Original Article

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Effect of platelet-rich plasma and bioactive glass in the treatment of intrabony defects - a split-mouth study in humans

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Abstract

Aim: To compare the efficacy of platelet rich plasma (PRP) associated with bioactive glass (BG) and BG alone in the treatment of periodontal intrabony defects. **Methods:** Ten patients participated of the study. Using a split-mouth design, interproximal bony defects were surgically treated with either PRP/BG or BG alone. The clinical parameters plaque index, gingival index, gingival bleeding index, pocket probing depth (PPD), clinical attachment level (CAL) and gingival recession were recorded, and the defect fill was evaluated radiographically at baseline and 3 and 6 months after surgery. **Results:** At 6 months after therapy, the PRP/BG group showed mean PPD reduction of 3.4±1.4 mm, CAL mean gain of 4.3±1.3 mm, and defect fill of 3.5±1.0 mm. The BG group showed mean PPD reduction of 2.6±1.1 mm, mean CAL gain of 3.3±1.3 mm, and defect fill of 3.1±1.2mm. There was statistically significant greater PPD reduction at 3 months and CAL gain at 6 months for PRP/BG compared to BG alone, but no significant difference was observed in defect fill. **Conclusions:** Both therapies resulted in significant PPD reduction, CAL gain and defect fill. The association of PRP with a BG graft material seemed to add some benefits to the improvement of the clinical parameters in the treatment of intrabony defects.

Keywords: intra-bony defect, periodontal regeneration, platelet-rich plasma, bioactive glass.

Introduction

The goal of any periodontal therapy is the control of active inflammation, the arrest of disease progression and the reconstruction of structures lost to disease, where appropriate[1]. Although the effectiveness of scaling and root planing or surgical access for root planing plus regular maintenance care in moderate to severe periodontal disease cases has been well established, the efficacy is judged based on the ability of the therapy to improve osseous lesions[2].

Periodontal regeneration is a multifactorial process and requires an orchestrated sequence of biological events including cell adhesion, migration, multiplication and differentiation which involves recruitment of locally derived progenitor cells to the site[3]. Therapies capable of achieving this goal include guided tissue regeneration (GTR), autografts, allografts, alloplasts, growth factors and combination of these techniques as well as osseous resective surgery[4-6]. Although observations from histological studies in humans and data from controlled clinical trials have demonstrated that some of the available grafting procedures may result in periodontal regeneration in intrabony defects, but complete reconstruction on a regular predictable basis has been difficult to achieve[7-8].

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Autogenous bone graft taken from intraoral or extraoral donor sites is a well accepted treatment option in the periodontal community, but it has disadvantages of limited availability of donor sites and requirement for an additional surgical site to obtain the graft material. The use of an allograft may have risk of disease transmission and questions have been raised to their osteogenic potential[9-10]. This has renewed interest in evaluating alloplastic bone substitutes in treatment of intraosseous defects.

Bioactive glass (BG) is a synthetic material composed of sodium and calcium salts, phosphates and silicon dioxide. It has been suggested that BG has advantages of forming strong bond with both bone and soft connective tissue and to having modulus of elasticity similar to that of bone thus preventing the formation of intervening fibrous connective tissue interface[11]. BG has an osteostimulatory effect in addition to its osteoconductive properties[12], and it has also shown to have antibacterial effect against subgingival and supragingival bacteria[13]. Low et al.[9] and Zamet et al.[14] reported good clinical results in intrabony defects in sites treated with a BG when compared to debridement.

As the knowledge of the fundamentals of bone regeneration has increased, Marx et al.[15] studied the growth factors contained within platelets in relation to bone regeneration. Several growth factors are deposited in the extracellular matrix where they are released during matrix degradation and they act as a part of a complex network of signals with mutual effects during tissue remodeling and regeneration. Platelet derived growth factor (PDGF), transforming growth factor-β (TGF-β) and insulin-like growth factor (IGF) may be available for this purpose[3,1516].

Platelet-rich plasma (PRP) is an autologous concentration of platelets in plasma developed by gradient density centrifugation and contains many growth factors, such as PDGF, TGF-β, vascular endothelial growth factor, and others. PRP also contains proteins known to act as cell adhesion molecules for osteoconduction and as a matrix for bone, connective tissue, and epithelial migration[15-17].

One major criterion for periodontal regeneration is the maintenance of a wound space for the periodontal ligament cells to migrate into. For growth factors to exert their potential, they require a medium that can provide this space, and thus cell induction and differentiation can be obtained by combining a bone graft with PRP in the wound space,. Marx et al.[15] demonstrated a 1.62-2.16-fold increase in bone maturation using autologous platelet preparation in combination with a bone autograft compared to bone autograft alone in large human mandibular continuity defects. However, few studies have focused on the application of PRP in combination with alloplastic graft materials, evaluating the treatment outcomes in intra-bony defects[18-20]. Therefore, the purpose of this study was to compare clinically and radiographically the regenerative potential of a combination of PRP and BG to that of BG alone in the treatment of periodontal intrabony defects.

Material and methods

Ten systemically healthy 25-45-year-old patients with moderate to advanced chronic periodontitis were selected from the Department of Periodontology and Implantology of the Meenakshi Ammal Dental College and Hospital, Chennai, India. The study design was approved by the University Institution Review Board. Informed consent was obtained from each patient following the information about the treatment plan in the form of a duly signed document prior to the surgical phase. The oral hygiene conditions of the patients were evaluated 2 weeks after the initial therapy (oral hygiene instructions and four quadrants of scaling and root planing, and occlusal adjustment if required).

Criteria for inclusion in the study were (1) having a good level of oral hygiene [plaque index (PI) <1 Loe 1967], (2) probing pocket depth (PD) $\geq$ 6mm (3) radiographic evidence of at least one vertical bony defect in bilateral sites of mandibular posterior teeth. The exclusion criteria comprised systemic disease, compromised immune system, tobacco smoking, history of antibiotics usage 6 months prior to surgery and treatment for periodontitis during the last 6 months and molars with furcation defects.

The selected sample included 20 sites (premolars and molars) in 10 patients where two interproximal sites were randomly (toss of a coin) assigned to the PRP/BG or BG groups in a split-mouth design. Ten sites in Group I were treated with PRP and BG graft (Perioglas®; US Biomaterials Corporation, Alachua, FL, USA) and 10 sites in Group II were treated with BG alone.

Clinical recordings

Customized acrylic stents were fabricated with guiding grooves to provide reproducible alignment for a periodontal probe and the cemento-enamel junction was used as a reference point (Figure 1). The outcome variables included PI (Silness and Loe 1964)[21], gingival index (GI) (Loe and Silness 1963)[22], gingival bleeding index (GBI) (Ainamo and Bay 1975)[23], clinical attachment level (CAL), probing pocket

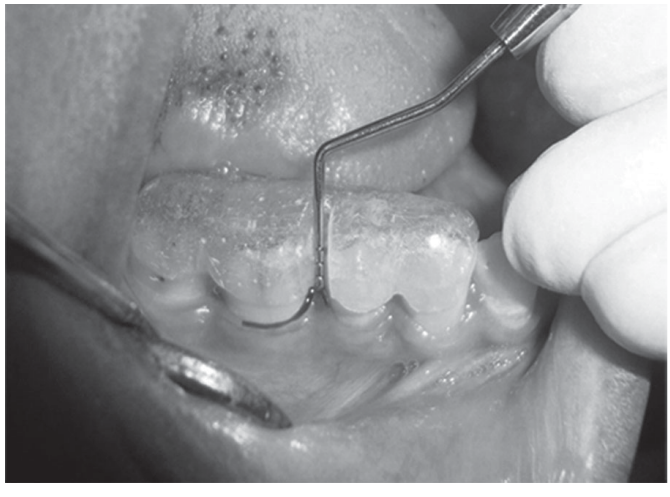

Fig. 1. Customized acrylic stent with guiding grooves.

depth (PPD) and gingival recession (GR). All measurements were made at six sites *per* tooth (mesio-facial, mid-facial, disto-facial, mesio-lingual, mid-lingual, distolingual). Measurements were performed with a Williams probe to the nearest millimeter. All clinical and radiographic measurements were performed by a single examiner.

Radiographic examination

A commercially available film holder device with putty impression material was used on bite blocks to index the dentition. Standardized reproducible digital radiographs using a standardized paralleling cone technique with positioning aids were taken at each treated site and imported into Sopro® imaging software (Satelec), which yields an accuracy of 0.1mm.

Defects were radiographically evaluated to measure defect fill: distance from the cementoenamel junction to base of the defect (CEJ-BD), alveolar crest height: distance from the cementoenamel junction to the alveolar crest (CEJ-CD). All clinical and radiographic measurements were performed by a single examiner at baseline, and 3 and 6 months after the surgical procedure. Percentage of defect resolution was calculated as preoperative minus postoperative defect depth/ preoperative defect depth $\times$ 100.

Preparation of PRP with BG

Nine milliliters of whole blood was drawn by venipuncture of the antecubital vein and collected into two 4.5ml blood collection tubes. These tubes contained 0.105mol/l buffered sodium citrate. The tubes were initially centrifuged at 200g for 10 min. and the plasma part was separated from the red blood cells (RBCs). The whole plasma portion and the top layer of the RBCs, which include fresh platelets, were transferred to a second sterile tube. Second centrifuge procedure was performed at 250 g for 10 min leaving the PRP at the bottom of the tube. The upper portion of the plasma, namely platelet-poor plasma (PPP), was discarded. Finally, 0.5 mL of PRP, 0.3 mL 0.025 M $CaCl_2$ and the bovine thrombin were mixed in the vial containing the BG graft and left for gelation.

Surgical procedure

A single surgeon performed all surgeries. After proper isolation of the surgical field, the operative sites were anaesthetized using 2% xylocaine hydrochloride with adrenaline (1:200,000). Intra-crevicular incisions were performed, extending to the neighboring teeth and full thickness flaps were raised retaining sufficient tissue to attain primary closure. No conditioning of the root surfaces was performed. Defect debridement and root planning were carried out carefully with hand and ultrasonic instruments to remove subgingival plaque, calculus and inflammatory granulation tissue.

The PRP preparation for Group I (PRP/BG) started 30 min before surgery. After debridement and pre-suturing, the combination of PRP and BG was placed in sites treated as group I(Figure 2), while BG alone was mixed with 4 to 6 drops saline, according to the manufacturer's instructions, and was placed in sites treated as group II (Figure 3). Graft was condensed to adapt the particles to configuration of defect taking care not to over fill the defect. Every effort was made to avoid contamination of debrided root surface with saliva and blood until the graft material was applied. After grafting, flaps were repositioned to their original levels and periodontal dressing was placed (Coe pak)™. Patients were instructed to avoid chewing in the surgical area during the first post-operative day and were recommended to refrain from mechanical plaque control, in and around the surgical area for 2 weeks. Systemic antibiotics (Amoxicillin 500 mg 3 times a day for 5 days) and (Ibuprofen 400 mg 3 times a day for 3 days) were prescribed to achieve an analgesic and anti-inflammatory effect with instructions to rinse the mouth twice daily with a solution of 0.2% chlorhexidine digluconate for 2 weeks to aid in plaque suppression. Sutures were removed 2 weeks following surgery. Recall appointments were scheduled once in 10 days for the 1st month and every 4 weeks thereafter. At every recall appointment oral hygiene was checked and reinforced. All clinical and radiographic measurements were repeated at 3 months and 6 months after the initial surgery.

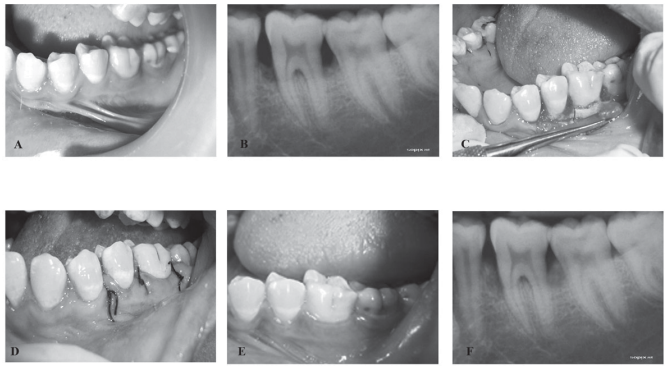

Fig. 2. Surgical procedure in Group I (PRP/BG). A. Preoperative clinical view; B. Preoperative radiograph with intrabony defects on the mesial and distal sides of tooth 36; C. Intraoperative view depicting graft placement after full-thickness flap elevation; D. Postoperative view (after flap closure); E. Postoperative view (6 months); F. Postoperative radiograph (6 months).

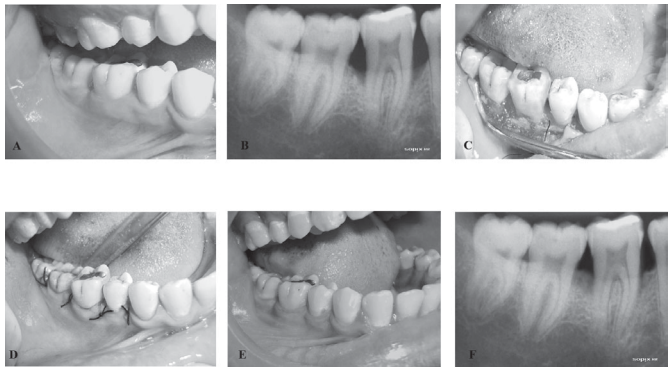

Fig. 3. Surgical procedure in Group II (BG alone). A. Preoperative clinical view; B. Preoperative radiograph with intrabony defects on mesial and distal sides of tooth 46; C. Intraoperative view depicting graft placement after full-thickness flap elevation; D. Postoperative view (after flap closure); E. Postoperative view (6 months); F. Postoperative radiograph (6 months)

Statistical analysis

Mean values (± standard deviations) were reported for the clinical and radiographic parameter. Taking into account the nature of changes from baseline to 6 months in each group, the Wilcoxon Signed Ranked test was employed to test mean and standard deviation of clinical and radiographic parameters for both groups at baseline, 3 months and 6 months. The Mann Whitney test was used to assess the significance of mean differences between groups from baseline to 3 months, baseline to 6 months, and 3 months to 6 months.

Results

All 10 patients completed the study. Healing was uneventful in all cases with no noted side effects or unusual complaints.

Oral hygiene level and infection control

A mean plaque index at baseline was 0.7 ± 0.1, with significant reduction to 0.4 ± 0.1 at 3 months and 6 months, thus demonstrating good compliance with oral hygiene instructions. Mean GI and bleeding on probing values showed significant reduction at both measurement points compared to baseline (Table1).

Probing pocket depth, clinical attachment level and gingival recession

The differences between groups at baseline for PPD and GR parameters were not statistically significant ($p > 0.05$); however, both groups showed significant changes for PPD, CAL and GR at different time intervals compared to baseline. Changes in PPD are reported in Table 2. The mean reduction in PPD was 3.7 ± 1.4 mm and 3.0 ± 1.2 mm from baseline to the 3rd month ($p = 0.10$), and 3.4 ± 1.4 mm and 2.6 ± 1.1 mm up to the 6th month ($p = 0.10$) for PRP/BG and BG group, respectively, with no statistically significant difference. There was a slight increase in mean PPD from 3 to 6 months for both groups ($p = 0.74$). CAL gain for PRP/BG and BG groups was 3.9 ± 1.4 mm and 3.5 ± 1.2 mm, respectively, from baseline to the 3rd month, and 4.3 ± 1.3 mm and 3.3 ± 1.3 mm up to 6th month, with statistically significant difference ($p = 0.03$). At 6 months, there was no significant change between study groups for PPD however there was significant gain in CAL in PRP/BG group compared to BG group (4.3 mm versus 3.3 mm), which was consistent with slightly higher preoperative CAL (9.7 mm versus 9.1 mm). Both groups showed a statistically significant reduction in GR with coverage of 0.9 ± 0.2 mm for PRP/BG group and 0.7 ± 1.0 mm BG group from baseline to 6th month, though without significant difference between among the groups ($p = 0.7$).

Radiographic measurements

Table 3 reports changes in defect fill and alveolar crest resorption for both groups. Both groups presented with resorption of alveolar crest adjacent to the defect (-0.2 ± 0.07 mm for PRP/BG group and -0.2 ± 0.08 mm for BG group from baseline to the 6th month), but the difference between the two groups was not statistically significant ($p = 0.90$).

The mean defect fill was similar for both groups at all time intervals. From baseline to the 6th month, PRP/BG and BG groups showed a mean defect fill of 3.5 ± 1.0 mm and 3.1 ± 1.2 mm, respectively, with no statistically significant difference ($p = 0.72$).

The mean percentage of defect resolution was slightly greater for PRP/BG group ($50.3 \pm 9.8\%$ and $52.0 \pm 11.2\%$) compared to the BG group with mean defect resolution of $45.1 \pm 14.0\%$ was 46.3 ± 13.7 from baseline to the 3rd month and baseline to the 6th month, respectively. Though the mean percentage of defect resolution was slightly higher for the PRP/BG group, but it was not statistically significant ($p = 0.44$).

Discussion

A wide array of new materials has been used for promoting periodontal regeneration in intraosseous defects. The bone replacement grafts provide regeneration through conductive or inductive processes and in combination with growth factors, have the potential to optimize the outcome of periodontal regeneration. Proliferation and migration of periodontal ligament cells and synthesis of extracellular matrix as well as differentiation of cementoblasts and osteoblasts is a prerequisite for obtaining periodontal regeneration and growth factors (PRP) may represent a

Table 1 - Changes in plaque index (PI), gingival index (GI), gingival bleeding index (GBI) scores*(full mouth).

Baseline		3 months	6 months	0-3 month	0-6 months	3-6 months
PI	0.7±0.1	0.4±0.1	0.4±0.1	0.28±0.09 p-value 0.005(S)	0.28±0.1) p-value 0.004(S)	0.0±0.03 p-value 0.31(NS)
GI	1.33±0.32	0.78±0.11	0.71±0.12	0.55±0.22 p-value 0.004(S)	0.62±0.22 p-value 0.004(S)	0.07±0.04 p-value 0.004(S)
GBI**	73.8±15.4	37.5±2.2	28.7±4.1	36.3±15.6 p-value 0.004 (S)	45.1±16.7 p-value 0.004 (S)	8.8±4.1 p-value 0.004(S)

*Mean ± standard error of the mean; **% of bleeding sites; Sig, significant; NS, not significant.

Table 2 Changes in probing pocket depth (PPD), clinical attachment level (CAL) and gingival recession (GR) in millimeters*.

		Baseline	3 months	6 months	0-3month	0-6 month	3-6 month
PPD	PRP+BG	8.1±1.4	4.4±0.5	4.7±0.8	3.7±1.4 0.005(S)	3.4±1.4 0.005(S)	-0.3±0.7 0.18(NS)
	BG	8.0±1.2	5.0±0.5	5.4±0.5	3.0±1.2 0.004(S)	2.6±1.1 0.004(S)	-0.4±0.5 0.046(S)
	Comparison p-value	1.00 (NS)	0.01 (S)	0.07 (NS)	0.10 (NS)	0.10 (NS)	0.74 (NS)
CAL	PRP+BG	9.7±1.3	5.8±1.3	5.4±1.1	3.9±1.4 0.004(S)	4.3±1.3 0.004(S)	0.4±0.8 0.16 (NS)
	BG	9.1±1.4	5.6±1.2	5.8±1.0	3.5±1.2 0.005(S)	3.3±1.3 0.005(S)	-0.2±0.8 0.41 (NS)
	Comparison p-value	0.03 (S)	0.61(NS)	0.21 (NS)	0.34(NS)	0.03 (S)	0.10(NS)
GR	PRP+BG	1.6±1.0	1.4±0.9	0.7±0.8	0.2±0.9 0.15(NS)	0.9±0.2 0.034(S)	0.7±0.2 0.059(NS)
	BG	1.1±1.2	0.6±1.0	0.4±1.2	0.5±0.2 0.059(NS)	0.7±1.0 0.02(S)	0.2±1.2 0.41(NS)
	Comparison p-value	0.24(NS)	0.06(NS)	0.51(NS)	0.3(NS)	0.7(NS)	0.41(NS)

*Mean ± standard deviation; S, significant; NS, not significant; PRP, platelet-rich plasma; BG, bioactive glass

Table 3 Changes in defect fill and in alveolar crest resorption in millimeters*

	Defect Fill			Alveolar Crest		
	PRP+BG	BG	Comparison p-value	PRP+BG	BG	Comparison p-value
Baseline	6.7±1.0	6.5±1.3	0.72(NS)	3.6±0.6	3.7±0.5	0.42(NS)
3 months	3.3±0.6	3.4±0.9	0.81(NS)	3.7±0.6	3.9±0.5	0.40(NS)
6 months	3.2±0.8	3.4±1.1	0.61(NS)	3.8±0.5	4.0±0.8	0.54(NS)
0-3months	3.4±1.0 0.005(S)	3.1±1.3 0.005(S)	0.68(NS)	-0.1±0.04 0.004(S)	-0.1±0.07 0.004(S)	0.42(NS)
0-6 months	3.5±1.0 0.005(S)	3.1±1.2 0.005(S)	0.72(NS)	-0.2±0.07 0.004(S)	-0.2±0.08 0.004(S)	0.90(NS)
3-6 months	0.1±0.3 0.26(NS)	0.02±0.2 0.71(NS)	0.44(NS)	-0.1±0.07 0.009(S)	-0.09±0.07 0.014(S)	0.53(NS)

*Mean ± standard deviation; S, significant; NS, not significant; DF, defect fill; AC, alveolar crest height

potential aid in attempts to regenerate the periodontium[15].

The present study was designed to evaluate the efficacy of associating PRP with a BG material. The lack of adverse reactions, abscesses, or rejection of implanted materials, suggested that BG and PRP used were tolerated well and in line with observation from previous studies[14,18-19,24] that failed to show any foreign body reaction during initial healing and thereafter in the 6-month evaluation period. PRP is an autogenous preparation, and is inherently safe and free from concerns over transmissible diseases. In the present study, each patient was placed on monthly recall visits, including supragingival scaling and patient motivation to maintain the hygienic conditions which led to significant change for PI, GI and GBI at 3 and 6 months after treatment. Cortellini et al.[25] suggested that monthly periodic controls enhanced patient cooperation and infection control.

The results of this study have demonstrated that treatment of deep intrabony defects with both, the combination of PRP/

BG and BG alone demonstrated significant reduction in various periodontal parameters. Froum et al.[26] compared BG graft material and open flap debridement in the treatment of 59 intrabony defects in a 12-month reentry study, and observed 4.26 mm PPD reduction, 2.96 mm CLA gain, and 3.28mm of defect fill. Lovelace et al.[27] observed that BG is capable of producing similar results to DFDBA in short term of 6 months in moderate to deep periodontal osseous defects. However, there is no histological evidence in humans that BG may promote true periodontal regeneration[10]. Nevins et al.[28] confirmed the new attachment at only one tooth out of five human intrabony defects that were treated with BG.

There are only a few studies comparing the clinical effects of PRP/graft combination with a graft material alone. In one of the study by Ouyang XY et al.[29] and Hanna et al.[30] revealed additional effectiveness of PRP + BPBM to BPBM in CAL gain in the treatment of intrabony defects. Okuda et al.[18] compared a combination of PRP and HA with a mixture of HA and saline in a 1-year study. Their study revealed a statistically significant CAL gain of 4.7 mm in PRP + HA group versus 3.7 mm with HA alone. However, the benefit of PRP to HA in reduction of bony defect depth measured on radiographs was not found in their study which was comparable to results of our study. The results of the present study demonstrated a mean PPD reduction of 3.4 ± 1.4mm, CAL gain of 4.3 ± 1.3 mm, GR coverage of 0.9 ± 0.2 mm, and a mean defect fill of 3.5 ± 1.0 mm in the PRP/BG group; and PPD reduction of 2.6 ± 1.1 mm, CAL gain of 3.3 ± 1.3 mm, GR coverage of 0.7 ± 1.0 mm, and a mean defect fill of 3.1 ± 1.2 mm for the BG group after 6 months. There was statistically significant reduction in PPD at 3 months and gain in CAL from baseline to 6 months with PRP/BG. GR coverage was statistically significant from baseline to 6 months in both the groups. In contrast to this, Demir et al.[20] compared the same graft materials and found a mean CAL gain of 3.1 ± 0.5 mm and a mean defect fill of 3.5 ± 0.5mm in the PRP/BG group and CAL gain of 2.9 ± 0.4mm and mean defect fill of 3.4 ± 0.6 in BG group, which failed to show significant difference in any of the investigated parameters. GR was 0.47 ± 0.19mm for the PRP/BG group and 0.43 ± 0.25 mm for the PRP/BG group, both of which were not statistically significant ($p > 0.05$). Using a similar study design, Harnack et al.[31] demonstrated no additional effect of PRP/ beta-TCP over beta-TCP alone in 22 patients with contralateral intrabony defects in a 6-month reentry study. This may be attributed to the use of a different study design.

The explanation for slightly higher mean gain in CAL for PRP/BG could be the potential of PRP to contribute in soft tissue healing[32]. Arikan et al.[33] and Caceres et al.[34] suggested the ability of PRP to stimulate gingival fibroblast and to modulate several cell responses potentially involved in wound healing, such as cell adhesion, cell migration, and myofibroblastic differentiation.

The method of PRP preparation is an important aspect to be considered. Different techniques of preparation have been known to yield substantially different amounts of cells, i.e., platelets and leukocytes as well as different levels of growth factors[35-36]. As periodontal defects are small in size, obtaining a large amount of blood from patients for PRP preparation is unnecessary, hence only 9 mL of venous blood was withdrawn. According to the technique suggested by Landesberg et al.[37], two centrifugal forces of 200 g for 10 min provide the maximum increase at platelet number. Dugrillon et al.[38] stated that an increased amount of g force decreases the amount of growth factor instead of increasing it. The number of platelets in PRP is another important issue in the literature about PRP, though many studies including a recent study by Creeper et al.[39] observed the effect of PRP on osteoblasts and periodontal ligament cell function to be concentration specific, Christgau et al.[40] showed only a weak correlation between the platelet counts or the growth factor levels and the clinical and radiographic regeneration outcomes.

It should also be kept in mind that PRP, as used in this study, may affect wound healing not only by a release of PGFs from platelets, but also due to its other physical and chemical properties. According to Obarrio et al.[41], PRP preparation assumes a sticky consistency, due to its high fibrin content, which works as a haemostatic and stabilizing agent and may aid the blood clot and bone graft immobilization in the defect area.

In conclusion, the present results indicate that at 6 months after surgery both therapies resulted in significant PPD reduction, CAL gain, GR coverage and defect fill. The association of PRP and BG group provided good soft tissue response with an additional effect on the CAL for intrabony defects. Future long-term clinical and histological studies should be undertaken to determine the efficacy of bioactive ceramics in combination with PRP in the treatment of intrabony defects.

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