph of the case treated with PRF–BPBM shown in Figs 2 and 4.

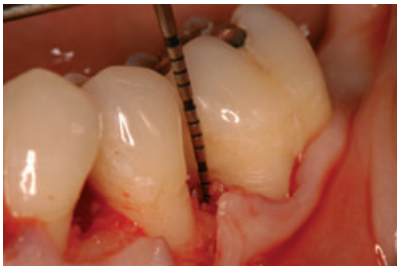

Fig. 7. Re-entry surgery of the case treated with PRF (Figs 1, 3 and 5).

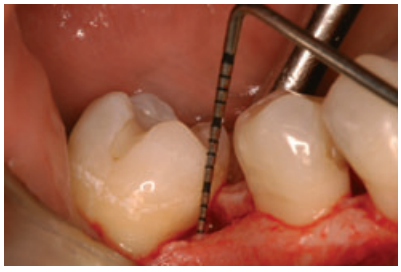

Fig. 8. Re-entry surgery of the case treated with PRF (Figs 2, 4 and 6).

were to remove all granules of the graft material that were surrounded by soft tissue or that were clearly loose and not fully incorporated into what clinically appeared to be bone. Therefore, granules of the graft material that were visible, but surrounded by hard tissue were regarded as bone upon clinical inspection and left undisturbed. If the tip of the periodontal probe touched any of the incorporated graft particles during any of the measurements, it was regarded as a legitimate landmark. All intrasurgical measurements taken at the time of the initial surgeries were repeated during the re-entry surgeries using the same acrylic stent. If residual defects were present in the treated areas, resective osseous surgery was employed for their elimination. Resective osseous surgery was also the therapy of choice for other defects in the quadrant of the study defect that, in the judgement of the therapist, could be improved by this modality of therapy.

Statistical analysis

Clinical measurements for each group were averaged (means ± SEM). The net difference between each pair of measurements (pre- and postoperative) was calculated, followed by computation of the difference between treatment groups. Intra- and intergroup comparisons were conducted using the chi-squared test. Values of $p \leq 0.05$ were regarded as statistically significant.

For the primary study outcomes, namely pocket depth, attachment level and defect fill, a sample size of 17 paired defects would confer power to identify intergroup differences of 1 mm as statistically significant at the 5% level (95% certainty).

Table 1. Changes in tissue healing index[a]

	PRF	PRF–BPBM	*p*-Value
7 d	3.53 ± 0.50	3.59 ± 0.49	> 0.05 (n.s.)
14 d	3.94 ± 0.54	3.88 ± 0.47	> 0.05 (n.s.)
21 d	4.12 ± 0.47	4.18 ± 0.51	> 0.05 (n.s.)
28 d	4.35 ± 0.48	4.41 ± 0.49	> 0.05 (n.s.)

Values are given as means ± SEM. n.s., not statistically significant.
[a] Tissue healing index of Landry *et al.* (36).

Table 2. Individual inbrabony defect location and morphology

Patient	Tooth number and surface	Treatment group	Defect type: number of walls	Bony walls present
1	4D	PRF–BPBM	3	DBL
	13D	PRF	3	DBL
2	14M	PRF–BPBM	2	ML
	3M	PRF	2	ML
3	22M	PRF–BPBM	3	MBL
	28D	PRF	2	DL
4	5M	PRF–BPBM	3	MBL
	11M	PRF	3	MBL
5	11D	PRF–BPBM	2	DL
	6M	PRF	2	ML
6	31M	PRF–BPBM	2	ML
	19D	PRF	3	DBL
7	22D	PRF–BPBM	3	DBL
	28D	PRF	2	DL
8	31M	PRF–BPBM	2	MB
	19M	PRF	2	ML
9	20M	PRF–BPBM	2	ML
	29D	PRF	2	DL
10	30D	PRF–BPBM	2	DL
	19D	PRF	2	DL
11	29D	PRF–BPBM	2	DL
	20D	PRF	3	DBL
12	20D	PRF–BPBM	2	DL
	30M	PRF	2	ML
13	21M	PRF–BPBM	2	ML
	29M	PRF	2	ML
14	5M	PRF–BPBM	3	MBL
	13M	PRF	2	ML
15	14M	PRF–BPBM	2	ML
	3M	PRF	2	ML
16	5D	PRF–BPBM	2	DL
	14D	PRF	2	DL
17	12M	PRF–BPBM	2	ML
	15M	PRF	2	ML

D, distal; B, buccal; L, lingual; M, mesial; PRF, platelet-rich fibrin; BPBM, bovine porous bone mineral

Results

All 17 patients completed the study. Healing in cases treated with either PRF or PRF–BPBM was uneventful. Table 1 shows the healing index for both groups during the first 28 postoperative days.

The individual characteristics of intrabony defects treated in both groups are listed in Table 2. In three patients (nos 12, 16 and 17), the study teeth included a molar and a nonmolar. The molar intrabony lesions were diagnosed in flat surfaces (no furcation invasions associated with the defect) and were therefore believed to be a fair comparison to intrabony lesions present on flat surfaces of nonmolar teeth.

There were no significant differences in pocket depth between the two groups at baseline. Changes is pocket depth are reported in Table 3. Both PRF and PRF–BPBM groups showed significant pocket depth reduction at 6 mo compared with baseline. Mean pocket reduction in the PRF group was 3.35 ± 0.68 mm on buccal and 3.24 ± 0.73 mm on lingual sites and in the PRF–BPBM group 4.47 ± 0.78 mm on buccal and 4.29 ± 0.82 mm on lingual sites. The differences observed between the two groups were

statistically significant in favor of the PRF–BPBM group.

Clinical attachment level changes for the two groups are reported in Table 4. The PRF group presented with a clinical attachment gain of 2.24 ± 0.73 mm on buccal sites and 2.12 ± 0.78 mm on lingual sites, while the gain for the PRF–BPBM group was of 3.82 ± 0.78 mm on buccal and 3.71 ± 0.75 mm on lingual sites. The differences in attachment gain observed between the two groups were significantly better in the PRF–BPBM group.

Postsurgical gingival recession was similar for both treatment groups, as shown in Table 4.

Table 5 reports the changes in defect fill for both groups. The PRF–BPBM group presented with significantly greater defect fill (4.06 ± 0.87 mm on buccal and 3.94 ± 0.73 mm on lingual sites) than the PRF group (2.21 ± 0.68 mm on buccal and 2.06 ± 0.64 mm on lingual sites). Both PRF and PRF–BPBM groups presented with resorption of the alveolar crest adjacent to the defect, but the differences between the two groups were not statistically significant (Table 5).

Plaque measurements and sulcular bleeding index were not significantly different between the two groups at baseline or at 6 mo (Table 6).

Table 3. Changes in pocket depth (in millimeters; means ± SEM) as measured from gingival margin (n = 17 paired defects)

	Site	PRF	PRF–BPBM	p-Value
Initial	Buccal	7.82 ± 1.10	7.94 ± 1.16	> 0.05 (n.s.)
	Lingual	7.76 ± 0.94	7.88 ± 1.02	> 0.05 (n.s.)
6 mo	Buccal	4.47 ± 0.70	3.47 ± 0.50	< 0.001*
	Lingual	4.53 ± 0.50	3.59 ± 0.60	< 0.001*
Mean reduction	Buccal	3.35 ± 0.68	4.47 ± 0.78	< 0.001*
	Lingual	3.24 ± 0.73	4.29 ± 0.82	< 0.001*

n.s., not statistically significant.
* Statistically significant.

Table 4. Changes in attachment level and gingival recession (in millimeters; means ± SEM) as measured from acrylic stent (n = 17 paired defects)

Site	PRF	PRF–BPBM	p-Value
Attachment level			
Mean gain (initial to 6 mo)			
Buccal	2.24 ± 0.73	3.82 ± 0.78	< 0.001*
Lingual	2.12 ± 0.68	3.71 ± 0.75	< 0.001*
Gingival recession			
Mean change (initial to 6 mo)			
Buccal	1.06 ± 0.42	0.65 ± 0.59	> 0.05 (n.s.)
Lingual	1.12 ± 0.32	0.59 ± 0.49	> 0.05 (n.s.)

n.s., not statistically significant.
* Statistically significant.

Table 5. Changes in defect fill (means ± SEM) and alveolar crest (in millimeters; means ± SEM) as measured from acrylic stent (n = 17 paired defects)

	Site	PRF	PRF–BPBM	p-Value
Mean fill (initial to 6 mo)	Buccal	2.21 ± 0.68	4.06 ± 0.87	< 0.001*
	Lingual	2.06 ± 0.64	3.94 ± 0.73	< 0.001*
Mean resorption (initial to 6 mo)	Buccal	1.06 ± 0.42	0.94 ± 0.23	> 0.05 (n.s.)
	Lingual	1.18 ± 0.38	1.12 ± 0.32	> 0.05 (n.s.)

n.s., not statistically significant.
* Statistically significant.

Table 6. Changes in plaque index[a] (means ± SEM) and gingival sulcus bleeding index[b] (means ± SEM)

	PRF	PRF–BPBM	p-Value
Plaque index			
Initial	0.62 ± 0.32	0.60 ± 0.30	> 0.05 (n.s.)
6 mo	0.57 ± 0.29	0.59 ± 0.28	> 0.05 (n.s.)
p-Value	> 0.05 (n.s)	> 0.05 (n.s.)	
Gingival sulcus bleeding index			
Initial	1.41 ± 0.77	1.35 ± 0.76	> 0.05 (n.s.)
6 mo	1.24 ± 0.73	1.17 ± 0.71	> 0.05 (n.s.)
p-Value	> 0.05 (n.s.)	> 0.05 (n.s.)	

[a] Plaque index of Silness and Löe (34).
[b] Gingival sulcus bleeding index of Mühlemann and Son (35).
n.s., not statistically significant.

Discussion

Autologous platelet concentrations have attracted the attention of researchers and clinicians as a way to accelerate and enhance wound healing in surgical wounds in both dentistry and medicine. There are several techniques that can be used to prepare autologous platelet concentrates (26). These techniques employ distinct preparation steps, and they result in products that differ in their content with respect to the amount of platelets and leukocytes, as well as fibrin density and its spatial organization. The reader is referred to a review by Dohan Ehrenfest *et al*. (26) for a comprehensive description and classification of platelet concentrates.

The main characteristics of PRF compared with other platelet concentrates, including platelet-rich plasma, are that it does not require any anticlotting or gellifying agent (37), the naturally forming PRF clot has a dense and complex three-dimensional architecture, and this type of clot concentrates not only platelets but also leukocytes. Based on these characteristics, PRF is

simpler and less expensive to prepare, as well as being less risky to patients because it does not expose them to animal-derived anticlotting agents. Owing to its dense fibrin matrix, PRF takes longer to be resorbed by the host, which results in the slower and sustained release of platelet- and leukocyte-derived growth factors (27,28) into the wound area. Finally, by virtue of containing leukocytes, PRF may exert an antibacterial effect in the wound (38) and work as an abundant source of vascular endothelial growth factor, which is a key player in angiogenesis (39). Overall, PRF has physical and biochemical attributes that make it attractive for application in periodontal wound healing, and for these reasons it was investigated as a potential regenerative agent for intrabony periodontal defects.

In the present study, the decision to utilize minced PRF membranes as defect fillers (alone or in combination with BPBM) was made because of its ease of manipulation and delivery to the surgical site. Once defects were filled, they were covered by a PRF membrane, which was sutured in the desired position. The intended role of the PRF membrane was to contain the BPBM and/or minced PRF in the intrabony defect in the early phase of healing. It is unlikely, although not impossible, for the PRF membrane to have exerted a guided tissue regeneration effect on the wound. Similar to a natural clot, the PRF membrane is resorbed in approximately 7 d (30), which is a substantially shorter period of time than the 4–6 wk necessary for guided tissue regeneration to occur (2).

Despite the fact that PRF is a denser and firmer agent than other biological preparations, such as platelet-rich plasma and enamel matrix proteins, it is still nonrigid to a degree that its space-maintaining ability in periodontal defects is not ideal. It has been reported that the combination of a mineralized, rigid graft material, such as BPBM, with a semi-fluid, nonrigid agent, such as enamel matrix proteins (8), significantly enhanced the clinical outcome of intrabony defects treated without the addition of BPBM. For that reason, we chose BPBM, hypothesizing that it could enhance the effects of PRF by maintaining the space for tissue regeneration to occur, as well as by exerting an osteoconductive effect in the intrabony defect area.

Results of this clinical trial showed that both treatment groups presented with high HI scores early after surgeries (first and second postoperative weeks). The HI improved even further during the third and fourth weeks after surgery, further supporting the potential positive effects of PRF in the healing process. This outcome may be related to the extremely high density of fibrin fibers detected in PRF. The high density of fibrin fibers provides additional stability to the wound and promotes rapid neo-angiogenesis (40). The HI improvements achieved with PRF could also be explained as being a result of elevated concentrations of various polypeptide growth factors in the surgical wound. Polypeptide growth factors may enhance soft-tissue healing by increasing the angiogenesis and matrix biosynthesis during the wound healing process.

Defects treated with PRF alone showed significant improvements in all clinical parameters compared with baseline. Clinical attachment gain and defect fill exceeded 2 mm (2.12–2.24 mm and 2.06–2.21 mm, respectively) and pocket depth reduction was in the range of 3.24–3.35 mm in defects that were severe at baseline, with pocket depths averaging 7.76–7.94 mm. Defects treated with PRF had an average 6 mo postoperative pocket depth in the range of 4.47–4.53 mm, which is not ideal but considered reasonably accessible to scaling and root planing during the maintenance phase of periodontal therapy (41). This clinical trial did not include a control group that was treated with open-flap debridement. Therefore, no definitive conclusions can be drawn with respect to the benefits of PRF beyond those associated with the surgical procedure alone. While comparisons between different studies must be made with care, our research group has treated intrabony defects with similar severity at baseline to the ones treated in this clinical trial with open flap debridement (9,42,43). The results of those studies revealed that changes in pocket depth, clinical attachment gain and defect fill were approximately 0.5–1 mm inferior to those observed with PRF treatment in this clinical trial. This interstudy comparison suggests that PRF used alone may exert a positive, yet modest effect when used as sole treatment for severe intrabony defects. This hypothesis needs to be tested, however, in an independent clinical trial, because interstudy comparisons can be unreliable.

Combining BPBM with PRF resulted in significantly greater pocket depth reduction, gain in clinical attachment and defect fill than PRF used alone. The differences observed between the two treatment groups could not be attributed to significantly different levels of gingival recession (Table 4), plaque or gingival inflammation (Table 6). Also, greater defect resolution in the PRF–BPBM group could not be attributed to a more accentuated resorption of the alveolar crest in the PRF group. Therefore, the differences in clinical parameters observed between the two treatment groups in this trial can be attributed to the use of BPBM with a high degree of certainty. As stated above, the possible reasons for the advantages observed with the combination of BPBM with platelet-rich plasma are that BPBM maintains the space for tissue formation to occur and works as a scaffold for the growth of mineralized tissue. Even though an attempt was made to remove all unincorporated particles of BPBM before the re-entry bone measurements were made, it cannot be fully assured that all such particles were removed; therefore, the possibility of BPBM granules influencing bone measurements in favor of the group that received the xenograft should not be discarded.

Autologous platelet-rich plasma shares some similarities with PRF, with the main one being the delivery of a great number of platelets and its associated elevated concentration of polypeptide growth factors to the surgical wound. In a human clinical trial, platelet-rich plasma failed to augment the effects of BPBM in intrabony periodontal defects (44). Obviously,

the design of the presemt study does not allow for an evaluation of the ability of platelet-rich plasma to enhance the regenerative effects of BPBM, and such a comparison deserves to be investigated in a separate trial. The reason not to have included a BPBM-alone group in the present study was the difficulty associated with recruiting patients with three similar intrabony defects instead of two. Evidently, a study with three arms would have been the ideal design and the best way to compare the regenerative capability of the xenograft in relation to PRF. That being stated, the effectiveness of BPBM used as monotherapy for intrabony defects has already been investigated (10,44). Based on these studies where intrabony defects were treated with BPBM, it can be speculated that this graft material possesses properties that make it highly effective in improving clinical parameters associated with periodontal regeneration of intrabony defects, and may therefore obfuscate any positive regenerative effect exerted by autologous platelet concentrates in the treatment process.

In conclusion, the data from this study suggest, firstly, that treatment of intrabony defects with PRF results in significant improvements of pocket depth, clinical attachment level and defect fill compared with baseline and, secondly, that BPBM significantly increases the regenerative effects observed with PRF in the treatment of human intrabony defects. The long-term results associated with both modalities of therapy, as well as the histological nature of newly formed tissues by either treatment, remains to be elucidated.

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