



RESEARCH ARTICLE

COMPARATIVE ANALYSIS OF POROUS HYDROXYAPATITE BONE GRAFT WITH AND WITHOUT PLATELET RICH PLASMA IN THE TREATMENT OF PERIODONTAL INTRABONY OSSEOUS DEFECTS

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ABSTRACT

Background: Today, regenerative attempts for treatment of periodontal disease focus on the introduction of a filler material into the defect in hope of inducing bone regeneration. The purpose of this study was to clinically and radiographically evaluate the use of porous hydroxyapatite bone graft with and without platelet-rich plasma (PRP) in the treatment of intrabony defects. **Materials and Methods:** The study was carried out in ten patients between 18 and 60 years. Patients with pocket depth ≥ 5 mm and radiographic evidence of vertical bone loss in the affected site were randomly assigned to treatment with a combination of PRP + Hydroxyapatite (HA) (test sites) or HA alone (control sites). The parameters were compared at baseline and 6 months postoperatively. **Results:** There was a statistically significant reduction in probing depth and gain in clinical attachment in both the groups individually (more in experimental group); however, on comparing the two groups, the net reduction was not significant. Radiographic assessment showed a decrease in the defect size in both the groups. **Conclusion:** PRP in addition to a bone graft in the treatment of intrabony defects is safe and shows improved defect fill as compared to the use of bone graft alone.

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INTRODUCTION

Periodontal therapy has always strived to control or eliminate periodontal disease in an attempt to restore the structures, integrity, and the function of tissues that have been lost as a result of inflammatory periodontal disease (Sunitha, 2010). A well-coordinated sequence of a number of biologic events including cell migration, adherence, multiplication, and differentiation has increased the predictability of periodontal regeneration (Arvidson *et al.*, 2011). Treatment of intrabony defects has often focused on the bony defect and this has led to the use of a number of grafting materials to stimulate bone repair (Carranza *et al.*, 2002). Bone grafting materials when retained in the defect site provide a structural framework for clot development, maturation, and remodeling that supports bone formation in osseous defects (Reynolds *et al.*, 2010). A wide array of bone graft substitutes is available today and has shown to produce greater clinical bone defect fill than flap debridement alone (Nasr, 1999). Bioceramic alloplasts primarily composed of calcium phosphate are available as tricalcium phosphate and hydroxyapatite. Hydroxyapatite became the ceramic of choice, producing predictable short-term and long-term results (Aichelmann-Reidy, 1998).

The graft material acts as a biocompatible material within the gingival tissue, and as it resorbs, it acts as a mineral reservoir and assists bone formation through osteoconductive mechanisms, resulting in clinically acceptable responses (Yukna, 2000; Wagner, 1989). A different approach used for periodontal regeneration in the present era is the use of growth factors that are a class of naturally occurring proteins which effectively stimulate the formation of mineralized as well as nonmineralized tissues (Plachokova *et al.*, 2008). Platelet-rich plasma (PRP) as introduced by Marx is defined as an autologous concentration of platelets in a small volume of plasma and is considered to be a rich source of autologous growth factors (Marx, 1998). In the field of dentistry, PRP has been used in different clinical procedures (i.e., sinus floor elevation, alveolar ridge augmentation, mandibular reconstruction, maxillary cleft repair, treatment of periodontal defects, gingival recession, and treatment of extraction sockets), where it has been applied alone or in addition to bone grafts (Carlson *et al.*, 2002; Alissa *et al.*, 2010; Boyapati, 2006; Rutkowski, 2010; Wojtowicz, 2007; Kumar, 2012). PRP once grafted into the defect site begins to release alpha granules within 10 min of clot development and secrete over 90% of their prepackaged growth factors within 1

h, thereby initiating a greater and faster initial cellular response than a normal blood clot. Platelet-rich-derived fibrin clot formation stimulates collagen synthesis in the periodontium and effectively promotes wound healing at sites of injury in periodontal tissue (Lacoste, 2003; Rodrigues, 2012). Thus, the purpose of this study was to clinically and radiographically evaluate the use of porous hydroxyapatite bone graft with and without PRP in the treatment of periodontal intrabony osseous defects.

MATERIALS AND METHODS

Patient selection: This randomized controlled study was carried out in the Department of Periodontics Govt Dental College Srinagar after approval by the Ethical Committee. The criteria for inclusion were systemically healthy individuals between age groups 18 and 60 years of either sex, no history of any medication affecting the periodontium in the past 6 months, and those who had not undergone any periodontal treatment in the past 6 months. Patients who had a clinical evidence of an intrabony defect with probing pocket depth (PD) ≥ 5 mm and radiographic evidence of angular bone loss in the affected site were included in the study. Patients excluded were individuals with systemic diseases (diabetes mellitus and platelet deficiencies), pregnant and lactating females, individuals with a present history of tobacco usage, and individuals on anticoagulant or immunosuppressive therapy.

Initial therapy: The patients were subjected to oral prophylactic procedures, occlusal equilibration, if required, and routine laboratory investigations before surgery. Patients oral hygiene status was evaluated by plaque Index (Silness and Loe) (Løe, 1967) and gingival index (Loe and Silness) (Loe, 1962). On reevaluation of Phase I therapy, only those patients who had attained a score of ≤ 1 were selected for the surgical phase.

Clinical parameters: To standardize the reproducibility of clinical measurements, occlusal acrylic stents for positioning the periodontal probe were fabricated on a cast obtained from an alginate impression (Camargo *et al.*, 2002). The following clinical parameters were recorded

Radiographic parameters: Preoperative radiographs were obtained at baseline and then at 3 and 6 months postsurgery. The size of the defect or defect fill was measured using depth of the intrabony component, and the bony defect width as described by Eickholz *et al.* (2004)

Group allocation: Before the commencement of the surgical procedure, the site to be treated was randomly allocated into experimental (PRP + Hydroxyapatite (HA)) or control (HA alone) study groups.

Platelet rich plasma procurement: Just before the surgery, 10 ml of blood was withdrawn from the antecubital vein of the patients and collected in tubes containing sodium citrate anticoagulant. The test tube was placed into the automated centrifuge machine always ensuring that the tubes were counterbalanced, as per the centrifuge manual. The first cycle of 2400 rpm for 10 min separated the whole blood into a platelet-poor plasma layer at the top, a white buffy coat in the middle, and a layer of red blood corpuscles (RBC) at the bottom. The upper two layers and the top 1–2 mm of RBC layer were expressed into another tube (without anticoagulant) and

centrifuged at 3600 rpm for 15 min that resulted in an upper portion of clear yellow supernatant with a very low concentration of platelets and a d-tinged bottom layer with highly concentrated platelets. At the time of application, PRP was combined with an equal volume of a sterile saline solution containing 10% calcium chloride (a citrate inhibitor) and human thrombin (an activator) which resulted in a sticky gel that was relatively easy to apply in surgical defects (Okuda, 2005; Tözüm, 2003)

Surgical procedure: A standardized conventional periodontal flap surgery was performed by a single operator. The site was anesthetized using adequate local anesthesia (2% lidocaine hydrochloride with adrenaline 1:80,000). Intra- and extra-oral incisions were given and full thickness mucoperiosteal flaps were elevated to expose the defect. A thorough debridement was carried out to ensure a clean site followed by thorough root planing. For the control site, adequate quantity of the graft (Biograft HA) was mixed with a few drops of saline to obtain a workable mass and the defect was filled (Figure 1). At the experimental site, HA graft was mixed with PRP gel in a proportion of 1:1 and was inserted up to the vertical height of the corresponding adjacent bone level (Figure 2). Flap was repositioned and sutured with 3-0 silk suture material (Ethicon) followed by a periodontal dressing. Postoperative instructions and medications were prescribed to the patients and were recalled after 10 days for suture removal. Postoperative care included reinforcement of oral hygiene and scaling when necessary. Patients were periodically monitored and the clinical and radiographic parameters were recorded at 3 and 6 months postsurgery.

Statistical analysis: The collected data were assessed for both the control and the experimental groups individually as well as compared with each other using SPSS v19. Baseline, 3 months and 6 months postoperative data were tabulated and analyzed statistically.

RESULTS

In the present study, twenty sites were selected from ten systemically healthy individuals (4 males 6 females; average 38.3 years) after fulfilling the inclusion criteria and were randomly allocated to control group or experimental group with the arch-wise distribution as shown in Table 1. There was a reduction in mean plaque and gingival index scores from baseline to 6 months in both the groups with no statistical difference between the groups at all time periods (Table 2). A statistically significant mean PD reduction was observed in the control group from baseline to 6 months (4.1 ± 1.66 ; $P = 0.000$) with a greater PD reduction in the experimental group (4.4 ± 1.35 ; $P = 0.000$). The difference between both the groups with respect to the mean PD reduction was statistically insignificant at different time intervals ($P > 0.05$) (Table 3). There was a greater CAL gain in the experimental group than in the control group at the end of 6 months (50% vs. 43.24%), although both of which were statistically significant ($P = 0.00$). On comparison between the groups, the change in the differences of their means from baseline to 3 and 6 months was not significant ($P > 0.05$) (Table 3). No significant difference in the amount of gingival recession was seen in both groups at any point of time ($P = 0.09$ in control group and $P = 0.46$ in the test group from

baseline to 6 months). The difference between the groups was also not statistically significant ($P = 0.34$) (Table 3).

Hence, the particle size of porous hydroxyapatite bone graft (Biograft HA) used in this study was 350–500 μm .

Table 1. Distribution of intrabony defect in relation to tooth type and treatment modality

Group	Arch	Anterior	Bicuspid	Molar	total
Test (10)	Maxilla mandible	31	11	22	64
Control (10)	Maxilla mandible	50	01	22	73

Table 2. Comparison of mean values of oral hygiene status within and between control and experimental groups

Parameter	Time interval	Control group		Experimental group		Control versus experimental group	
		Mean \pm SD	Difference from baseline (P)	Mean \pm SD	Difference from baseline (P)	Difference in difference of means	P
Plaque index	Baseline	0.53 \pm 0.22	-	0.7 \pm 0.37	-	-0.17 \pm 0.50	0.21
	3 month	0.45 \pm 0.26	0.07 \pm 0.26 (0.39)	0.5 \pm 0.31	0.2 \pm 0.23 (0.02)*	-0.125 \pm 0.33	0.27
	6 month	0.25 \pm 0.20	0.27 \pm 0.28 (0.01)*	0.2 \pm 0.19	0.5 \pm 0.26 (0.00)*	-0.225 \pm 0.38	0.07
Gingival index	Baseline	0.85 \pm 0.27	-	0.725 \pm 0.34	-	0.125 \pm 0.27	0.37
	3 month	0.42 \pm 0.26	0.425 \pm 0.43 (0.01)*	0.425 \pm 0.21	0.30 \pm 0.28 (0.00)*	0.125 \pm 0.42	0.45
	6 month	0.22 \pm 0.20	0.625 \pm 0.39 (0.00)*	0.2 \pm 0.19 0.525 \pm	0.29 (0.00)*	0.1 \pm 0.26	0.53

*Significant $P < 0.05$. Value for difference between groups indicates that experimental group value is higher than control group. SD: Standard deviation

Table 3. Comparison of mean values of various parameters within and between control and experimental groups

Parameter	Time interval	Control group		Experimental group		Control versus experimental group	
		Mean \pm SD	Difference from baseline (P)	Mean \pm SD	Difference from baseline (P)	Difference in difference of means	P
Pocket probing depth	Baseline	6.5 \pm 1.43	-	- 7.6 \pm 1.35	-	-1.1 \pm 2.02	0.09
	3 month	3.1 \pm 1.29	3.4 \pm 1.95 (0.0003)*	4.2 \pm 0.92	3.4 \pm 1.07 (0.0000)*	0 \pm 2.58	1.0
	6 month	2.4 \pm 1.17	4.1 \pm 1.66 (0.000)*	3.2 \pm 0.92	4.4 \pm 1.35 (0.0000)*	-0.3 \pm 2.54	0.66
Gingival recession	Baseline	0.9 \pm 0.74	-	- 0.6 \pm 0.84	-	- 0.3 \pm 0.82	0.40
	3 month	1.8 \pm 1.55	-0.9 \pm 1.37 (0.06)	1.0 \pm 1.05	-0.4 \pm 0.96 (0.22)	-0.5 \pm 1.50	0.35
	6 month	1.8 \pm 1.62	-0.9 \pm 1.52 (0.09)	0.9 \pm 1.10	-0.3 \pm 1.25 (0.46)	-0.6 \pm 1.57	0.32
Defect size/ defect fill	Baseline	62.22 \pm 20.5	-	- 91.95 \pm 22.21	-	-29.73 \pm 29.22	0.06
	3 month	36.74 \pm 16.3	25.4 \pm 16.26 (0.007)*	46.93 \pm 30.22	45.02 \pm 13.28 (0.01)*	-19.55 \pm 16.15	0.008*
	6 month	26.81 \pm 14.6	35.4 \pm 22.41 (0.007)*	39.38 \pm 24.64 0.06	52.5 \pm 14.92 (0.000)*	-17.15 \pm 22.90	0.06

*Significant $P < 0.05$. -: Value for difference between groups indicates that experimental group value is higher than control group. SD: Standard deviation

In the control group, the amount of defect fill from base line to 6 months post treatment was 56.91% ($P = 0.0007$). For the experimental group, greater defect fill was observed from baseline to 6 months (57.16%; $P = 0.00$, respectively). When a comparison was made between the groups, statistically significant difference ($P = 0.008$) was seen in the differences of means between the groups from baseline to 3 months. However, from baseline to 6 months, the difference was not significant ($P = 0.06$) (Table 3).

DISCUSSION

The management of periodontal osseous defects including the destruction of the periodontium has always been a challenge in clinical periodontics. A plethora of literature is available substantiating the use of bone graft materials along with growth factors with an aim to optimize the outcome of periodontal regeneration by assisting the proliferation, migration, and differentiation of periodontal ligament cells, cement oblasts, and osteoblasts (Pradeep, 2009) This study combined PRP with porous hydroxyapatite bone graft (Biograft HA®) to enhance the regenerative potential of the graft used. Various bone grafting materials have been used to fill periodontal intrabony defects with particle size between 300 and 500 μm in diameter, which has resulted in clinically acceptable responses (Yukna, 2000; Mellonig, 1992)

It has been seen that porous HA bone grafts have excellent bone conductive properties which permit outgrowth of osteogenic cells from existing bone surfaces into adjacent bone graft material (Stahl, 1987) Since there are no organic components contained in HA, this bone graft material does not induce any allergic reaction; however, true periodontal regeneration is not achieved because the healing which occurs is a connective tissue encapsulation of the graft with a long junctional epithelium (Meffert, 1985; Sculean, 2004). Different techniques of PRP preparation have been known to yield substantially different amounts of cells, i.e., platelets and leukocytes as well as different levels of growth factors (Weibrich, 2002). As periodontal defects are small in size, only 8–10 ml of venous blood was withdrawn with a preparation time of about 30 min that is performed simultaneously during the surgery, thereby not increasing the chair-side time. The method of procurement of PRP used in this study was similar to that used in the study performed by Okuda *et al.* (2005) According to de Obarrio *et al.* (2000) PRP preparation assumes a sticky consistency, due to high fibrin content, making it a hemostatic and stabilizing agent that aids bone graft immobilization and has been suggested as an important event in wound healing. PRP is an autogenous preparation and is inherently safe and free from concerns over transmissible diseases (Pradeep, 2009). In the present study also, the lack of adverse reactions, abscesses, or

rejection of implanted materials suggested that HA and PRP used were well tolerated and failed to show any foreign body reaction during the entire study period. Improvement in plaque and gingival scores in the present study can be attributed to the fact that only those patients who showed maintenance of optimal oral hygiene were included in the study, and this level was maintained throughout the study period by reinforcement of plaque control measures and oral hygiene instructions at various recall periods. These results are in accordance with the results of Hanna *et al.* (2004) and Okuda *et al.* (2005) who reported that all patients enrolled for the study maintained very low mean plaque and gingival index scores at baseline and 6 months demonstrating high compliance with oral hygiene instructions. The change in probing depth and clinical attachment level could not be attributed to any significant difference in the levels of oral hygiene between both the groups. Periodontal pocket is considered as a pathognomonic sign of periodontal disease and reduction in PD is one of the requisites for successful periodontal therapy. When both experimental and control groups were assessed individually, the mean reduction in the probing depth from baseline to 6 months showed statistically significant results. This reduction can be attributed to the decrease in inflammation, shrinkage of the pocket wall, change in the tissue tone and the placement of graft material into defect, that may modify the gingival tissue consistency thereby impeding the penetration of periodontal probe.(34,35).

Results of the present study are in conformity with the triple combination therapy including PRP, bovine porous bone mineral (BPBM), and guided tissue regeneration (GTR) carried out by Lekovic *et al.*, (2002) who reported a slightly greater reduction of PD (4.19 ± 0.81) in the test group (PRP + BPBM + GTR) when compared to 3.98 ± 1.02 in the control group (PRP + BPBM) implying that GTR adds no clinical benefit to PRP + BPBM. Okuda *et al.* (2005) reported that the test group (PRP + HA) exhibited statistically significant changes compared to the control sites (HA alone) which differ from the results of the present study due to a longer duration of evaluation, although the mean PD reduction of both groups was comparable to the present study. The mean gain in the clinical attachment levels was greater in the experimental group than the control group 6 months Post treatment. On comparing the two groups, the results were found insignificant during any of the time intervals. The explanation for slightly higher mean gain in CAL for PRP + HA could be the potential of PRP to contribute in tissue healing. Arikan *et al.* (2007) and Cáceres *et al.* (38) 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 results of this study are comparable with the studies of Yilmaz *et al.* (2010) and Demir *et al.*(2007). The amount of gingival recession increased with time in both the groups; however, it was higher in the control group and was statistically not significant between the two groups at 6 months ($P = 0.34$). The results of the present study are in conformity with the results of the study carried out by Kaushick *et al.*, (2011) who reported no significant change in the levels of gingival margin between the groups at the end of 6 months. Following periodontal therapy, the reduction in the probing depth was due to a combination of gingival recession and gain in the attachment levels. Hence, the levels of the gingival margins were not significant. The defect fill from baseline to 3 months was

greater in the experimental group than in the control group with a significant difference on the intergroup comparison.

This can be interpreted as an increased remodeling of the graft due to addition of PRP which delivers a highly concentrated source of autologous platelets containing a variety of biological mediators and improves the handling properties of the graft material with which it is combined, facilitating graft placement and stability (Kaushick *et al.*, 2011). The bone gain by PRP + HA observed in this study is in accordance with that of Marx *et al.*,(10) who reported that the addition of PRP to grafts evidenced a radiographic maturation rate 1.62–2.16 times that of grafts without PRP. In the present study, there was no significant difference between the groups at the end of 6 months. The results are in accordance with the results of Okuda *et al.*,(2005) who observed no statistical difference in the mean radiographic intrabony defect gain between the PRP + HA group and saline + HA group at 12 months although better results were seen in the test group (PRP + HA was 70% and saline + HA was 56%).

These findings were explained by the fact that both treatments have the ability to retain HA granules in intrabony defects for a period of 12 months or longer, suggesting a longer period of monitoring to determine whether the end result is true regeneration rather than repair. Defect resolution following bone grafting can be a result of connective tissue encapsulation of the graft and the long junctional epithelium formation or because of remodeling of the graft and replacement by host bone.(Kaushick, 2011) The varying results from different studies may be derived from the use of different graft materials, the varying morphology of the initial defects, and/or study designs. The explanations of the reason for the lack of additive effect of PRP will be speculative due to the limitations of the present study which were small sample size, shorter follow-up period, absence of re-entry, and histological examination. Furthermore, the potential mechanisms of PRP for bone formation were not tested. No blood parameters were evaluated which might have led to the production of PRP with low platelet counts.

Conclusion

Both treatment modalities demonstrated a significant improvement in the probing depth, clinical attachment level, and radiographic size of the defect at 6 months. Within the limitations of the current study, it can be concluded that PRP addition to a bone graft in the treatment of periodontal intrabony osseous defects shows improved defect fill as compared to the use of bone graft alone. The synergistic effect of PRP is safe and effective in treatment of periodontal intrabony osseous defects. However, long-term clinical trials with larger sample size are needed to evaluate the individual role of PRP along with the regenerative potential when used in combination with bone substitutes.

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